COMPETENT AUTHORITY REPORT

1,2-Benzisothiazol-3-(2*H*)-one (BIT) (PT 13)

Document III-A

Active Substance

Rapporteur Member State: Spain
February 2022

Section A1 Applicant

Annex Point IIA, I 1

				Official use only
1.1	**Applicant**	Name:	[redacted]	**X1**
		Address:	Troy Chemical Company B.V. Uiverlaan 12E 3145 XN Maassluis The Netherlands	
		Telephone:	[redacted]	
		Fax number:	[redacted]	
		E-mail address:	[redacted]	
1.2	**Manufacturer of Active Substance** (if different)	See Doc. III, Business Confidential Information		**X2**
1.3	**Manufacturer of Product(s)** (if different)	Not applicable, since the product defined in this dossier is a theoretical product.		

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *May 2020*

Conclusion *The applicant's version is adopted with clarifications.*

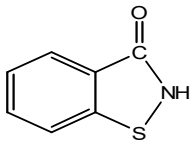
Remarks *(X1) The applicant changed his contact details in 2019. The new contact details are:*

Name	*Troy Chemical Company B.V.*
Address	*Poortweg 4C* *2612 PA* *Delft* *The Netherlands*
Contact person	[redacted]
Telephone	[redacted]
Fax number	--
Mobile	[redacted]
E-mail address	[redacted]

(X2) This information is included in the Doc IIIA.

Section A2 Identity

Annex point IIA, II 2 **Identity of Active Substance**

Subsection (Annex Point)			Official use only
2.1	**Common name (IIA, II)**	1,2-Benzisothiazol-3-(2*H*)-one	
2.2	**Chemical name (IIA, II 2.2)**	1,2-Benzisothiazol-3-(2*H*)-one	
2.3	**Manufacturer´s development code number(s) (IIA, II 2.3)**	Not applicable	
2.4	**CAS No and EC numbers (IIA, II 2.4)**		
2.4.1	CAS-No	2634-33-5	
2.4.2	EC-No	220-120-9 (EINECS)	
2.4.3	Other	Not applicable	
2.5	**Molecular and structural formula, molecular mass (IIA, II 2.5)**		
2.5.1	Molecular formula	C_7H_5NOS	
2.5.2	Structural formula		
2.5.3	Molecular mass	151.19 g/mol	
2.6	**Method of manufacture of the active substance (IIA, II 2.6)**	Please refer to Doc. III-A, Appendix 2 - Business Confidential Information.	**X1**
2.7	**Specification of the purity of the active substance, as appropriate (IIA, II 2.7)**	g/kg: > 986; g/L: ; % w/w: > 98.6; % v/v:	**X2, X3**

Section A2 Identity

Annex point IIA, II 2 **Identity of Active Substance**

		The above figures are calculated values for dry material derived from the results of a six-batch analysis performed with wet material containing ca. 30% w/w of water. The six-batch analysis is to be found in Doc IV as DocIIIA_02_07_01.pdf The calculation of the specification based on the results of the six-batch analysis is also to be found in Doc IV: DocIIIA_02-07-02.pdf	
2.8	**Identity of impurities and additives, as appropriate (IIA, II 2.8)**	Please refer to Doc. III-A, Appendix 2 - Business Confidential Information.	**X1**
2.8.1	Isomeric composition	Not relevant	
2.9	**The origin of the natural active substance or the precursor(s) of the active substance (IIA, II 2.9)**	Not applicable for 1,2-Benzisothiazol-3-(2*H*)-one as it is not a natural active substance	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

April 2014

May 2020

December 2020

Conclusion

The applicant's version is adopted with clarifications.

Remarks

No further remarks.

(X1) This information is included in the Doc IIIA confidential.

(X2) According to the information submitted by the applicant the source specifications are:

Name	*5-bacht analyses (g/kg) – dry matter*	*5-bacht analyses (g/kg) – wet matter*
	Minimum purity	*Minimum purity*
Source 1	*≥ 985.2*	*≥ 716.2*

Section A2 Identity

Annex point IIA, II 2 **Identity of Active Substance**

(X3) The applicant has submitted a new 5-batch analyses and therefore the new specifications are:

Name	*5-bacht analyses (g/kg) – dry matter*	*5-bacht analyses (g/kg) – wet matter*
	Minimum purity	*Minimum purity*
Source 1	*≥ 976.9*	*≥ 799.4*

Section A2 Identity

Subsection A2.10

Annex Point IIA2.10

EXPOSURE DATA IN CONFORMITY WITH ANNEX VIIA TO COUNCIL DIRECTIVE 92/32/EEC (OJ NO L, 05.06.1992, P. 1) AMENDING COUNCIL DIRECTIVE 67/548/EEC

Subsection		Official use only
2.10.1 Human exposure towards active substance		
2.10.1.1 Production		
i) Description of process	Not applicable for 1,2-Benzisothiazol-3-(2*H*)-one as it is manufactured outside Europe.	
ii) Workplace description	Not applicable for 1,2-Benzisothiazol-3-(2*H*)-one as it is manufactured outside Europe.	
iii) Inhalation exposure	Not applicable for 1,2-Benzisothiazol-3-(2*H*)-one as it is manufactured outside Europe.	
iv) Dermal exposure	Not applicable for 1,2-Benzisothiazol-3-(2*H*)-one as it is manufactured outside Europe.	
2.10.1.2 Intended use(s)		
1. Professional Users		**X**
i) Description of application process	Please refer to Document II-B (PT 13) for the different uses: paints, glues, cleaning fluids, textile production and fuels.	
ii) Workplace description	Please refer to Document II-B (PT 13) for the different uses: paints, glues, cleaning fluids, textile production and fuels.	
iii) Inhalation exposure	The estimated inhalation exposures calculated following the models established in the TNsG Part 2 are shown in Document II-B (PT 13).	
iv) Dermal exposure	The estimated dermal exposures calculated following the models established in the TNsG Part 2 are shown in Document II-B (PT 13).	
2. Non-professional Users including the general public		**X**
(i) via inhalational contact	This is not relevant since metalworking fluids are not indicated for non-professional users.	
(ii) via skin contact	This is not relevant since metalworking fluids are not indicated for non-professional users.	

Section A2 Identity

Subsection A2.10 EXPOSURE DATA IN CONFORMITY WITH ANNEX VIIA TO COUNCIL DIRECTIVE 92/32/EEC (OJ NO L, 05.06.1992, P. 1) AMENDING COUNCIL DIRECTIVE 67/548/EEC

Annex Point IIA2.10

(iii) via drinking water	As the recommended uses have no potential for water contact indirect exposure *via* drinking water is considered negligible.
(iv) via food	As the recommended uses have no potential for food contact indirect exposure *via* food is considered negligible.
(v) indirect via environment	The estimated secondary exposures are shown in Document II-B (PT13).

2.10.2 Environmental exposure towards active substance

2.10.2.1 Production

(i) Releases into water	It is proposed that this data point is not relevant as BIT is produced outside of the EU.
(ii) Releases into air	It is proposed that this data point is not relevant as BIT is produced outside of the EU.
(iii) Waste disposal	It is proposed that this data point is not relevant as BIT is produced outside of the EU.

2.10.2.2 Intended use(s)	PT 13 (Metalworking fluid) - [redacted] is intended for use as a preservative, which is effective against a range of microbes. [redacted] and the end use product to which it is added, are intended for use by professional applicators in indoor scenarios. [redacted] is also used post-addition in re-circulating systems in order to maintain bacterial control.
Affected compartment(s):	A Level I Mackay Fugacity Model was used to assess the distribution of BIT in the various environmental compartments: McAteer, N. (2007b) Environmental distribution of 1,2-benzisothiazol-3(2*H*)-one (Mackay Level I fugacity model), report no.: RI2007/07/13 (unpublished).
water	97.77 %
sediment	0.05 %
air	0.0004 %
soil	2.2 %
Predicted concentration in the affected compartment(s)	

Section A2	**Identity**
Subsection A2.10 **Annex Point IIA2.10**	**EXPOSURE DATA IN CONFORMITY WITH ANNEX VIIA TO COUNCIL DIRECTIVE 92/32/EEC (OJ NO L, 05.06.1992, P. 1) AMENDING COUNCIL DIRECTIVE 67/548/EEC**

water	Please refer to Doc. II-B
sediment	Please refer to Doc. II-B
air	Please refer to Doc. II-B
soil	Please refer to Doc. II-B

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*July 2010* *March 2015*
Materials and methods	*The applicant's version is adopted.*
Conclusion	*The applicant's version is adopted*
Reliability	
Acceptability	
Remarks	***X:*** *The assessment of human exposure was performed according to the TNsG on Human Exposure to Biocidal Products (2002, 2007) taking into account the User Guidance to report 2002 and the HEEG opinion on Human exposure assessment to biocidal products used in metalworking fluids (PT 13), (Ispra, 22/09/2008). Work place description and exposure data is described in Doc. II-B.* March 2015: Human Exposure Assessment will be revised to take into account comments received from MS and applicant after 2012 initial submission as well as ECHA BPWG's agreements.

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density (IIA, III 3.1)								
3.1.1 Melting point	US EPA 63-5	Purity: > 97% BIT (1,2-Benzisothiazol-3-(2*H*)-one); Batch number: CN-306-111 / Specification given in Doc. III-A, 2/2	Result: 159.5 - 160ºC Pressure: Not documented		Y	1	[redacted] 1994 A3.1.1	**X1**
	US EPA 830.6303	Purity: > 85% BIT (1,2-Benzisothiazol-3-(2*H*)-one); Batch number:200600114-56 / Specification given in Doc. III-A 3.1.2	Result: 155.3 – 155.8ºC Pressure: Not documented		Y	1	[redacted] 2007a *A3.1.2*	
3.1.2 Boiling point	OECD 103	Purity: 99.6% BIT (1,2-Benzisothiazol-3-(2*H*)-one); Batch number: A0213295001 / Specification given in Doc. III-A, 2/1	Result: 249.5 ± 0.6ºC Pressure: Not documented		Y	1	[redacted] 2007a A3.1.2	**X2** **X24**

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1.3 Bulk density/ relative density	US EPA 63-7	Purity: > 97% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: CN-306-111 / Specification given in Doc. III-A, 2/2	Result: 1.361 ± 0.02 g/mL at 20 ± 1ºC		Y	1	[redacted], 1994 *A3.1.1*	**X3**
	CIPAC method MT159	Purity: > 85% BIT (1,2-Benzisothiazol-3-(2*H*)-one); Batch number:200600114-56 / Specification given in Doc. III-A 3.1.2	Result: Pour density 0.412 g/mL Result: Tap density 0.616 g/mL		Y	1	[redacted], 2007a *A3.1.2*	
3.2 Vapour pressure and Henry`s Law Constant (IIA, III 3.2)								
Vapour pressure	US EPA 63-9	Pure BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: CN-306-111-2	Result: 1.5 x 10^{-4} Pa Temperature: 25 ± 1ºC		Y	1	[redacted], 1994 *A3.1.1*	**X4** **X5**
	US EPA 63-9 OECD 104 EC A4	Purity: > 97.42% [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: BT 17301/ Specification given in Doc. III-A, 2/1	Result: 3.02 x 10^{-3} Pa Temperature: 20ºC Result: 8.91 x 10^{-3} Pa Temperature: 25ºC		Y	1	[redacted] 2003 A3.2-2	**X6**

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
Henry´s Law Constant			Measured/calculated: calculated Result: 4.08 x 10^{-4} Pa x m^3 x mol^{-1}		NA	0 Calculation method		**X7** **X22**
3.3 Appearance (IIA, III 3.3)								
3.3.1 Physical state 1	Visual inspection	Purity: > 97% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: CN-306-111 / Specification given in Doc. III-A, 2/2	Result: Crystalline solid		Y	1	[redacted], 1994 *A3.1.1*	
3.3.1 Physical state 2	Visual inspection	Purity: > 85% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number:200600114-56 / Specification given in Doc. III-A 3.1.2	Result: Solid Powder		Y	1	[redacted], 2007a *A3.1.2*	
3.3.2 Colour 1	Visual inspection	Purity: > 97% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: CN-306-111 /	Result: Off-white		Y	1	[redacted], 1994 A3.1.1	

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
		Specification given in Doc. III-A, 2/2						
3.3.2 Colour 2	Visual inspection	BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number:200600114-56 / Specification given in Doc. III-A 3.1.2	Result: Ivory		Y	1	[redacted], 2007a A3.1.2	
3.3.3 Odour 1	Olfactory inspection	Purity: > 97% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: CN-306-111 / Specification given in Doc. III-A, 2/2	Result: Faint odour		Y	1	[redacted], 1994 *A3.1.1*	
3.3.3 Odour 2	Olfactory inspection	BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number:200600114-56 / Specification given in Doc. III-A 3.1.2	Result: Mild Musty to Odourless		Y	1	[redacted], 2007a *A3.1.2*	
3.4 Absorption spectra (IIA, III 3.4)								
UV/VIS	Directive 98/8/EC,	Purity: 83.5% BIT [1,2-Benzisothiazol-3-(2*H*)-one];	Result: pH < 2 at 5.05 µg/mL	Methanol solvent	Y	1	[redacted] 2007b A3.4-1	

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
	Annex IIA, III 3.4.1	Batch number: 2006-00114-56	λ_{max} 226 nm; ε 21.3 L mol^{-1} cm^{-1} λ_{max} 318 nm; ε 5.37 L mol^{-1} cm^{-1} pH < 2 at 10.1 µg/mL λ_{max} 226 nm; ε 21.8 L mol^{-1} cm^{-1} λ_{max} 318 nm; ε 5.58L mol^{-1} cm^{-1} pH 7 at 5.05 µg/mL λ_{max} 224 nm; ε 13.9 Lmol^{-1} cm^{-1} λ_{max} 246 nm; ε 10.8 Lmol^{-1} cm^{-1} λ_{max} 319 nm; ε 4.68 L mol^{-1} cm^{-1} pH 7 at 10.1 µg/mL λ_{max} 224 nm; ε 13.8 L mol^{-1} cm^{-1} λ_{max} 245 nm; ε 9.47 L mol^{-1} cm^{-1} λ_{max} 319 nm; ε 4.69 L mol^{-1} cm^{-1}					
	OECD 101	100 % pure BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: 060309/01	pH < 2 at 104 g/L λ_{max} 226 nm; ε 20200 L mol^{-1} cm^{-1}		Y	1	[redacted]	**X8**

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
			λ_{max} 236 nm; ε 9320 L mol^{-1} cm^{-1} pH < 2 at 259 g/L λ_{max} 246 nm; ε 5800 L mol^{-1} cm^{-1} λ_{max} 261 nm; ε 3420 L mol^{-1} cm^{-1} λ_{max} 316 nm; ε 4590 L mol^{-1} cm^{-1} pH 7-8 at 104 g/L λ_{max} 226 nm; ε 17500 L mol^{-1} cm^{-1} λ_{max} 236 nm; ε 8840 L mol^{-1} cm^{-1} pH 7-8 at 259 g/L λ_{max} 245 nm; ε 6360 L mol^{-1} cm^{-1} λ_{max} 262 nm; ε 3430 L mol^{-1} cm^{-1} λ_{max} 316 nm; ε 4610 L mol^{-1} cm^{-1} pH > 12 at 104 g/L λ_{max} 222 nm; ε 11000 L mol^{-1} cm^{-1} λ_{max} 248 nm; ε 11600 L mol^{-1} cm^{-1}				[redacted] 2006a A3.4-2	

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
			pH > 12 at 259 g/L λ_{max} 287 nm; ε 2930 L mol^{-1} cm^{-1} λ_{max} 321 nm; ε 4100 L mol^{-1} cm^{-1}					
IR	Information not available	100 % pure BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: 060309/01	Result: The major absorbances are obtained at 743, 1148, 1316, 1324, 1443, 1592, 1637, 2685, 2907, 3056 cm^{-1}	The infrared spectrum was consistent with the proposed chemical structure	Y	1	[redacted], 2006a *A3.4-2*	**X8**
NMR	Information not available	Purity: 96% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: 4067-2 / Specification given in Doc. III-A, 2/2	Result: The ^{13}C NMR spectrum shows the following shifts: 120.61, 123.88, 124.31, 124.72, 129.54, 147.84 and 164.73 ppm	The results of the NMR analyses agrees very closely with the literature values of BIT	Y	1	[redacted] 1997 A3.4-3	**X9**
MS	Information not available	Purity: > 86% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: T03626, T03627, T03628, T03629, T03630. Specification given in Doc. III-A, 2/2	Result: Molecular peak at m/z: 152, which confirms the identity of the test substance	The results of the MS analyses indicate that the spectrum is consistent with the proposed	Y	1	[redacted], 2005 A3.4-4 (see confidential data file)	**X10**

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
				structure of BIT				
3.5 Solubility in water (IIA, III 3.5)	US EPA 63-8	Purity: > 97% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: CN-306-111 / Specification given in Doc. III-A, 2/2	Result: 1.118 g/L Temperature: 20ºC pH: Not documented		Y	1	[redacted] *A3.1.1*	**X11** **X12**
3.6 Dissociation constant (-)	US EPA 63-10	Pure BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: CN-306-111-2	Result: pKa = 7.04		Y	1	[redacted], 1994 *A3.1.1*	
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA, III 1)	US EPA 63-8	Purity: >97% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: CN-306-111 / Specification given in Doc. III-A, 2/2	Result: 135 mg/L in heptane 11.6 g/L in ethyl acetate Temperature: 20ºC		Y	1	[redacted] 1994 *A3.1.1*	**X13** **X14**

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA, III 2)		Purity: > 97% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: CN-306-111 and pure BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: CN-306-111-2 / Specification given in Doc. III-A, 2/2	Result: Stable in deionised water, heptane, ethyl acetate and 1-octanol at ambient temperature for 24, 48 and 120 h	This test is not required since the active substance as manufactured does not contain an organic solvent.	Y	1	[redacted], 1994 *A3.1.1*	
		[redacted] Purity: 19.3% BIT [1,2-Benzisothiazol-3-(2*H*)-one] Lot number: 0306-8692	Result: [redacted] is stable after 12 months of storage at room temperature. [redacted] is stable after storage for 30 days at 50ºC. Since [redacted] contains the organic solvents used in the biocidal product., the stability of BIT in [redacted] shows that BIT is stable in the organic solvents used in the biocidal product		Y	1	[redacted] [redacted] 2006	**X15**

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.9 Partition coefficient n-octanol/water (IIA, III 3.6)	US EPA 63-11	Pure BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: CN-306-111-2	Result: 1.40 ± 0.06 Temperature: 21ºC pH: Not documented		Y	1	[redacted] 1994 *A3.1.1*	**X16**
3.10 Thermal stability, identity of relevant breakdown products (IIA, III 3.7)								

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
Termal stability 1	US-EPA 63-17	Not documented; Batch number: 4067-2	Result: Stable following storage at 25°C and a relative humidity of 50% for 12 months		Y	1	[redacted], 1994 A3.1.1	
Termal stability 2	US EP 63-13	Purity: >97% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: CN-306-111 / Specification given in Doc. III-A, 2/2	Result: Stable following storage at 55 ± 1ºC for 14 days		Y	1	[redacted]., 1994 A3.1.1	
Termal stability 3	US EPA 63-17	Purity: ~ 96% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: 4067-2 / Specification given in Doc. III-A, 2/2	Result: Stable following storage at 25°C and a relative humidity of 50% for 12 months		Y	1	[redacted] 1997 A3.4-3	
Termal stability 4	OECD 113	Purity: 89.8% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: 2005-051	Result: Stable at room temperature		Y	1	[redacted] 2006a A3.4-2	
Termal stability 5	OECD 113 CIPAC Method MT46	Purity: 85.3% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: 2005-051	Result: Stable following storage at 54 ± 2 ºC for 14 days		Y	1	[redacted] 2006b A3.10-4	

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
Termal stability 6	U.S. OPPTS 830.6317 U.S. OPPTS	Purity: 83.5% BIT [1,2-benzisothiazolin-3-(2*H*)-one]; Batch number: 2006-00114-56	Result: Stable and not corrosive to high density polyethylene (HDPE) following storage at 2, 25 and 35 ± 1.4ºC for a simulated one-year		Y	1	[redacted] 2007c	
3.11 Flammability, including auto-flammability and identity of combustion products (IIA, III 3.8)								
Flammability 1	EC A10	Purity: 89.8% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: 2005-051	Result: Not highly flammable		Y	1	[redacted]., 2006a *A3.4-2*	
Flammability 2	EC A10 US EPA 63-15	Purity: 83.5% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: 2006-00114-56	Result: Not highly flammable		Y	1	[redacted] 2007c A3.11-2	
Phyrophoric Properties	EC A13			Pyrophoric compounds	Y	1	[redacted]	

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
				generally comprise active metals, organometalics, and metal hybrides; alkyl boranes and phosphines are also pyrophoric. 1,2-Benzisothiaz ol-3-(2*H*)- one (BIT) is chemically unrelated to such materials And should not be classed among them.			[redacted]., 2006b	
Auto-flamability	EC A16	Purity: 89.8% BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: 2005-051	Result: No relative self-ignition temperature below its melting point		Y	1	[redacted] 2006a *A3.4-2*	
Auto-flamability 2	EC A16	Purity: 83.5% BIT [1,2-Benzisothiazol-3-(2*H*)-one];	Result: No relative self-ignition temperature below its melting point		Y	1	[redacted]	

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
		Batch number: 2006-00114-56					[redacted] 2007	
3.12 Flash-point (IIA, III 3.9)				The flash-point is not required since BIT is a solid				**X17**
3.13 Surface tension (IIA, III 3.10)	EC Method A5 OECD 115	100 % pure BIT [1,2-Benzisothiazol-3-(2*H*)-one]; Batch number: 060309/01	Result: 71.5 mN/m (0.881 g/L solution) Temperature: 21.6 ± 0.5 ºC	BIT is not considered to be a surface-active material	Y	1	[redacted] 2006a *A3.4-2*	**X18**
3.14 Viscosity (-)				The viscosity is not required since BIT is a solid				**X19**
3.15 Explosive properties (IIA, III 3.11)				The molecular structure of BIT indicates that the substance has no explosive properties. Therefore, a study is not required.				**X20**

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.16 Oxidizing properties (IIA, III 3.12)				The molecular structure of BIT indicates that the substance has no oxidising properties. Therefore, a study is not required.				**X21** **X23**
3.17 Reactivity towards container material (IIA, III 3.13)								
Reactivity towards container material 1	US EPA 63-20	Not documented; Batch number: 4067-2	No signs of corrosion or discoloration were observed in a piece of polyethylene after being in contact with the test substance for approximately 14 days		Y	1	[redacted]., 1994 *A3.1.1*	
Reactivity towards container material 2	U.S. OPPTS 830.6317	Purity: 83.5% BIT [1,2-benzisothiazolin-3-(2*H*)-one]; Batch number: 2006-00114-56	Result: not corrosive to high density polyethylene (HDPE) following storage at 2, 25 and 35 ± 1.4ºC for a simulated one-year period		Y	1	[redacted] 2007c	

Section A3 Physical and Chemical Properties

Annex point IIA, III 3 PHYSICAL AND CHEMICAL PROPERTIES OF ACTIVE SUBSTANCE

Subsection (Annex point)	Method	Purity / Specification	Results	Remarks / Justification	GLP (Y/N)	Reliability	Reference	Official use only
	U.S. OPPTS 830.6320							

Section A3 **Flash point (II.3.9)**		
Annex Point 3.12		
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	It is not required since BIT is a solid.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*July 2010*	
Evaluation of applicant's justification	*The non-submission of data is justified*	
Conclusion	*Acceptable*	
Remarks	*No further remarks*	

Section A3 **Annex Point 3.14**	**Viscosity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	It is not required since BIT is a solid.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*July 2010*	
Evaluation of applicant's justification	*The non-submission of data is justified*	
Conclusion	*Acceptable*	
Remarks	*No further remarks*	

Section A3 **Annex Point 3.15**	**Explosive Properties**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	The molecular structure of BIT indicates that the substance has no explosive properties. Therefore, a study is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*July 2010*	
Evaluation of applicant's justification	*The non-submission of data is justified*	
Conclusion	*Acceptable*	
Remarks	*No further remarks*	

Section A3 **Annex Point 3.15**	**Oxidising Properties**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	The molecular structure of BIT indicates that the substance has no oxidising properties. Therefore, a study is not required.	X
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*July 2010* *August 2021*	
Evaluation of applicant's justification	*The non-submission of data is justified*	
Conclusion	*Acceptable*	
Remarks	*No further remarks* *(X) According to the ECHA Guidance on the Application of the CLP criteria (version 5.0, July 2017), the classification procedure for organic substances does not need to be applied if:* *a) the substance or mixture does not contain oxygen, fluorine or chlorine; or* *b) the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen* *The active substance contains oxygen but it is chemically bonded to carbon and no classification as oxidizing solid is to be expected.*	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

October 2011

August 2021

Applicant´s Comment

Evaluation of data submitted under section A3

3.1.1. Melting point (1 & 2)

Materials and Method: (X1) The method used for the determination of the melting point was the capillary method.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable. (X24) After the BIT RCOM 2015, the submitted study by Troy is not acceptable because the purity used is not consistent with the stated specification.

3.1.2. Boiling point

Materials and Method: (X2) The method according to Siwoloboff was used to determine the boiling point, the Melt Temp II was apparatus used. The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

3.1.3. Relative density

Materials and Method: (X3) The pycnometer method was used to determine the relative density of the test substance by determining the amount of water displaced by BIT. The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable.

3.2. Vapour pressure

Vapour pressure 1

Materials and Method: (X4) This parameter was determined by the gas saturation method. The applicant's version is adopted.

(X5) The vapour pressure must be studied at two temperatures (at 20ºC and 25ºC) or a vapour pressure curve must be determined. The applicant has determined the vapour pressure at one temperature (25ºC).

Results: The applicant should have determined this parameter at least at two temperatures.

Reliability: 2 The applicant has presented the vapour pressure for one temperature.

Acceptability: Acceptable with objections.

Vapour pressure 2

Materials and Method: (X6) This parameter was determined by the gas saturation method. The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable.

3.2.1. Henry's Law Constant

Materials and Method: The applicant's version is adopted. (X7). The value obtained by Epiweb is $2.4 \cdot 10^{-5}$ Pa m^3 mol^{-1}. (X22) Taking into account the Troy comment in the BIT RCOM 2015: Based on a vapour pressure of 1.5E-04 Pa at 25°C and a water solubility of 1118 mg/L at 20°C (corresponding to 1200 mg/L at 25°C) the calculated Henry's Law Content is 1.89E-05 Pa x m3 x mol-1.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

3.3. Appearance

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

3.4. Absorption spectra, and mass spectrum

3.4.1. UV/VIS

UV/VIS 1

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

UV/VIS 2

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

3.4.2. IR

Materials and Method: (*X8) The applicant has indicated that method was not available but the test report presented (Woolley and Mullee, 2006) include a description of the method in accordance with Council Directive 98/8/EC, Annex IIA, III 3.4. The applicant's version is adopted.*

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

3.4.3. NMR

Materials and Method: (X9) The applicant has indicated that method was not available but the test report presented (Polson, 1997) include a description of the method. The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

3.4.4. MS

Materials and Method: (X10) The applicant has indicated that method was not available but the test report presented (Thomson, 2005) include a description of the method. The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

3.5. Water solubility

Materials and Method: (X11) The solubility was determined with the flask method. The applicant's version is adopted.

Results: (X12) The water solubility must be studied at different temperatures and pH.

Reliability: 2. The study must include the effect of temperature and pH on solubility.

Acceptability: Acceptable with objections.

3.6. Dissociation constant

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1.

Acceptability: The method and result are acceptable

3.7. Solubility in organic solvents

Materials and Method: (X13) The solubility was determined with the flask method. The applicant's version is adopted.

Results: (X14) The solubility in organic solvents must be studied at different temperatures.

Reliability: 2. The study must include the effect of temperature on solubility.

Acceptability: Acceptable with objections.

3.8. Stability in organic solvents used in b.p.

Materials and Method: (X15) The applicant indicating that as the active substance does not include an organic solvent. However the biocidal product is formulated with organic solvents.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

3.9 Partition coefficient

Materials and Method: The applicant's version is adopted..

Results: (X16) The partition coefficient must be studied at different temperatures and pH.

Reliability: 1.

Acceptability: The method and result are acceptable

3.10 Thermal stability

Thermal stability 1

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

Thermal stability 2

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

Thermal stability 3

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

Thermal stability 4

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

Thermal stability 5

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

Thermal stability 6

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

3.11 Flammability

Flammability 1

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

Flammability 2

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

Auto-Flammability 1

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

Auto-Flammability 2

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

3.12. Flash point

(X17) This property is not required for solid active substances therefore, the non submission of data is justified.

3.13. Surface tension

Materials and Method : (X18) The surface tension was determined by ring method. The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

3.14. Viscosity

(X19) This property is not required for solid active substances therefore, the non submission of data is justified.

3.15. Explosive properties

(X20) The molecular structure of BIT indicates that the substance has no explosive properties. Therefore, a study is not required.

3.16. Oxidizing properties

(X21) The molecular structure of BIT indicates that the substance has no oxidizing properties. Therefore, a study is not required.

(X23) According to the ECHA Guidance on the Application of the CLP criteria (version 5.0, July 2017), the classification procedure for organic substances does not need to be applied if:

a) the substance or mixture does not contain oxygen, fluorine or chlorine; or

b) the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen

The active substance contains oxygen but it is chemically bonded to carbon and no classification as oxidizing solid is to be expected.

3.17. Reactivity towards the container

Materials and Method: The applicant's version is adopted.

Results: The applicant's version is adopted.

Reliability: 1

Acceptability: The method and result are acceptable

Section A4 Analytical Methods for Detection and Identification

Subsection A4.1/1

Annex Point IIA, IV 4.1 (a)

ANALYTICAL METHOD FOR THE DETERMINATION OF 1,2-BENZISOTHIAZOL-3-(2*H*)-ONE IN THE ACTIVE SUBSTANCE AS MANUFACTURED

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2005), Preliminary analysis, Product Safety Laboratories 2394 Highway 130, Dayton, New Jersey 08810, USA, unpublished report no.: 16800 Date of experimental: February 10 – February 17, 2005	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access	Not applicable	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was conducted according to US EPA guideline OPPTS 830.1700 (August 1996) which is comparable to the guideline SANCO/3030/99 rev. 4.	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	Yes, the following deviation was noted: The calibration curve information was not documented. This deviation is minor and is not considered to have affected the scientific validity of the study or the interpretation of the results.	
		3 MATERIALS AND METHODS	
3.1	**Preliminary test**		
3.2.1	Enrichment	Not applicable as samples were only diluted in methanol and further dilution with methanol/water before analysis.	
3.2.2	Cleanup	Not applicable	
3.2	**Detection**		

Section A4 Analytical Methods for Detection and Identification

Subsection A4.1/1

Annex Point IIA, IV 4.1 (a)

ANALYTICAL METHOD FOR THE DETERMINATION OF 1,2-BENZISOTHIAZOL-3-(2*H*)-ONE IN THE ACTIVE SUBSTANCE AS MANUFACTURED

3.2.1	Separation Method	HPLC Column: Thermo, Betasil C-18, 3u, 150 x 2.1 mm Column temperature: 40°C Mobile phase: methanol/acetic acid, 100/0.1 : water/methanol/acetic acid, 95/5/0.1 (30 : 70) Flow rate: 200 µL/min Injection volume: 10 µL Run time: 10 min
3.2.2	Detector	UV, wavelength 254 nm
3.2.3	Standard(s)	External standard (Nipacide BIT 99.9%)
3.2.4	Interfering susbtance(s)	None identified

3.3 Linearity

3.3.1	Separation method	Not documented

Section A4 **Analytical Methods for Detection and Identification**

Subsection A4.1/1 **ANALYTICAL METHOD FOR THE DETERMINATION OF 1,2-BENZISOTHIAZOL-3-(2*H*)-ONE IN THE ACTIVE SUBSTANCE AS MANUFACTURED**

Annex Point IIA, IV 4.1 (a)

3.3.2	Detector	Not documented
3.3.3	Standard(s)	Correlation coefficient was 0.9944
3.4	**Specificity: interfering substances**	Any compound with identical retention times to this of BIT will potentially interfere with the analysis. The chromatograms provided do not show any interference on the BIT peak. Identity was confirmed by LC-MS (m/z = 152).
3.5	**Recovery rates at different levels**	In the test system employed, there were no interfering substances in the soil.
3.5.1	Relative standard deviation	
3.6	**Limit of determination**	Not required
3.7	**Precision**	Not required
3.7.1	Repeatability	Not required
3.7.2	Independent laboratory validation	

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	The objective of the study was to validate a method for the determination of 1,2-Benzisothiazolin-3-one of the active substance as manufactured. Samples were dissolved in methanol before analysis by HPLC-UV at 254 nm. This study was conducted according to EPA guideline OPPTS 870.1700 which is comparable to the SANCO/3030/99 as described under point 3. The following deviation was noted: The calibration curve information was not documented.

Section A4	**Analytical Methods for Detection and Identification**
Subsection A4.1/1 **Annex Point IIA, IV 4.1 (a)**	**ANALYTICAL METHOD FOR THE DETERMINATION OF 1,2-BENZISOTHIAZOL-3-(2*H*)-ONE IN THE ACTIVE SUBSTANCE AS MANUFACTURED**

		However, this deviation is minor and is not considered to have affected the scientific validity of the study or the interpretation of the results.
4.2	**Conclusion**	The method presented allows for the determination of 1,2-Benzisothiazolin-3-one. Although there is a deviation, the method is considered acceptable.
4.2.1	Reliability	1
4.2.2	Deficiencies	One deviation was noted and is outlined under point 3.3. However, it does not compromise the scientific validity of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*July 2010*
Materials and methods	*The RMS requested validated analytical methods for the determination of 1,2-Benzisothiazol-3-(2H)-one in the active substances. The applicant should have included the calibration range, recovery and limits of detection data in the summary of the method.*
Conclusion	*The results are insufficiently reported.*
Reliability	*2*
Acceptability	*The method and result are acceptable*
Remarks	*No further remarks*

Section A4 Analytical Methods for Detection and Identification

Subsection A4.1/2

Annex Point IIA, IV 4.1 (a)

ANALYTICAL METHOD FOR THE DETERMINATION OF 1,2-BENZISOTHIAZOL-3-(2*H*)-ONE IN THE ACTIVE SUBSTANCE AS MANUFACTURED

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2013), BIT Method Validation for Assay of Test Substance, Huntingdon Life Sciences, Occold, Eye, Suffolk, IP23 7PX, United Kingdom, report no.: ZNB0111 Date of experimental: April 10 – April 18, 2013	
1.2	**Data protection**	Yes	
1.2.4	Data owner	Troy Chemical Company BV	
1.2.5	Companies with letter of access	None	
1.2.6	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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was conducted according to guideline SANCO/3030/99 rev. 4.	
2.2	**GLP**	Yes, certified by Department of Health of the Government of the United Kingdom	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Preliminary test**		
3.2.1	Enrichment	Not applicable as samples were only diluted in methanol and further dilution with methanol/water before analysis.	
3.2.2	Cleanup	Not applicable	
3.2	**Detection**		
3.2.1	Separation Method	HPLC Column: Betasil C18, 15 cm x 4.6 mm internal diameter Column temperature: 40°C Mobile phase: water/methanol/acetic acid (66.5:33.5:0.1 v/v/v) Flow rate: 1 mL/min Injection volume: 10 µL Run time: 40 min	

Section A4	**Analytical Methods for Detection and Identification**
Subsection A4.1/2 **Annex Point IIA, IV 4.1 (a)**	**ANALYTICAL METHOD FOR THE DETERMINATION OF 1,2-BENZISOTHIAZOL-3-(2*H*)-ONE IN THE ACTIVE SUBSTANCE AS MANUFACTURED**

3.2.2 Detector	UV, wavelength 254 nm
3.2.3 Standard(s)	External standard (BIT 99.4%, Sigma-Aldrich)
3.2.4 Interfering susbtance(s)	None identified
3.3 Linearity	
3.3.4 Calibration range	340 – 1700 mg/L
3.3.5 Number of measurements	5
3.3.6 Linearity	Correlation coefficient was 1.0000
3.4 Specificity: interfering substances	Specificity of the method was confirmed by a retention time match of a test substance sample and the analytical standard. No interfering peaks could be detected. Representative chromatograms of the test substance, analytical standard and the diluent are presented in the study report. The specificity of the method was also confirmed by analysis of a test substance sample and the analytical standard using diode array detection (DAD). Typical spectra are presented in the study report.

Section A4	**Analytical Methods for Detection and Identification**
Subsection A4.1/2 **Annex Point IIA, IV 4.1 (a)**	**ANALYTICAL METHOD FOR THE DETERMINATION OF 1,2-BENZISOTHIAZOL-3-(2*H*)-ONE IN THE ACTIVE SUBSTANCE AS MANUFACTURED**

3.5	**Recovery rates at different levels**	Not required
3.5.1	Relative standard deviation	Not required
3.6	**Limit of determination**	Not required
3.7	**Precision**	
3.7.1	Repeatability	Five replicate injections of two lots of the test substance produced relative standard deviations of 0.53% and 1.33%, respectively. The recommended value calculated with the modified Horwitz equation is 1.40%, i.e. higher than the repeatability obtained, which indicates that the repeatability for the method is acceptable.
3.7.2	Independent laboratory validation	Not required.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	The objective of the study was to validate a method for the determination of 1,2-Benzisothiazolin-3-one of the active substance as manufactured. Samples were dissolved in methanol:water (50:50 v/v) before analysis by HPLC-UV at 254 nm. This study was conducted according to SANCO/3030/99 rev. 4.
4.2	**Conclusion**	The method presented allows for the determination of 1,2-Benzisothiazolin-3-one.
4.2.1	Reliability	1
4.2.2	Deficiencies	None

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*April 2014*
Materials and methods	*The applicant's version is adopted.*
Conclusion	*The applicant's version is adopted.*
Reliability	*1*
Acceptability	*The method and result are acceptable.*

Section A4 Analytical Methods for Detection and Identification

Subsection A4.1/2

Annex Point IIA, IV 4.1 (a)

ANALYTICAL METHOD FOR THE DETERMINATION OF 1,2-BENZISOTHIAZOL-3-(2*H*)-ONE IN THE ACTIVE SUBSTANCE AS MANUFACTURED

Remarks	*No further remarks.*

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2a/1

Annex Point IIA, IV 4.2 (a)

ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2007c), Validation of a residue analytical method for the determination of 1,2-Benzisothiazolin-3-one (BIT) in soil, RCC Ltd, Zelgliweg 1, CH-4452 Itingen, Switzerland, unpublished report no.: A89458 Date of experimental work: September 6, 2006 – October 27, 2006	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	ROHM & HAAS	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to guideline requirements SANCO/3029/99 rev. 4 and SANCO/825/00 rev 7.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2a/1 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Annex Point IIA, IV 4.2 (a)

3.1.1	Enrichment	Samples were extracted twice using methanol:water (3:1 v/v) and acetic acid.
3.1.2	Extraction	Samples were centrifuged and decanted. The clear supernatants obtained in the two extraction process were diluted with methanol:water (2:3 v/v)

3.2 Detection

3.2.1 Separation method

HPLC

Column: Zorbax SB Phenyl (Agilent): 5µm (50 mm x 2.1 mm)

Mobile phase A: methanol:water (5:95 v:v) containing 0.01% ammonium formate (5 mmol/L)

Mobile phase B: methanol:water (95:5 v:v) containing 0.01% ammonium formate (5 mmol/L)

Gradient program:

Time (minutes)	A (%)	B (%)
0.0	80	20
3.0	40	60
3.1	0	100
3.5	0	100
3.6	80	20
5.0	80	20

Flow rate: 300 µL/min

Injection volume: 50 µL

Mode: Gradient

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2a/1 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Annex Point IIA, IV 4.2 (a)

3.2.2	Detector	MS/MS Ionization mode: Pneumatically and thermally associated electrospray Nebuliser gas: Air Heater gas: Air Curtain gas: Nitrogen Collision gas: Nitrogen Ion Source: Sciex Turbo-V-Source Heater Gas Temperature: 500 °C Spray voltage: 2500 V Scan mode: MRM Ion polarity: Positive Precursor Ion: m/z 152 Product Ion: m/z 105 Collision energy: 33 V
3.2.3	Standard(s)	External standard 1,2-Benzisothiazolin-3-one (100% purity).
3.2.4	Interfering substance(s)	None identified
3.3	**Linearity**	
3.3.1	Calibration range	0.05 – 5.0 ng/mL.
3.3.2	Number of measurements	Single determinations at 5 concentration levels.
3.3.3	Linearity	Correlation coefficient (r) was determined as greater than or equal to 0.99. The equation of the line was determined on 4 different occasions. The results are: y = 2006 + 310251x, y = 10683 + 195365x, y = 13441 + 189094x, y = 3247 + 221175x, where y is the peak area and x the concentration of analyte in ng/ml.
3.4	**Specificity: interfering substances**	There was no determinable signal found in the control samples at the retention time of BIT. Therefore, there were no interferences at the retention time of the analyte above 30% of the LOQ. Although not required, the technique was confirmed by using a second mass transition (m/z = 152 → 109).

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2a/1 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Annex Point IIA, IV 4.2 (a)

3.5	**Recovery rates for soil**	Recovery was determined at two fortification levels. The results are summarised in Table A4.2-1. The mean recovery for 1,2-Benzisothiazolin-3-one at fortification level 0.05 mg/kg was 70%. The mean recovery for 1,2-Benzisothiazolin-3-one I at fortification level 0.50 mg/kg was 77%. All of these within the SANCO/3029/99 rev.4 guideline requirements (70 – 110%). Two control samples were analysed.
3.6	**Relative standard deviation**	Refer to Table A4.2-1
3.7	**Limit of determination**	The LOQ, defined as the lowest concentration at which a recovery of 70-110% with relative standard deviation of ≤ 20% is obtained**,** for 1,2-Benzisothiazol-3-one was 0.05 mg/kg. The LOQ result is acceptable according to SANCO/825/00 rev.7 since it is not greater than 0.05 mg/kg.
3.8	**Precision**	
3.8.1	Repeatability	Repeatability was determined as the RSD calculated from five determinations at each fortification level. The mean RSD for 1,2-Benzisothiazolin-3-one was 5 and 3% at each fortification level, respectively with overall RSD of 6%. All of these were within the specification (i.e. ≤ 20%).
3.8.2	Independent laboratory validation	Not required
		4 APPLICANT'S SUMMARY AND CONCLUSION
4.1	**Materials and methods**	The analytical method was validated for the determination of 1,2-Benzisothiazolin-3-one in soil. Soil Samples were extracted in methanol:water (3:1 v/v) and acetic acid. Extracts were centrifuged and decanted. Quantitation was performed using HPLC-MS/MS. This study was conducted according to SANCO/3029/99 rev.4 and SANCO/825/00 rev.7. No deviations were found.
4.2	**Conclusion**	The method appears to be specific for the determination of 1,2-Benzisothiazolin-3-one in soil since no interferences were observed. The mean recovery and RSD were within the guideline requirements.

Section A4	**Analytical Methods for Detection and Identification**
Subsection A4.2a/1 **Annex Point IIA, IV 4.2 (a)**	**ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL**

		The method is suitable for the determination of 1,2-Benzisothiazolin-3-one in soil.
4.2.3	Reliability	1
4.2.4	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*July 2010* *May 2020*
Materials and methods	*The applicant's version is adopted*
Conclusion	*The applicant's version is adopted*
Reliability	*1*
Acceptability	*The method and result are acceptable*
Remarks	*No further remarks* *(X) The study year has been changed from 2007c to 2007 to harmonise all submitted data.*

Table A4.2.a-1: Validation data for the analytical determination of ,2-Benzisothiazolin-3-one in soil

Fortification level (mg/kg)	n	Recovery		RSD (%)
		Range (%)	Mean (%)	
0.05	5	64 – 72	70	5
0.50	5	75 – 80	77	3
Overall	10	64 - 80	73	6

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2a/2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Annex Point IIA, IV 4.2 (a)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2008), Development and Validation of a Residue Analytical Method for the Determination of BIT in Soil. RCC Ltd, Zelgliweg 1, 4452 Itingen, Switzerland, unpublished report no.: B94948 Date of experimental work: June 11, 2008 – June 20, 2008	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV and Dow Benelux BV	
1.2.2	Companies with letter of access	Not applicable	
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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to guideline requirements SANCO/3029/99 rev. 4 and SANCO/825/00 rev 7.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	5.0 g soil was weighed into an 11-mL-ASE (accelerated solvent extraction) extraction tube. 1-2g Isolute were added and the solution was mixed well. Samples were diluted in methanol (9:1; v/v)	
3.1.2	Extraction	Not applicable samples were centrifuged	

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2a/2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Annex Point IIA, IV 4.2 (a)

3.2 Detection

3.2.1 Separation method

LC

Column: Zorbax SB Phenyl (Agilent): 5µm (50 mm x 2.1 mm)

Mobile phase A: 5mM Ammonium Formate with water / Methanol / Formic acid (95:5:0.1; v/v/v)

Mobile phase B: 5mM Ammonium Formate with water / Methanol / Formic acid (5:95:0.1; v/v/v)

Gradient program:

Time (minutes)	A (%)	B (%)
0.0	80	20
3.0	40	60
3.1	0	100
3.5	0	100
3.6	80	20
5.0	80	20

Flow rate: 300 µL/min

Injection volume: 20 µL

Mode: Gradient

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2a/2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Annex Point IIA, IV 4.2 (a)

3.2.2	Detector	MS/MS Ionization mode: Pneumatically and thermally assisted ESI Nebuliser gas: Air Heater gas: Air Curtain gas: Nitrogen Collision gas: Nitrogen Ion Source: Sciex Turbo-V-Source Heater Gas Temperature: 500 ºC Spray voltage: 2500 V Scan mode: MRM Ion polarity: Positive Precursor Ion: m/z 152.0 – 105.0 CE33 primary method Product Ion: m/z 152.0 – 109.0 CE32 secondary method Collision energy: 33 V
3.2.3	Standard(s)	External standard 1,2-Benzisothiazolin-3-one (100% purity).
3.2.4	Interfering substance(s)	Conclusively, the method is sufficiently specific for the determination of BIT in soil
3.3	**Linearity**	
3.3.1	Calibration range	0.102 – 2.04 ng/mL.
3.3.2	Number of measurements	Single determinations at 7 concentration levels.
3.3.3	Linearity	Correlation coefficient (r) was determined as greater than or equal to 0.99 by injecting calibration solution of seven levels ranging from 0.1 ng/mL to 2.0 ng/mL. The equation of the line was determined on 2 different occasions. The results are: y = 84566 * x + 157 and y = 115940 * x + 578, where y is the peak area and x the concentration of analyte in ng/mL.
3.4	**Specificity: interfering substances**	The retention times of BIT signals in the specimen extracts match the retention time of the standard solution. Interferences above 30% of the LOQ were not observed at the retention time of the analyte.

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2a/2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Annex Point IIA, IV 4.2 (a)

3.5	**Recovery rates for soil**	Recovery was determined at two fortification levels. The results are summarised in Table A4.2-1. Primary method: The mean recovery for 1,2-Benzisothiazolin-3-one at fortification level 0.05 mg/kg was 88%. The mean recovery for 1,2-Benzisothiazolin-3-one at fortification level 0.51 mg/kg was 88%. Secondary method: The mean recovery for 1,2-Benzisothiazolin-3-one at fortification level 0.05 mg/kg was 87%. The mean recovery for 1,2-Benzisothiazolin-3-one at fortification level 0.51 mg/kg was 87%. All of these within the SANCO/3029/99 rev.4 guideline requirements (70 – 110%).
3.5.1	Relative standard deviation	Refer to Table A4.2-1
3.6	**Limit of determination**	The LOQ, defined as the lowest concentration at which a recovery of 70-110% with relative standard deviation of ≤ 20% is obtained**,** for 1,2-Benzisothiazol-3-one was 0.05 mg/kg. The LOQ result is acceptable according to SANCO/825/00 rev.7 since it is not greater than 0.05 mg/kg.
3.7	**Precision**	
3.7.1	Repeatability	Repeatability was determined as the RSD calculated from five determinations at each fortification level. The primary mean RSD for 1,2-Benzisothiazolin-3-one was n = 5 and 4% for lower fortification level and n = 5 and 1% for the higher level, with overall RSD of n = 10 and 3%. The confirmatory mean RSD for 1,2-Benzisothiazolin-3-one was n = 5 and 3% for lower fortification level and n = 5 and 2% for the higher level, with overall RSD of n = 10 and 2%. All of these were within the required specification (i.e. RSD ≤ 20%).
3.7.2	Independent laboratory validation	Not required

4 APPLICANT'S SUMMARY AND CONCLUSION

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2a/2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Annex Point IIA, IV 4.2 (a)

4.1	**Materials and methods**	The analytical method was validated for the determination of 1,2-Benzisothiazolin-3-one in soil. Soil Samples were extracted in methanol. Samples were centrifuged and diluted in methanol (9:1; v/v). Quantification was performed using LC-MS/MS. This study was conducted according to SANCO/3029/99 rev.4 and SANCO/825/00 rev.7. No deviations were found.
4.2	**Conclusion**	The method is specific for the determination of 1,2-Benzisothiazolin-3-one in soil since no interferences were observed with a LOQ of 0.05 mg/kg. The mean recovery and RSD were within the guideline requirements. The method is suitable for the determination of 1,2-Benzisothiazolin-3-one in soil.
4.2.1	Reliability	1
4.2.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*July 2010*
Materials and methods	*The applicant's version is adopted*
Conclusion	*The applicant's version is adopted*
Reliability	*1*
Acceptability	*The method and result are acceptable*
Remarks	*No further remarks*

Table A4.2-1: Validation data for the analytical determination of 1,2-Benzisothiazolin-3-one in soil

Fortification level (mg/kg) Primary method	n	Recovery		RSD (%)
		Range (%)	Mean (%)	
0.05	5	84 – 95	88	4

0.51	5	87 – 90	88	1
Overall	10	84 - 95	88	3
Fortification level (mg/kg) Secondary method	**n**	**Recovery**		**RSD (%)**
		Range (%)	**Mean (%)**	
0.05	5	84 – 91	87	3
0.51	5	85 – 89	87	2
Overall	10	84 - 91	87	2

Figure A4.2-1: Representative calibration curve of BIT in soil (Primary method)

y = 84566 * x + 157
r = 0.9980

c[ng/mL]	Area [counts]	Bias [%]
0.102	8785	0.0
0.204	16939	-2.7
0.510	41607	-3.9
0.816	67547	-2.3
1.02	88140	2.0
1.53	136686	5.5
2.04	167565	-3.0

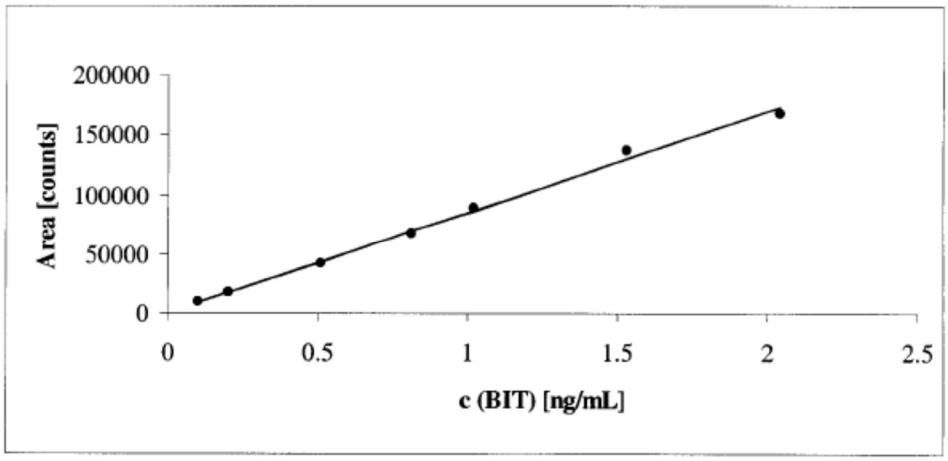
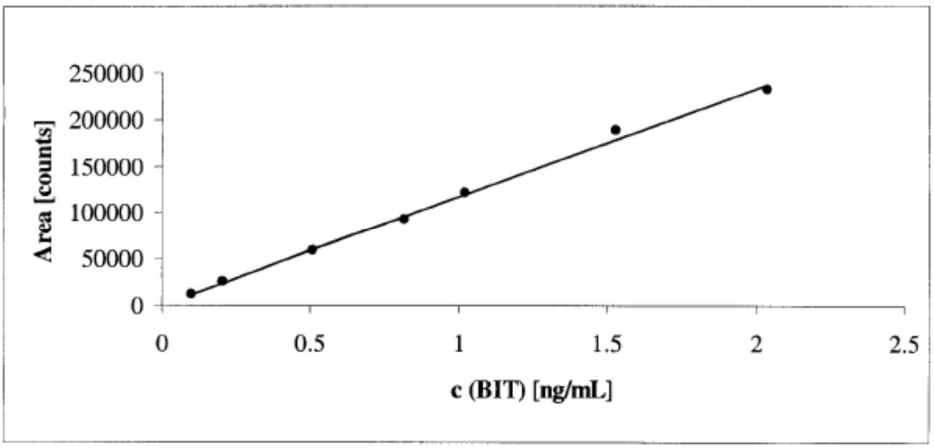


Figure A4.2-2: Representative calibration curve of BIT in soil (Confirmatory method)

y = 115940 * x + 578
r = 0.9983

c[ng/mL]	Area [counts]	Bias [%]
0.102	12512	0.9
0.204	24419	0.8
0.510	57909	-3.0
0.816	91755	-3.6
1.02	120507	1.4
1.53	187163	5.2
2.04	231161	-2.5



Section A4 Analytical Methods for Detection and Identification

Subsection A4.2b/1 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Annex Point IIA, IV 4.2 (b)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2007a): Validation of a residue analytical method for the determination of 1,2-Benisothiazolin-3-one (BIT) in air. RCC Ltd, Zelgliweg 1, CH-4452 Itingen, Switzerland, unpublished report no A78311. Date of experimental work: October 3, 2006 – May 24, 2007.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	ROHM & HAAS	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to the Residual Analytical Method, Guideline 96/46/EC, SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 7.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, the following deviation was noted: 1. The mean recovery value for the lower fortification level was below the requirements. This deviation is minor and is not considered to compromise the scientific validity of the study.	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Air samples were adsorbed on silica gel tubes. Back and front sections of the tubes were extracted with methanol/water mixture (4:1 v/v) in an ultrasonic bath for 15 minutes.	
3.1.2	Cleanup	Not applicable	
3.2	**Detection**		

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2b/1 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Annex Point IIA, IV 4.2 (b)

3.2.1	Separation method	HPLC:	
		Column:	Zorbax SB Phenyl (Agilent); 5µm, (50 mm X 2.1 mm)
		Mobile phase:	Eluent A: 95:5:0.1 water:methanol:formic acid and 5mM ammonium formate
			Eluent B: 5:95:0.1 water:methanol:formic acid and 5 mM ammonium formate
		Flow rate:	300 µL/min
		Injection volume: 50 µL	
		Mode:	gradient
3.2.2	Detector	MS/MS	
		Nebuliser gas:	Air
		Heater gas:	Air
		Curtain gas:	Nitrogen
		Collision gas:	Nitrogen
		Ionization mode:	Pneumatically and thermally associated electrospray
		Ion Source:	Sciex Turbo-Ion Spray
		Temperature:	500 °C
		Spray voltage:	2500 V
		Scan mode:	MRM
		Ion polarity:	Positive
		Precursor Ion:	m/z 152
		Product Ion:	m/z 105
		Collision energy:	33 V
3.2.3	Standard(s)	External standard 1,2-Benzisothiazolin-3-one	
3.2.4	Interfering substance(s)	None identified	
3.3	**Linearity**		
3.3.1	Calibration range	0.2 – 20 ng/mL.	
3.3.2	Number of measurements	Single determinations at 7 levels.	

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2b/1 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Annex Point IIA, IV 4.2 (b)

3.3.3	Linearity	Correlation coefficient (r) was determined as greater than or equal to 0.998. The equation of the line was determined in 8 different days. The results are: y = -1082 + 205547x, y = 867 + 225223x, y = 1360 + 217338x, y = 3319 + 253576x, y = 2223 + 234068x, y = 2461 + 245613x, y = 741 + 25242x and y = 1191 + 85277x, where y is the peak area and x the concentration of analyte in ng/ml.
3.4	**Specificity: interfering substances**	There was no determinable signal found in the control samples at the retention time of BIT. There were no interferences at the retention time of the analyte above 30% of the LOQ. The technique was confirmed by using a second mass transition (m/z = 152 → 109).
3.5	**Recovery rates at different levels**	Recovery was determined at three fortification levels. Results are summarised in Tables A4.2b/1-1, A4.2b/1-2. The mean recovery for 1,2-Benzisothiazolin-3-one at fortification level 6.0 µg/m^3 at 20 ºC and 40 - 69% relative humidity was 56%. The mean recovery for 1,2-Benzisothiazolin-3-one at fortification level 60 µg/m^3 at 20 ºC and 40 - 69% relative humidity was 82%. The mean recovery for 1,2-Benzisothiazolin-3-one at fortification level 600 µg/m^3 at 20 ºC and 40 - 69% relative humidity was 94%. The overall recovery for 1,2-Benzisothiazolin-3-one at 20 ºC and 40 - 69% relative humidity was 77%. The mean recovery for 1,2-Benzisothiazolin-3-one at fortification level 6.0 µg/m^3 at 35 - 36 ºC and 81 - 88% relative humidity was 87%. The mean recovery for 1,2-Benzisothiazolin-3-one at fortification level 60 µg/m^3 at 35 - 36 ºC and 81 - 88% relative humidity was 94%. The mean recovery for 1,2-Benzisothiazolin-3-one at fortification level 600 µg/m^3 at 35 - 36 ºC and 81 - 88% relative humidity was 95%. The overall recovery for 1,2-Benzisothiazolin-3-one at 35 - 36 ºC and 81 - 88% relative humidity was 92%. The overall recovery for 1,2-Benzisothiazolin-3-one was 84%. The mean recovery value of 56% for the lower fortification level at enhanced conditions go below the limit of 70 – 110%. The rest of these results are within the SANCO/3029/99 rev.4 guideline requirements (70 – 110%, RSD ≤ 20%). Two control samples were analysed.
3.5.1	Relative standard deviation	Refer to Tables A4.2b/1-1 and A4.2b/1-2.
3.6	**Limit of determination**	The LOQ, defined as the lowest concentration at which a recovery of 70-110% with relative standard deviation of ≤ 20% is obtained**,** for 1,2-Benzisothiazol-3-one was 6.0 µg/m^3.

Section A4 **Analytical Methods for Detection and Identification**

Subsection A4.2b/1 **ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR**

Annex Point IIA, IV 4.2 (b)

An AOEL of 0.06 mg/kg bw/day was set based on a teratogenicity study in New Zealand white rabbits. Using this AOEL produced a concentration (C) of 0.018 mg/m^3. Therefore, LOQ value is within the required specification (equal or lower than C) according to SANCO/825/00 rev.7.

3.7 Precision

3.7.1 Repeatability

Repeatability was determined as the RSD calculated from five determinations at each fortification level. One recovery value was marked as outlier (test by Grubbs) at three fortification levels and was not taken into account. Results are summarised in Tables A4.2b/1-1 and A4.2b/1-2.

The mean RSD for 1,2-Benzisothiazolin-3-one was 8, 4, 4, 2, 9 and 5%, respectively with overall RSD of 18%.

All of these results are within the specification (i.e. ≤ 20%).

3.7.2 Independent laboratory validation

Not required

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

The analytical method was validated for the determination of 1,2-Benzisothiazolin-3-one in air. Air samples were adsorbed in OVS silica gel adsorption tubes. The flow rate was adjusted to 0.5 L/min for a sampling period of 6 h. The test was performed at ambient conditions and normal temperature as well as enhanced temperature and humidity (35 ºC approximately and minimum 80% RH) Samples were then extracted using methanol:water 4:1 v/v. The mixture was allowed to settle and cool down at room temperature and the clear supernatant was taken and diluted using methanol:water 1:9 v/v. Quantitation was performed using HPLC-MS/MS.

The following deviation was found according to the guidance documents requirements SANCO/3029/99 rev. 4 and SANCO/825/00 rev 7:

1. The mean recovery value for the lower fortification level at 20 ºC and 40-69% RH was below the requirements.

However, this deviation is minor and is not considered to compromise the scientific validity of the study.

4.2 Conclusion

The method appears to be specific for the determination of 1,2-Benzisothiazolin-3-one in air since no interferences were observed. The mean recovery and RSD were within the guideline requirements. The method is acceptable for the determination of 1,2-Benzisothiazolin-3-one in air.

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2b/1 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Annex Point IIA, IV 4.2 (b)

4.2.1	Reliability	1
4.2.2	Deficiencies	One deviation was noted and is outlined under point 3.5. However, this does not compromise the scientific validity of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*July 2010*
Materials and methods	*The applicant's version is adopted*
Conclusion	*The applicant's version is adopted*
Reliability	*1*
Acceptability	*The method and result are acceptable*
Remarks	*No further remarks*

Table A4.2b/1-1: Validation data for the analytical determination of 1,2-Benzisothiazol-3-(2*H*)-one in air at 20 ºC and 40 - 69% relative humidity.

Fortification level (µg/m^3)	n	Recovery		RSD (%)
		Range (%)	Mean (%)	
6.0	5	52 - 63	56	8
60	4*	79 – 87	82	4
600	5	88 – 98	94	4
Overall	14	52-98	77	22

*One recovery value was marked as outlier (test by Grubbs) and was not taken into account

Table A4.2b/1-2: Validation data for the analytical determination of 1,2-Benzisothiazol-3-(2*H*)-one in air at 35 - 36 °C and 81 - 88% relative humidity.

Fortification level (µg/m³)	n	Recovery		RSD (%)
		Range (%)	Mean (%)	
6.0	4*	85 – 89	87	2
60	4*	81 – 100	94	9
600	5	88 – 100	95	5
Overall	13	81-100	92	7

*One recovery value was marked as outlier (test by Grubbs) and was not taken into account

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2b/2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Annex Point IIA, IV 4.2 (b)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2007), Development and validation of an industrial hygiene method for the collection and analysis of 1,2-Benzisothiazol-3-(2*H*)-one, Global Industrial Hygiene Expertise Center, The Dow Chemical Company, unpublished report no.: IHAL 40473. Date of experimental work: Not documented.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, but the validation was conducted according to requirements comparable to guideline requirements SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 7.	
2.2	**GLP**	No	**X1**
2.3	**Deviations**	Yes, the following deviations were noted: 1. The temperature of the air was 33.3 °C rather than 35 °C as recommended by the guideline 2. Four samples were analysed at each fortification level rather than five as recommended by the guideline 3. No control samples were tested as required by the guideline. These deviations are minor and are not considered to compromise the scientific validity of the study.	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Air samples are collected using a sampling train consisting of a 37 mm Teflon filter with cellulose support pad followed by a 1.3-g silica gel back-up tube. Air was pulled through each sampling media at a flow rate of approx. 2 L/min for 15 min or 1 L/min for 8 hours.	
3.1.2	Cleanup	Sample media is desorbed in 50:50 methanol:water. Aliquots are transferred to autosampler vials.	
3.2	**Detection**		

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2b/2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Annex Point IIA, IV 4.2 (b)

3.2.1	Separation method	HPLC: Column: Zorbax Rx-C18 HPLC reverse phase column, 250 x 4.6 mm ID, 5µm pore size Mobile phase: 50:50 methanol:Milli-Q water (v/v) Flow rate: 1.5 mL/min Injection volume: 75 µL Temperature: 25 °C Mode: Isocratic
3.2.2	Detector	Ultraviolet Absorption Detector, UV 274 nm
3.2.3	Standard(s)	External standard 1,2-Benzisothiazol-3-(2*H*)-one
3.2.4	Interfering substance(s)	None identified
3.3	**Linearity**	
3.3.1	Calibration range	2.8 - 280 µg/10mL approximately.
3.3.2	Number of measurements	Single determinations at 5 levels
3.3.3	Linearity	Correlation coefficient (r) was determined as greater than 0.999. The equation of the line was y = 2.36316x, where y is the peak area and x the concentration of analyte in µg/10mL.
3.4	**Specificity: interfering substances**	No significant response in the retention time and no significant matrix effects were observed. Identity was confirmed by the use of a confirmatory technique that used a different mobile phase and a different separation column in the analysis.
3.5	**Recovery rates at different levels**	Recovery was determined at different fortification levels. Results are summarised in Tables A4.2b/2-1, A4.2b/2-2, A4.2b/2-3, A4.2b/2-4 and A4.2b/2-5. The mean recovery for 1,2-Benzisothiazol-3-(2*H*)-one at fortification

Section A4 **Analytical Methods for Detection and Identification**

Subsection A4.2b/2 **ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR**

Annex Point IIA, IV 4.2 (b)

		level 0.0168 mg/m^3 at 33.3 ºC and 94% relative humidity was 96.95%. The mean recovery for 1,2-Benzisothiazol-3-(2*H*)-one at fortification level 0.27067 mg/m^3 at 25.6 ºC and 25% relative humidity was 93.35%. The mean recovery for 1,2-Benzisothiazol-3-(2*H*)-one at fortification level 6.567 mg/m^3 at 25.6 ºC and 25% relative humidity was 96.30%. The mean recovery for 1,2-Benzisothiazol-3-(2*H*)-one at fortification level 0.27067 mg/m ^{3}at 27.8 ºC and 78% relative humidity was 90.12%. The mean recovery for 1,2-Benzisothiazol-3-(2*H*)-one at fortification level 0.68 mg/m^3 at 27.8 ºC and 78% relative humidity was 93.87%. The mean recovery for 1,2-Benzisothiazol-3-(2*H*)-one at fortification level 6.567 mg/m^3 at 27.8 ºC and 78% relative humidity was 93.65%. The mean recovery for 1,2-Benzisothiazol-3-(2*H*)-one at fortification level 0.043125 mg/m^3 at 33.3 ºC and 88% relative humidity was 89.95%. The mean recovery for 1,2-Benzisothiazol-3-(2*H*)-one at fortification level 0.402083 mg/m^3 at 33.3 ºC and 88% relative humidity was 92.88 %. The mean recovery for 1,2-Benzisothiazol-3-(2*H*)-one at fortification level 2.643 mg/m^3 at 33.3 ºC and 88% relative humidity was 89.43%. The mean recovery for 1,2-Benzisothiazol-3-(2*H*)-one at fortification level 4.53 mg/m^3 at 33.3 ºC and 88% relative humidity was 94.83%. The overall recovery for 1,2-Benzisothiazol-3-(2*H*)-one was 93.1% All of these results are within the SANCO/3029/99 rev.4 guideline requirements (70 – 110%, RSD ≤ 20%). No control samples were analysed.
3.5.1	Relative standard deviation	Refer to Tables A4.2b/2-1, A4.2b/2-2, A4.2b/2-3, A4.2b/2-4 and A4.2b/2-5.
3.6	**Limit of determination**	The LOQ, defined as the lowest concentration at which a recovery of 70-110% with relative standard deviation of ≤ 20% is obtained**,** for 1,2-Benzisothiazol-3-(2*H*)-one was 0.0168 mg/m^3. An AOEL of 0.06 mg/kg bw/day was set based on a teratogenicity study in New Zealand white rabbits. Using this AOEL produced a concentration (C) of 0.018 mg/m^3. Therefore, LOQ value is within the required specification (equal or lower than C) according to SANCO/825/00 rev.7.
3.7	**Precision**	

Section A4	**Analytical Methods for Detection and Identification**
Subsection A4.2b/2 **Annex Point IIA, IV 4.2 (b)**	**ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR**

3.7.1	Repeatability	Repeatability was determined as the RSD calculated from four determinations at each fortification level. Results are summarised in Tables A4.2b/2-1, A4.2b/2-2, A4.2b/2-3, A4.2b/2-4 and A4.2b/2-5. The mean RSD for 1,2-Benzisothiazol-3-(2*H*)-one was 1.01, 1.92, 0.54, 9.15, 4.06, 6.34, 1.89, 2.13, 8.06 and 1.91 % with overall RSD of 4.5%. All of these results are within the specification (i.e. ≤ 20%).	
3.7.2	Independent laboratory validation	Not required	

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	The analytical method was validated for the determination of 1,2-Benzisothiazol-3-(2*H*)-one in air. Spikes were prepared on a sampling train consisting of a 37-mm Teflon filter in series with a 1.3-g silica gel tube over a target mass range of ~8 - 200 µg BIT/sample. Air at different conditions of temperature and relative humidity was passed through the tubes at a rate of approximately. 2 L/min for 15 min or 1 L/min for 8 hours. Residual 1,2-Benzisothiazol-3-(2*H*)-one were extracted from the sorbent material with 50:50 methanol:water. Quantitation was performed using HPLC-UV at 274 nm with the following deviations according to guideline requirements SANCO/3029/99 rev. 4 and SANCO/825/00 rev 7: 1. The temperature of the air was 33.3 ºC rather than 35 ºC as recommended by the guideline 2. Four samples were analysed at each fortification level rather than five as recommended by the guideline 3. No control samples were tested as required by the guideline. However, these deviations are minor and are not considered to compromise the scientific validity of the study.	
4.2	**Conclusion**	The method appears to be specific for the determination of 1,2-Benzisothiazol-3-(2*H*)-one in air since no interferences were observed. The mean recovery and RSD were within the guideline requirements. The method is acceptable for the determination of 1,2-Benzisothiazol-3-(2*H*)-one in air.	
4.2.1	Reliability	2	
4.2.2	Deficiencies	Three deviations were noted and are outlined under point 3.5, 3.7.1 and 4.1. However, they do not compromise the scientific validity of the study.	

Evaluation by Competent Authorities

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2b/2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Annex Point IIA, IV 4.2 (b)

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*July 2010*
Materials and methods	*(X1) The method is acceptable, however the applicant has not followed the GLP principles.*
Conclusion	*The applicant's version is adopted*
Reliability	*1*
Acceptability	*The method and result are acceptable*
Remarks	*No further remarks*

Table A4.2b/2-1: Validation data for the analytical determination of 1,2-Benzisothiazol-3-(2*H*)-one in air at 33.3 ºC and 94% relative humidity at a flow rate of 1.0 L/min for a duration of 8 hours.

Fortification level (mg/m^3)	n	Recovery		RSD (%)
		Range (%)	Mean (%)	
0.0168	4	96.3 – 98.4	96.95	1.01

Table A4.2b/2-2: Validation data for the analytical determination of 1,2-Benzisothiazol-3-(2*H*)-one in air at 25.6 ºC and 25% relative humidity at a flow rate of 2.0 L/min for a duration of 15 minutes.

Fortification level (mg/m^3)	n	Recovery		RSD (%)
		Range (%)	Mean (%)	
0.27067	4	90.9 – 95.2	93.35	1.92
6.567	4	95.9 – 97.0	96.30	0.54

Table A4.2b/2-3: Validation data for the analytical determination of 1,2-Benzisothiazol-3-(2*H*)-one in air at 27.8 ºC and 78% relative humidity at a flow rate of 2.0 L/min for a duration of 15 minutes.

Fortification level (mg/m^3)	n	Recovery	RSD (%)

		Range (%)	Mean (%)	
0.27067	4	78.7 – 97.3	90.12	9.15
0.68	4	88.2 – 96.1	93.87	4.06
6.567	4	84.8 – 97.5	93.65	6.34

Table A4.2b/2-4: Validation data for the analytical determination of 1,2-Benzisothiazol-3-(2*H*)-one in air at 33.3 ºC and 88% relative humidity at a flow rate of 1.0 L/min for a duration of 8 hours.

Fortification level (mg/m^3)	n	Recovery		RSD (%)
		Range (%)	Mean (%)	
0.043125	4	87.4 – 90.8	89.95	1.89
0.402083	4	90.2 – 94.8	92.88	2.13

Table A4.2b/2-5: Validation data for the analytical determination of 1,2-Benzisothiazol-3-(2*H*)-one in air at 33.3 ºC and 88% relative humidity at a flow rate of 2.0 L/min for a duration of 15 minutes.

Fortification level (mg/m^3)	n	Recovery		RSD (%)
		Range (%)	Mean (%)	
2.643	4	78.7 – 93.8	89.43	8.06
4.53	4	92.6 – 96.3	94.83	1.91

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2c ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Annex Point IIA, IV 4.2 (c)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2007b), Development and validation of a residue analytical method for the determination of 1,2-Benzisothiazolin-3-one (BIT) in drinking, surface and sea water, RCC Ltd, Analytics, Zelgiweg 1, CH-4452 Itingen, Switzerland, unpublished report no: A78276 Date of experimental work: August 11 – August 28, 2006	
1.2	**Data protection**	Yes	
1.2.1	Data owner	ROHM & HAAS	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted in accordance with the SANCO/3029/99 rev. 4 and SANCO/825/00 rev 7.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, with the following deviation: 1. Validation using ground water as a matrix was not performed as required by the guideline However, this deviation does not compromise the scientific validity of the study.	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	None.	
3.1.2	Cleanup	None	
3.2	**Detection**		

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2c ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Annex Point IIA, IV 4.2 (c)

3.2.1 Separation method

HPLC

Column: Zorbaz SB Phenyl (Agilent); 5 µm, (50 mm x 2.1mm)

Mobile phase: Eluent A: 95:5:0.1 (water:methanol:formic acid) and 5mM ammonium formate

Eluent B: 5:95:0.1 (water:methanol:formic acid) and 5 mM ammonium formate

Gradient:

Time (min.)	A %	B %
0	80	20
3.0	40	60
3.1	0	100
3.5	0	100
3.6	80	20
5.0	80	20

Flow rate: 300 µL/min

Injection volume: 50 µL

3.2.2 Detector

MS/MS

Ionisation mode: pneumatically and thermally associated electrospray ionisation (ESI)

Neguliser gas: air

Heater gas: air

Curtain gas: nitrogen

Collision gas: nitrogen

Heater gas temperature: 500 ºC

Spray voltage: 2500 V

Ion monitoring details: Precursor ion *m/z* 152, product ion m/z 105

(primary method), 109 (confirmatory method)

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2c ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Annex Point IIA, IV 4.2 (c)

3.2.3	Standard(s)	External standard 1,2-Benzisothiazolin-3-one: 100% purity; Lot no. 220904
3.2.4	Interfering substance(s)	None identified
3.3	**Linearity**	
3.3.1	Calibration range	0.025 – 1.0 ng/mL
3.3.2	Number of measurements	Single determinations at 6 levels
3.3.3	Linearity	Correlation coefficient (r) was determined as > 0.992. The equation of the calibration curve was y = 1006 + 173943x, where y is the peak area and x the concentration of the analyte in ng/mL.
3.4	**Specificity: interfering substances**	A HPLC-MS/MS method with a different daughter ion was used as confirmatory technique. However, this is not necessary due to the high specificity of the technique. It was found in the control samples that there was no interference with other compouds at the retention time of 1,2-Benzisothiazolin-3-one above 30% of the Limit of Quantitation (LOQ).
3.5	**Recovery rates at different levels**	Recovery was determined at two fortification levels for each water type. The mean recovery found in drinking water was 97% (with a range of 92-106%) and 101% (with a range of 96-105%) at fortification level 0.05 µg/L and 0.5 µg/L, respectively. The mean recovery found in surface water was 107% (with a range of 94-114%) and 109% (with a range of 107-109%) at fortification level 0.05 µg/L and 0.5 µg/L, respectively. The mean recovery found in sea water was 97% (with a range of 85-106%) and 77% (with a range of 70-82%) at fortification level 0.05 µg/l and 0.5 µg/L, respectively. The overall mean recovery for 1,2-Benzisothiazolin-3-one was 99% for drinking water, 108% for surface water and 87% for sea water. All these mean and overall mean values are within the SANCO/3029/99 rev.4 guideline requirements (70 – 110%). Refer to Table IIA 4.2c-1. Two controls were used as required by SANCO/825/00 rev.7 guideline requirements.

Section A4 Analytical Methods for Detection and Identification

Subsection A4.2c ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Annex Point IIA, IV 4.2 (c)

3.5.1	Relative standard deviation	Refer to Table IIA 4.2c-1.
3.6	**Limit of determination**	LOQ, defined as the lowest concentration at which a recovery of 70-110% with relative standard deviation of ≤ 20% is obtained, was 0.05 µg/L. The LOQ meets the requirements of Council Directive 80/778/EEC and SANCO/825/00 rev. 7 for drinking water, since the method can detect concentrations lower to 0.1 µg/L. According to the SANCO/825/00 rev. 7, the LOQ must be equal or lower than the concentration that has an impact on non-target organisms. The NOEL was established to be 1.13 mg/L for algae, 2.44 mg/L for daphnia and 4.9 mg/L for fish, which is greater than the LOQ, and therefore, acceptable.
3.7	**Precision**	
3.7.1	Repeatability	Repeatability was determined as the RSD calculated at two fortification levels for each water type. The RSD values were 4 – 6% for drinking water, 2 – 8% for surface water and 6 – 8% for sea water, which were within the required specification (i.e. RSD ≤ 20%).
3.7.2	Independent laboratory validation	Not required

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	An analytical method was validated for the determination of Benzisotiazolin-3-one in drinking water, surface water and sea water. Analyses were conducted by HPLC-MS/MS with a HPLC-MS/MS method using a different daughter ion as a confirmatory method. This study was conducted in accordance with the guidelines SANCO/3029/99 rev. 4 and SANCO/825/00 rev 7 with the following deviations: 1. Validation using ground water as a matrix was not performed as required by the guideline. However, this deviation does not compromise the scientific validity of the study.
4.2	**Conclusion**	The method is specific for the determination of 1,2-Benzisothiazolin-3-one in water. The mean recovery and RSD values were within the specified limits. The method is acceptable for the determination of 1,2-Benzisothiazolin-3-one in water.
4.2.1	Reliability	1

Section A4	**Analytical Methods for Detection and Identification**
Subsection A4.2c **Annex Point IIA, IV 4.2 (c)**	**ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER**

4.2.2	Deficiencies	One deviation was noted and is outlined under point 4.1. However, it does not compromise the scientific validity of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*July 2010*
Materials and methods	*The applicant's version is adopted*
Conclusion	*The applicant's version is adopted*
Reliability	*1*
Acceptability	*The method and result are acceptable*
Remarks	*No further remarks*

Table A4.2c-1: Validation data for the analytical determination of 1,2-Benzisothiazolin-3-one in water

Sample matrix	Fortification level (µg/L)	n	Recovery		RSD (%)
			Range (%)	Mean (%)	
Drinking water	0.05	5	92 – 106	97	6
Drinking water	0.50	5	96 – 105	101	4
Overall	-	10	92 – 106	99	5
Surface water	0.05	5	94 – 114	107	8
Surface water	0.50	5	107 – 112	109	2
Overall	-	10	94 - 114	108	5
Sea water	0.05	5	85 – 106	97	8
Sea water	0.50	5	70 – 82	77	6

Overall	-	10	70 – 106	87	14
Total	-	30	70 - 114	98	12

Section A4 **Subsection A4.2** **Annex Point IIA, IV 4.2 (d)**	**Analytical Methods for Detection and Identification** **ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN ANIMAL AND HUMAN BODY FLUIDS AND TISSUES**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	This is point is not relevant to 1,2-Benzisothiazol-3-(2*H*)-one as it is not classified as toxic or highly toxic according to acute mammalian toxicity studies.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*July 2010*	
Evaluation of applicant's justification	*The non-submission of data is justified*	
Conclusion	*Acceptable*	
Remarks	*No further remarks*	

Section A4 (4.3) Annex Point IIA, IV 4.3	Analytical Methods for Detection and Identification RESIDUE IN/ON FOOD OR FEEDSTUFFS	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	It is proposed that this point is not relevant as, according to the recommended use of 1,2-Benzisothiazol-3(2*H*)-one, the product is used in in-can preservatives (PT 6) and is not intended for spraying, aerosol use, etc. around food or feedstuffs. Therefore, the product will not be in contact with food or feedstuffs. No studies are therefore presented to address this point.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*June 2010*	
Evaluation of applicant's justification	*The non-submission of data is justified*	
Conclusion	*Acceptable*	
Remarks	*No further remarks*	

Section A5 Effectiveness against target organisms and intended uses

Subsection (Annex Point)			Official use only
5.1	**Function (IIA5.1)**	Preservative BIT is a preservative which is effective against a wide range of microbes.	
5.2	**Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)**	-	
5.2.1	Organism(s) to be controlled (IIA5.2)	**Organisms to be controlled (including but not limited to):** *Bacillus subtilis, Enterobacter aerogenes, Escerichia coli, Pseudomonas aeruginosa, Methylobacterium extorquens, Proteus vulgaris, Alcaligenes faecalis, Klebsiella pneumoniae, Aspergillus niger, Penicillium sp., Staphylococcus aureus, Rhizopus stolonifer, Aspergillus pencilloides, Alternaria radicina, Tricophyton mentagrophytes, Salmonella typhosa, Bacillus megaterium* and *Saccharomyces cerevisiae.*	
5.2.2	Products, organisms or objects to be protected (IIA5.2)	To be protected (including but not limited to): Titanium dioxide slurries, modified silica polymer dispersions, acrylic emulsion polymers, latex resin emulsions, water-based adhesives, paints and coatings, aqueous slurries, home cleaning and car care products, laundry detergents, fabric softeners, stain removers, polishes, soap, wax, tarnish agents, air fresheners, carpet shampoos, starch solutions, oil in water emulsions, latices, casein/rosin dispersions, textile spin-finish solutions, leather processing solutions, fuels, glues, caulks, sealants, grouts, spackling, ready-mixed cements, ready-mixed wallboard compounds, concrete mixtures and mortar.	X1
5.3	**Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)**		
5.3.1	Effects on target organisms (IIA5.3)	BIT disrupts membrane-mediated functions (including respiration, nutrient transport and waste excretion) leading to eventual cell death.	

Section A5 Effectiveness against target organisms and intended uses

5.3.2	Likely concentrations at which the A.S. will be used (IIA5.3)	
	PT13	20 % BIT [redacted] is either used by the manufacturer of the MWF concentrate and diluted in it at 1% to 2% (i.e. 0.2 - 0.4% BIT in the concentrate), which is then diluted with water 1:20 in the end MWF fluid (i.e. 0.01% - 0.02% BIT), or it can be directly used as it by the end-user and is incorporated in the MWF to get the same BIT concentration in it (i.e. 0.05% - 0.1% of [redacted] resulting in 0.01% - 0.02% BIT).
5.4	**Mode of action (including time delay) (IIA5.4)**	-
5.4.1	Mode of action	BIT attacks the thiol group-containing proteins found on the cytoplasmic membranes of bacteria, yeast and fungi. The mode of action is non-specific and is therefore not restricted to a certain enzyme or protein. Inactivation of membrane active proteins by BIT disrupts membrane-mediated functions (including respiration, nutrient transport and waste excretion) leading to eventual cell death. BIT acts an oxidising agent. The ring sulphur oxidises the thiol sulphur to form a disulfide bond. In the process, the ring nitrogen is reduced. If the reacted BIT encounters another free thiol, the free thiol is again oxidised to form a disulfide bond and the ring sulphur is reduced to a thiol. This results in the formation of mercaptobenzamide. If the reacted BIT encounters mercaptobenzamide, the thiol group on the mercaptobenzamide is oxidised to form a disulfide bond. This results in the formation of a dimer – dithiobisbenzamide.
5.4.2	Time delay	Information not available.
5.5	**Field of use envisaged (IIA5.5)**	
	MG02: Preservatives	Product Type PT 13
	Further specification	BIT is a preservative which is effective against a wide range of microbes.
5.6	**User (IIA5.6)**	
	Professional	[redacted] is intended for use by professionals in indoor scenarios.
	General public	PT 13 biocidal products are not intended for non professional use.

Section A5		Effectiveness against target organisms and intended uses	
5.7	**Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA5.7)**	-	
5.7.1	Development of resistance	No resistant strains have been shown in the efficacy trials conducted.	X2
5.7.2	Management strategies	Not applicable	
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	See Doc. III Appendix 2 – Business Confidential Information	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2011* *March 2015* *May 2020*
Materials and Methods	
Conclusion	*We do not have efficacy tests with the active substance but we have studies with the product in Doc. IV-B and Doc. III-B. We think that the efficacy of the active substance is demonstrated with the data of the product because of it contains 20% BIT and no other active substances are presented. For this reason, the summary studies in tabular format about efficacy of the active substance are in Doc. III-B. :*
Reliability	*2*
Acceptability	*Applicant's version accepted.*

Section A5 Effectiveness against target organisms and intended uses

Remarks	***X1**: the use for this product type is only refers to metal working fluid (PT13)* ***X2**: The applicant submitted the following information:* *"The active substance 1,2-benzisothiazol-3(2H)-one (BIT) belongs to the chemical class of isothiazolones. Several different active substances within this class have been used since many years as bactericides and fungicides for various applications. No development of resistance has been reported so far since the mode of action of BIT is unspecific. The FRAC Code List 2015 lists commercial available fungicides and some most important bactericides according to their mode of action and resistance risk. In this list isothiazolones and benzisothiazole are classified as active substances with no known resistance (http://www.frac.info/publications/downloads)* *Development of resistance is not likely since the reaction of BIT with membrane proteins is unspecific and membrane proteins with different functions will be inactivated upon contact with BIT thus lowering the risk of selection for resistant strains."*

Table A5.1: Summary table of experimental data on the effectiveness of the active substance against target organisms at different fields of use envisaged, where applicable

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
Preservative	MG02	As given in section 2	*Pseudomonas aeruginosa, Proteus vulgaris, Alcaligenes faecalis, Klebsiella pneumoniae, Penicillium notatum, Aspergillus niger* and *Saccharomyces cerevisiae.*	[redacted]	Samples were taken weekly, prior to inoculation. Fungal plates were incubated at 22 °C for 7 days and bacterial plates at 37 °C for 48 hours. Counts were recorded.	[redacted] containing 20% BIT, which is equivalent to the product being supported passed all three tests i.e. had bacterial counts less than 10^5 per ml and fungal counts less than 10^3 per ml at week 6.	[redacted] (2005) (Doc IVB 5.10_2)
Preservative	MG02	As given in section 2	The bacteria used were obtained	[redacted]	Bacterial counts were recorded weekly, prior to	In the synthetic metalworking fluid containing n-butyl ethanolamine, the sample	[redacted] (2004)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
			from contaminated coolant samples.	[redacted]	inoculation. The agar plates were incubated at 37 °C for 48 hours.	containing 500 ppm [redacted] failed after the 7th inoculum. The sample containing 900 ppm of [redacted] showed low bacterial counts after the 7th inoculum onwards, but in fact never met the criteria for failure even after 18 inoculations. In the synthetic fluid containing amino methyl propanol, the sample containing 500 ppm [redacted] failed after the 10th inoculum. The sample containing 900 ppm [redacted] showed variable and low bacterial counts from the 5th inoculum onwards, but in fact never met the criteria for failure even after 18 inoculations.	(Doc IVB 5.10_1)

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.1/1 Acute Toxicity

Annex Point IIA VI.6.1.1 6.1.1 Acute oral toxicity in rats (LD_{50} test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: July 15 – October 1, 1993	
1.2	**Data protection**	Yes	
1.1	Data owner	Troy Chemical Company BV	
1.2	Companies with letter of access	DOW	
1.3	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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to US EPA Guideline 81-1 which is equivalent to OECD Guideline 401.	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazol-3-(2*H*)-one ([redacted])	
3.1.1	Lot/Batch number	060793	
3.1.2	Specification	Please refer to Doc. III-A 2/2	
3.1.2.1	Description	Tan powder	
3.1.2.2	Purity	99.29%	
3.1.2.3	Stability	Not relevant (single dose only)	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Crl:CD®BR	
3.2.3	Source	Charles River Laboratories	

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.1/1 Acute Toxicity

Annex Point IIA VI.6.1.1 6.1.1 Acute oral toxicity in rats (LD_{50} test)

3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Not documented 220 – 299g
3.2.6	Number of animals per group	Range finding study: 1/sex/dose Definitive study: 5 rats/sex/dose
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Postexposure period	14 days
		Oral
3.3.2	Type	Gavage
3.3.3	Doses	Range finding 500, 1000, 2500 and 5000 mg/kg bw Definitive study Male: 600, 1200 and 1500 mg/kg bw Female: 600, 900 and 1200 mg/kg bw
3.3.4	Vehicle	Distilled water
3.3.5	Concentration in vehicle	Not documented
3.3.6	Total volume applied	10 mL/kg bw
3.3.7	Control	None
3.4	**Examinations**	Clinical observations, mortality, and gross necropsy examinations
3.5	**Method of determination of LD_{50}**	Modified Behrens-Reed-Muench cumulant method
3.6	**Further remarks**	None

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.1/1 Acute Toxicity

Annex Point IIA VI.6.1.1 6.1.1 Acute oral toxicity in rats (LD_{50} test)

4 RESULTS AND DISCUSSION

4.1	**Mortality**	Two females died in the 900 mg/kg bw group. Two males died in the 1200 mg/kg bw group. Please refer to Table A6.1.1/1-1
4.2	**Clinical signs**	Hypoactivity, staggered gait, absence of pain or righting reflex, hunched posture, prostration, mydriasis, miosis, lacrimation, red- or dark-stained face, excessive salivation, dyspnea, liquid or soft stool, dark or yellow stained urogenital area and death
4.3	**Pathology**	Lesions of the gastrointestinal tract in particular the stomach
4.4	**Other**	No body weight changes in surviving animals
4.5	**LD_{50}**	Definitive study Male: 1246 mg/kg bw Female: 944 mg/kg bw Sexes combined: 1010 mg/kg bw

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute oral toxicity of [redacted] was investigated by administering single doses of 600, 1200 and 1500 mg/kg bw to 3 groups of males (5/group) and 600, 900 and 1200 mg/kg bw to 3 groups of females (5/group). Initially, eight acclimated rats (one/sex/dose level) were used for each of four dose levels (500, 1000, 2500 and 5000 mg/kg bw) in range finding study in order to determine to definitive dose levels for the main study. Food and water were available ad libitum during the study, except for approximately 17 to 20 hours before the test material administration, when food, but not water, was withheld. This study was conducted according to US EPA Guideline 81-1 which is equivalent to OECD Guideline 401 and is described under point 3 with no deviations.

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.1/1 Acute Toxicity

Annex Point IIA VI.6.1.1 6.1.1 Acute oral toxicity in rats (LD_{50} test)

5.2	**Results and discussion**	Range-finding study: The male and female animal treated at 500 mg/kg bw and the male treated at 1000 mg/kg bw survived to termination. The female animal treated at 1000 mg/kg bw and all males and females treated at 2500 and 5000 mg/kg bw died within 3 days of treatment. Main study: All the males treated at 1500 mg/kg bw died. Two out of 5 males died at 1200 mg/kg bw and no male died at 600 mg/kg bw. All the females that received 1200 mg/kg bw test substance were found dead. Two out of 5 females died at 900 mg/kg bw and no female died at 600 mg/kg bw. Clinical signs of toxicity such as hypoactivity, staggered gait absence of pain or righting reflex, hunched posture, prostration, mydriasis, miosis, lacrimation, red- or dark-stained face, excessive salivation, dyspnea, liquid or soft stool, dark or yellow stained urogenital area were reported. There were no changes in body weight in surviving animals. In animals that died, the most predominant finding at necropsy was gastrointestinal lesions. The glandular mucosa of the stomach was diffusely red or dark red. The stomach and intestines contained material of variable color and consistency which could be a mixture of ingesta, autolysis or both. Animals that survived until study termination displayed adhesions involving the serosa of the gastrointestinal tract and the capsular surface of the liver. The mucosal surface of the stomach in some of these animals appeared thickened, had eroded areas, and had variable color changes. A tan semisolid material was noted in the stomachs of some animals. These findings were deemed to be treatment related. Please refer to Table A6.1.1/1-1.
5.3	**Conclusion**	The oral LD_{50} of the test substance was found to be 1246 mg/kg bw for males, 944 mg/kg bw for females and 1010 mg/kg bw for combined sexes. In accordance with Council Directive 2001/59/EEC (28th ATP), [redacted] is classified as harmful if swallowed and the symbol "Harmful" – Xn, and the risk phrase "R22", harmful if swallowed are required.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.1/1 Acute Toxicity

Annex Point IIA VI.6.1.1 **6.1.1 Acute oral toxicity in rats (LD_{50} test)**

Date	*August 2008*
Materials and Methods	*Applicant version is adopted*
Results and discussion	*Applicant's version is accepted except data displayed in Table A6.1.1/1-1, where is stated that in both (males and females) the mortality of animals treated with 1200 mg/kg bw was 0%, when indeed the record was 100%. There is also a mistake in the description of mortalities (first paragraph of section 5.2). An appropriate description of mortalities caused by BIT might be: "All the males treated at 1500 mg/kg bw died. Two out of 5 males and all females died at 1200 mg/kg bw. Two of five females that received 900 mg/kg bw were found dead.* *There were no mortalities in animals (males and females) treated with 600 mg BIT/kg bw.*
Conclusion	*Applicant version is adopted*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	*The purity of the tested substance (99.29% according to section 3.1.2.2) was not described in the Doc. IV, where is stated that this is responsibility of the sponsor.*

Table A6.1.1/1-1: Mortality data of [redacted]

Dose (mg/kg bw)	Sexes	Number of dead / number of investigated	Time of death	Observations
600	Male	0/5	-	-
600	Female	0/5	-	-
900	Female	2/5	Before day 7	-
1200	Male	2/5	Before day 7	-
1200	Female	5/5	Before day 7	-
1500	Male	5/5	Before day 7	-
LD_{50}	Male: 1246 mg/kg bw Female: 944 mg/kg bw Sexes combined: 1010 mg/kg bw			

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.1/2 Acute Toxicity

Annex Point IIA VI.6.1.1 **6.1.1 Acute oral toxicity in rats (LD_{50} test)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
		Dates of experimental work: July 15 – August 12, 2002	
1.2	**Data protection**	Yes	
1.1	Data owner	Dow Benelux BV	
1.2	Companies with letter of access	Troy Chemical Company BV	
1.3	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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD Guideline 401.	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazol-3-(2*H*)-one (BIT)	
3.1.1	Lot/Batch number	BT 17301	
3.1.2	Specification	Please refer to Doc. III-A 2/1	
3.1.2.1	Description	Beige to light brown coloured powder	
3.1.2.2	Purity	97.42% (Dry basis) 72.50% (Wet basis)	
3.1.2.3	Stability	Not relevant (single dose only)	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar	
3.2.3	Source	Breeding facility, Jai Research Foundation	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.1/2	**Acute Toxicity**
Annex Point IIA VI.6.1.1	**6.1.1 Acute oral toxicity in rats (LD_{50} test)**

3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	10-11 weeks at the time of dosing 158 – 248 g
3.2.6	Number of animals per group	Range finding study: 3 groups of 2 animals/sex Definitive study: 6 groups of 5 animals/sex
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Postexposure period	14 days
		Oral
3.3.2	Type	Gavage
3.3.3	Doses	Range finding 500, 1000 and 1500 mg/kg bw Definitive study 0, 450, 600, 698, 900 and 1350 mg/kg bw
3.3.4	Vehicle	0.5 % Carboxy methyl cellulose (CMC) solution
3.3.5	Concentration in vehicle	Not documented
3.3.6	Total volume applied	10 mL/kg bw
3.3.7	Control	CMC solution
3.4	**Examinations**	Mortality was recorded at 1, 2, 3, 4 hours and once thereafter after oral gavage on the day of dosing, and twice a day for a period of 14 days after dosing. Clinical observations were recorded at the same times on the day of dosing, and once a day during the study. Individual body weight was recorded prior to dosing (day 0) and days 7 and 14 following dosing. All animals were subjected to gross pathological examinations.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.1/2	**Acute Toxicity**
Annex Point IIA VI.6.1.1	**6.1.1 Acute oral toxicity in rats (LD_{50} test)**

3.5	**Method of determination of LD_{50}**	Probit analysis (Finney, 1971)
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Mortality**	3, 4, 7, 10 and 8 animals were found dead at the dose level of 450, 600, 689, 900 and 1350 mg/kg bw respectively. Please refer to Table A6.1.1/2-1
4.2	**Clinical signs**	Lethargy, abdominal breathing, gasping, nostril discharge and piloerection were observed in animals of the treated groups.
4.3	**Pathology**	Prominent lesions in lungs, liver, intestine, stomach and trachea were observed in animals of the treated groups.
4.4	**Other**	Normal increase in the body weight was observed in all groups with the exception of one animal at 600 mg/kg bw and one animal at 1350 mg/kg bw, which showed a slight decrease on day 7 and 14, and day 7 respectively.
4.5	**LD_{50}**	597.40 mg/kg bw
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The acute oral toxicity of BIT was investigated by administering single doses of 450, 600, 698, 900 and 1350 mg/kg bw to 5 groups of rats. This study was conducted according to OECD guideline 401 and is described under point 3 with no deviations.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.1/2	**Acute Toxicity**
Annex Point IIA VI.6.1.1	**6.1.1 Acute oral toxicity in rats (LD_{50} test)**

5.2	**Results and discussion**	All the females and three out of 5 males treated at 1350 mg/kg bw died. All the animals that received 900 mg/kg bw test substance were found dead. Three males and four females died at 698 mg/kg bw, two animals of each sex at 600, and 3 females at 450 mg/kg bw. Clinical signs of toxicity such as lethargy, abdominal breathing, gasping, nostril discharge and piloerection were reported in animals of treated groups. Those in the control group were found normal throughout the observation period. There were no abnormal changes in body weight in surviving animals. In animals that died, the predominant findings at necropsy were lungs and liver lesions. There was prominent haemorrhage/congestion oedema and emphysema in lungs, mottling/haemorrhage/congestion and pale foci in liver, mucus exhudation in intestine, haemorrhage/ulceration in stomach and haemorrhage in trachea. These lesions could be considered test substance related. Animals that survived until study termination displayed various inflammatory/vascular changes with low level of occurrence, which were considered unrelated with test substance. Please refer to Table A6.1.1/2-1.
5.3	**Conclusion**	The oral LD_{50} of BIT in Wistar rats was found to be 597.40 mg/kg bw and the 95% fiducial limits were 482.39 to 739.82 mg/kg bw. In accordance with Council Directive 2001/59/EEC (28th ATP), BIT is classified as harmful if swallowed and the symbol "Harmful" – Xn, and the risk phrase "R22", harmful if swallowed are required.
5.3.3	Reliability	1
5.3.4	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*August 2008*
Materials and Methods	*Applicant version is adopted*
Results and discussion	*Applicant version is adopted*
Conclusion	*Applicant's conclusion is adopted, although LD_{50} values must be adjusted for BIT purity (see sections of remarks).*

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.1/2 Acute Toxicity

Annex Point IIA VI.6.1.1 **6.1.1 Acute oral toxicity in rats (LD_{50} test)**

Reliability	*1*
Acceptability	*Acceptable*
Remarks	*The test material displayed a purity of 97.42% on the dry basis. The purity must be taken into consideration for adjusting the doses. Thus, the real doses were: 438, 585, 680, 877 and 1315 mg BIT/kg bw. In conclusion, the LD_{50} was 582 mg BIT/kg bw and the 95% fiducial limits were 470 to 721 mg/kg bw.* *Minor discrepancy with comments about body weight changes described in third paragraph of section 5.2. This point will be more properly described as follows: Normal increase in the body weight was observed in all groups with the exception of one animal at 600 mg/kg bw and one animal at 1350 mg/kg bw, which showed a slight decrease on day 7 and 14, and day 7 respectively.*

Table A6.1.1/2-1: Acute oral toxicity of BIT in rats

Dose (mg/kg bw)	Number of animals used	Mortalities/sex		Time of death	Mortalities %
		Male	Female		
0	10	0	0	-	0
450	10	0	3	Before day 7	30
600	10	2	2	Before day 7	40
698	10	3	4	Before day 7	70
900	10	5	5	Before day 7	100
1350	10	3	5	Before day 1	80
LD_{50}	597.40 mg/kg bw				

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.2/1 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit Test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
		Dates of experimental work: August 15 – August 29, 2001	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access	DOW	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD Guideline 402	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one Technical	
3.1.1	Lot/Batch number	BT 11600	
3.1.2	Specification	Please refer to Doc. III-A 2/2	
3.1.2.1	Description	Off white solid	
3.1.2.2	Purity	98%	
3.1.2.3	Stability	Not relevant, single dose only	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley derived, albino	

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.2/1 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit Test)

3.2.3	Source	Ace
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	9 – 10 weeks Males: 245 – 268 g Females: 207 – 222 g
3.2.6	Number of animals per group	10 (5/sex)
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Dermal
3.3.1	Postexposure period	14 days
3.3.2	Area covered	10% of body surface
3.3.3	Occlusion	Occlusive
3.3.4	Vehicle	Administered as supplied
3.3.5	Doses	Not applicable
3.3.6	Total volume applied	Not applicable. The test substance was applied as a dry paste (80% w/w mixture in distilled water).
3.3.7	Duration of exposure	24 hours
3.3.8	Removal of test substance	At the end of the exposure period, the pads were removed and the test sites were gently wiped with water and a clean towel to remove any residual test substance.
3.3.9	Controls	Not applicable

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.2/1 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit Test)

3.4	**Examinations**	Mortality was recorded at 1 and 4 hours after application and at least once daily thereafter for 14 days Signs of gross toxicity, behavioural changes at 1 and 4 hours after application and at least once daily thereafter for 14 days. Individual body weights were recorded prior to test substance application, on days 7 and 14 Gross necropsy: tissue and organs of the thoracic and abdominal cavities were performed on all animals on day 14.
3.5	**Method of determination of LD_{50}**	Not applicable
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	No signs of gross toxicity, adverse pharmacologic effects, abnormal behaviour, or dermal irritation were observed at 2000 mg/kg bw. All animals survived. Please refer to Table A6.1.2/1-1.
4.2	**Pathology**	No gross abnormalities were observed at necropsy
4.3	**Other**	All animals gained weight and appeared healthy during the study.
4.4	**LD_{50}**	Males > 2000 mg/kg bw Females > 2000 mg/kg bw Combined > 2000 mg/kg bw No lethal effect at maximal dose
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The acute dermal toxicity of 1,2-Benzisothiazolin-3-one Technical was investigated by applying a single dose of 2000 mg/kg bw topically to Sprague-Dawley rats. The study was conducted according to OECD Guideline 402 and is described under point 3 with no deviations.
5.2	**Results and discussion**	No gross signs of toxicity, adverse pharmacologic effects, abnormal behaviour, or dermal irritation were observed. All animals survived. Please refer to Table A6.1.2/1-1. No gross abnormalities were observed at necropsy. All animals gained weight and appeared healthy during the study.

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.2/1 Acute Toxicity

Annex Point IIA VI.6.1.2 **6.1.2 Acute dermal toxicity in rats (Limit Test)**

5.3	**Conclusion**	In accordance with Council Directive 2001/59/EEC (28th ATP), 1,2-Benzisothiazolin-3-one Technical remains unclassified and requires no symbols or risk phrases.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*August 2008*
Materials and Methods	*Applicant version is adopted.*
Results and discussion	*Applicant version is adopted.*
Conclusion	*Applicant version is adopted.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.1.2/1-1: Mortality data of 1,2-Benzisothiazolin-3-one Technical

Dose (mg/ kg bw)	Males Mortality	Time of death – Days (no of animals)	Dose (mg/kg bw)	Females Mortality	Time of death – Days (no of animals)
2000	0/5	-	2000	0/5	-
LD_{50} value	> 2,000 mg/kg		LD_{50} value	> 2,000 mg/kg	

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.2/2 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit Test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
		Dates of experimental work: July 01– July 15, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD Guideline 402	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazol-3-(2*H*)-one (BIT)	
3.1.1	Lot/Batch number	BT 17301	
3.1.2	Specification	Please refer to Doc. III-A 2/1	
3.1.2.1	Description	Beige to light brown coloured powder	
3.1.2.2	Purity	97.42% (Dry basis) 72.50% (Wet basis)	
3.1.2.3	Stability	Not relevant, single dose only	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar	

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.2/2 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit Test)

3.2.3	Source	Breeding facility, Jai Research Foundation
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Not documented 213-285 g
3.2.6	Number of animals per group	Range finding study: 2 groups of 2 animals/sex Main study: 2 groups of 5 animals/sex
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	**Dermal**
3.3.1	Postexposure period	14 days
3.3.2	Area covered	10% of body surface
3.3.3	Occlusion	Semi-occlusive: porous gauze dressing and surgical tape
3.3.4	Vehicle	Distilled water
3.3.5	Doses	Not documented
3.3.6	Total volume applied	Not applicable. Substance was moistened with distilled water
3.3.7	Duration of exposure	24 hours
3.3.8	Removal of test substance	At the end of the exposure period, the dressings were removed and the residual test substance was removed using cotton moistened with distilled water.
3.3.9	Controls	Distilled water

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.2/2 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit Test)

3.4	**Examinations**	Mortality was recorded at 1, 2, 3 and 4 hours and 40 minutes after application and twice daily thereafter for 14 days. After dosing, clinical signs were recorded at the same time as above on day 0, and once a day after application for 14 days. Individual body weights were recorded prior to test substance application, on days 7 and 14. Gross necropsy examination consisting of an external examination and opening of the thoracic and abdominal cavities was performed on all animals on day 14.
3.5	**Method of determination of LD_{50}**	Not applicable
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	All animals survived. No signs of gross toxicity were observed at 2000 mg/kg bw. Please refer to Table A6.1.2/2-1.
4.2	**Pathology**	No pathological lesions were observed at necropsy.
4.3	**Other**	The mean body weight of treated animals was comparable to that of control group animals.
4.4	**LD_{50}**	> 2000 mg/kg bw No lethal effect at maximum dose
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The acute dermal toxicity of BIT was investigated by applying a single dose of 2000 mg/kg bw topically to one group of 5 Wistar rats/sex. The study was conducted according to OECD Guideline 402 and is described under point 3 with no deviations.
5.2	**Results and discussion**	No mortalities were observed in the control as well as the group treated with BIT at the dose level of 2000 mg/kg bw. No clinical signs were observed in any of the groups. All animals gained weight and appeared healthy during the study. Post-mortem examination of terminally sacrificed animals from both groups, control and treated, showed lesions in lungs, liver and

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.2/2	**Acute Toxicity**
Annex Point IIA VI.6.1.2	**6.1.2 Acute dermal toxicity in rats (Limit Test)**

		congestion in kidneys. These were considered unrelated to the test substance. Please refer to Table A6.1.2/2-1.
5.3	**Conclusion**	The dermal LD_{50} of BIT in Wistar rats was found to be > 2000 mg/kg bw. In accordance with the provisions of Commission Directive 2001/59/EEC (28th ATP), BIT remains unclassified and requires no symbols or risk phrases.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*August 2008*
Materials and Methods	*Applicant version is adopted.*
Results and discussion	*Applicant version is adopted.*
Conclusion	*Applicant version is adopted.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.1.2/2-1: Acute dermal toxicity of BIT in rats

Dose (mg/ kg bw)	Males Mortality	Time of death – Days (no of animals)	Dose (mg/kg bw)	Females Mortality	Time of death – Days (no of animals)
0	0/5	-	0	0/5	-
2000	0/5	-	2000	0/5	-

LD_{50} value	> 2,000 mg/kg	LD_{50} value	> 2,000 mg/kg

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.1.3	**ACUTE INHALATION STUDY**	
Annex Point IIA, 6.1.3		
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Prior to carrying out the acute inhalation study, a feasibility test was performed on the generation of a BIT aerosol. According to the report by [redacted] (2002b), fourteen pre-test generation trials were conducted in an attempt to generate a suitable atmosphere for four hours at a chamber concentration of 2 mg/L for the acute inhalation test. However, due to the nature of the test substance, it was not possible to produce an aerosol at this concentration and with a particle size having a Mass Medium Aerodynamic Diameter of less than 4 µm. A number of attempts were carried out to aerosolise the test substance such as using a carbide blade and dissolving the test substance in water. The experiments are described in the report by [redacted] (2002b). Therefore, it was concluded that such a study was not feasible and that no further work would be carried out. For most use patterns of BIT, the inhalation route is not expected to be a major route of exposure and no inhalation classification is appropriate.	
Undertaking of intended data submission [ ]	Not relevant	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*August 2008*
Evaluation of applicant's justification	*BIT has proved to be irritant (corrosive to eye) and also has caused stomach irritation in acute oral toxicity test. Therefore, this irritant-corrosive nature of BIT might also be also manifested on the respiratory tract, causing severe toxic effects after acute exposure by inhalation. This is the main reason because the acute inhalation toxicity study would be desirable despite the mass medium aerodynamic diameter were slightly higher than 4 µm.*
Conclusion	*Applicant's justification is accepted due to described technical reasons, although due to the lack of information the adoption of protective measures to minimize exposure by inhalation might be desirable.*

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.3	**ACUTE INHALATION STUDY**
Annex Point IIA, 6.1.3	
Remarks	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.4/1	**Acute Dermal Irritation**
Annex Point IIA VI.6.1.4	**6.1.4 Acute dermal irritation**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: August 14 – August 17, 2001	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Company with letter of access	DOW	
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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to US EPA guideline OPPTS 870.2500 which is equivalent to OECD Guideline 404.	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	Yes, with the following deviations: 1. The bodyweights of the animals at start and conclusion of test were not provided. 2. It is not clearly stated if animals were clipped on two test sites for which the untreated area serves as the control. These deviations are not considered to compromise the scientific validity of the study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one Technical	

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.4/1 Acute Dermal Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute dermal irritation

3.1.1	Lot/Batch number	#BT 11600
3.1.2	Specification	Please refer to Doc. III-A 2/2
3.1.2.1	Description	Off white solid
3.1.2.2	Purity	98%
3.1.2.3	Stability	The test substance is expected to be stable for the duration of testing (single application).
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand albino
3.2.3	Source	Davidson`s Mill Farm, South Brunswick, NJ, USA
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Young adult
3.2.6	Number of animals per group	1 group of 3 males
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Dermal
3.3.1	Application	
3.4.1.1	Preparation of test substance	Test substance was prepared by mixing 0.5 g of test substance with distilled water to achieve a dry paste by preparing an 80% w/w mixture.
3.4.1.2	Test site and Preparation of Test Site	Twenty-four hours prior to the treatment, the test area (approximately 6 cm^2 of skin on the dorsal area and the trunk) of each rabbit was clipped free of fur.
3.3.2	Occlusion	Semiocclusive The pad and trunk of the animals was wrapped with semi-occlusive 3-inch Micropore tape to avoid dislocation of the pad.
3.3.3	Vehicle	Distilled water

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.4/1 Acute Dermal Irritation

Annex Point IIA VI.6.1.4 **6.1.4 Acute dermal irritation**

3.3.4	Concentration in vehicle	80% w/w mixture
3.3.5	Total volume applied	Not relevant (the substance is a solid, and 0.5 g was applied)
3.3.6	Removal of test substance	At the end of the exposure period, the wrappings were removed and the skin was gently rinsed of any residual test substance with distilled water.
3.3.7	Duration of exposure	4 hours
3.3.8	Postexposure period	72 hours
3.3.9	Controls	Not documented
3.4	**Examinations**	
3.4.1	Clinical signs	Animals were observed for signs of gross toxicity and behavioural changes at least once daily during the test period. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhoea and coma.
3.4.2	Dermal examination	Yes
3.4.3	Scoring system	Draize method
3.4.4	Examination time points	60 min, 24 hours, 48 hours and 72 hours
3.4.5	Other examinations	None
3.5	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Average score**	
4.1.1	Erythema	The average scores for all animals were 1.67 at 60 min, 0.67 at 24 h, 0 at 48 h and 0 at 72 h. The mean score (24-72 hours) was 0.22. Please refer to Table A6.1.4/1-1

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.4/1 Acute Dermal Irritation

Annex Point IIA VI.6.1.4 6.1.4 Acute dermal irritation

4.1.2	Oedema	The average scores for all animals at 60 minutes, 24, 48 and 72 hours was 0 in all cases. The mean score (24-72 hours) was 0. Please refer to Table A6.1.4/1-1
4.2	**Reversibility**	Yes Erythema noted at 30 - 60 minutes was reversed by the 48 h timepoint.
4.3	**Other examinations**	All animals appeared active and healthy during the study. Apart from the dermal irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behaviour.
4.4	**Overall result**	The test substance was determined to be non-irritating to rabbit skin.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute dermal irritation of 1,2-Benzisothiazolin-3-one Technical was investigated by applying a single dose of the test substance to the skin of a group of 3 White New Zealand rabbits for four hours. The study was conducted according to Guidelines OPPTS 870.2500 and OECD Guidelines 404 and is described under point 3 with the following deviations: 1. The bodyweights of the animals at start and conclusion of test were not provided. 2. It is not clearly stated if animals were clipped on two test sites for which the untreated area serves as the control. These deviations are not considered to compromise the scientific validity of the study.
5.2	**Results and discussion**	All animals appeared active and healthy during the study. Apart from the dermal irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behaviour. There was no oedema observed at any treated site during this study. Within an hour after patch removal, very slight to well defined erythema was noted for all three treated dose sites. The overall incidence and severity of irritation decreased with time. All animals were free from dermal irritation within 48 hours. The average scores for erythema for all animals were 1.67 at 60 min, 0.67 at 24 h, 0 at 48 h and 0 at 72 h. The mean score (24-72 hours) was 0.22 and 0 for erythema. Results are summarised in Table A6.1.4/1-1. The average scores for oedema for all animals at 60 minutes, 24, 48 and 72 hours was 0 in all cases. The mean score (24-72 hours) was 0 for oedema. Please refer to Table A6.1.4/1-1.
5.3	**Conclusion**	On the basis of reactions observed in this study and the criteria defined in Council Directive 2001/59/EC (28th ATP), 1,2-Benzisothiazolin-3-

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.4/1	**Acute Dermal Irritation**
Annex Point IIA VI.6.1.4	**6.1.4 Acute dermal irritation**

		one Technical is not classified as a skin irritant. No symbol or risk phrase is required.
5.3.1	Reliability	1
5.3.2	Deficiencies	Two deviations were noted and are outlined under point 2.3 and 5.1. However, these deviations are not considered to compromise the scientific validity of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*August 2008*
Materials and Methods	*Applicant version is adopted*
Results and discussion	*Applicant version is adopted*
Conclusion	*Applicant version is adopted*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.1.4.a/01-1. Dermal Irritation scores for 1,2-Benzisothiazolin-3-one Technical treated sites

	Erythema			**Oedema**		
Animal no.	5849 M	5850 M	5851 M	5849 M	5850 M	5851 M
After 24 h	1	1	0	0	0	0
After 48 h	0	0	0	0	0	0
After 72 h	0	0	0	0	0	0
Mean score 24-72 h	0.22			0.0		

* M : male

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.4/2 Acute Eye Irritation

Annex Point IIA VI.6.1.4.b/01 **6.1.4 Acute eye irritation (a)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
		Dates of experimental work: August 22 – August 29, 2001	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Company with letter of access	DOW	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes, the study was conducted according to US EPA guideline OPPTS 870.2400 which is equivalent to OECD Guideline 405.	
2.3	**GLP**	Yes (self-certified)	
2.4	**Deviations**	Yes, the following deviation was noted: The weight of the animals was not mentioned at study initiation. This deviation is minor and is not considered to compromise the scientific validity of this study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one Technical	
3.1.1	Lot/Batch number	BT 11600	
3.1.2	Specification	Please refer to Doc. III-A 2/2	
3.1.2.1	Description	Off white solid	
3.1.2.2	Purity	98%	
3.1.2.3	Stability	Not relevant, single dose only	

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.4/2 Acute Eye Irritation

Annex Point IIA VI.6.1.4.b/01 **6.1.4 Acute eye irritation (a)**

3.2 Test Animals	
3.2.1 Species	Rabbit
3.2.2 Strain	New Zealand albino
3.2.3 Source	Davidson's Mill Farm, South Brunswick, NJ, USA
3.2.4 Sex	2 males and 1 female
3.2.5 Age/weight at study initiation	Young adult/Not documented
3.2.6 Number of animals per group	1 group of 3 animals
3.2.7 Control animals	The left eye of each rabbit served as a control
3.3 Administration/ Exposure	
3.3.1 Preparation of test substance	Test substance was used as delivered
3.3.2 Amount of active substance instilled	0.08-0.09 g/ treated eye (0.1mL of the test substance)
3.3.3 Exposure period	Acute/single dose
3.3.4 Postexposure period	7 days
3.4 Examinations	
3.4.1 Ophthalmoscopic examination	Yes Ocular irritation was evaluated using a high-intensity white light (Mag Lite) in accordance with the Draize *et al* method.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.4/2	**Acute Eye Irritation**
Annex Point IIA VI.6.1.4.b/01	**6.1.4 Acute eye irritation (a)**

3.4.2 Scoring system	The irritant response was scored following the method of Draize *et al*. Observation were carried out for signs of ocular irritation at 1, 24, 48 and 72 hours and at 4 and 7 days after instillation. At 24 hours, one drop of 2% ophthalmic fluorescein sodium was instilled into the eyes of each rabbit. 30 seconds later the eyes were rinsed with physiological saline (0.9% NaCl) a Blak-Ray® Lamp was then used to evaluate the extent of corneal damage and any gross abnormalities. Observations of the cornea, iris, conjunctivae and any other observed lesions were noted.
3.4.3 Examination time points	1, 24, 48 and 72 hours and at 4 and 7 days after instillation.
3.4.4 Other examinations	Signs of gross toxicity, behavioural changes, gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour pattern, tremors, convulsions, salivation, diarrhoea and coma.
3.5 Further remarks	None
	4 RESULTS AND DISCUSSION
4.1 Clinical signs	No abnormalities or signs of gross toxicity, other than eye irritation, were recorded in any of the three rabbits.
4.2 Average score	
4.2.1 Cornea	The average score for all animals was 2.0 at 24 h, 2.33 at 48 h, 2.67 at 72 h, 3 at 4 days and 4 at 7 days. The mean score (24-72 hours) was 2.33. Please refer to Table A6.1.4/2-1. More than half the cornea was affected by opacity.
4.2.2 Iris	The average score for all animals was 1 at 24 h, 48 h, 72 h, 4 days and 7 days. The mean score (24-72 hours) was 1. Please refer to Table A6.1.4/2-1.
4.2.3 Conjunctiva	
4.2.3.1 Redness	The average score for all animals was 3 at 24 h, 48 h, 72 h and 4 days and 2 at 7 days. The mean score (24-72 hours) was 3. Please refer to Table A6.1.4/2-1.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.4/2	**Acute Eye Irritation**
Annex Point IIA VI.6.1.4.b/01	**6.1.4 Acute eye irritation (a)**

4.2.3.2 Chemosis	The average score for all animals was 3 at 24 h and 48 h, 3.33 at 72 h and 4 days and 3 at 7 days. The mean score (24-72 hours) was 3.11. Please refer to Table A6.1.4/2-1.
4.3 Reversibility	No The overall severity of irritation increased with time.
4.4 Other	Blanching and light red discharge was recorded in various animals at random time points.
4.5 Overall result	All animals appeared active and healthy during the study. Apart from the eye irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behaviour. One hour following test substance instillation, all treated eyes exhibited conjunctivitis, cornea opacity and iritis. The mean score (24-72 hours) was 2.33, 1, 3 and 3.11 for corneal opacity, iritis, conjunctival redness and conjunctival chemosis, respectively. Irritation was irreversible and the overall severity of irritation increased with time.
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	The acute eye irritation of 1,2-Benzisothiazolin-3-one Technical was investigated by instilling a single dose of 1,2-benzisothiazolin-3-(2*H*)-one into the conjunctival sac of one eye of a group of three New Zealand rabbits. Ocular irritation was evaluated in accordance with Draize *et al*. The study was conducted according to US EPA guideline OPPTS 870.2400 which is equivalent to OECD Guideline 405 and is described under point 3. The following deviation was noted: The weight of the animals was not mentioned at study initiation. However, this deviation is minor and is not considered to compromise the scientific validity of this study.

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.4/2 Acute Eye Irritation

Annex Point IIA VI.6.1.4.b/01 **6.1.4 Acute eye irritation (a)**

5.2	**Results and discussion**	All animals appeared active and healthy during the study. Apart from the eye irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behaviour. One hour following test substance instillation, all treated eyes exhibited conjunctivitis, cornea opacity and iritis. At 24 h, the average score was 2.0, 1, 3 and 3 for corneal opacity, iritis, conjunctival redness and conjunctival chemosis, respectively. At 48 h, the average score was 2.33, 1, 3 and 3 for corneal opacity, iritis, conjunctival redness and conjunctival chemosis, respectively. At 72 h, the average score was 2.67, 1, 3 and 3.33 for corneal opacity, iritis, conjunctival redness and conjunctival chemosis, respectively. The mean score (24-72 hours) was 2.33, 1, 3 and 3.11 for corneal opacity, iritis, conjunctival redness and conjunctival chemosis, respectively. At 4 days, the average score was 3, 1, 3 and 3.33 for corneal opacity, iritis, conjunctival redness and conjunctival chemosis, respectively. At 7 days, the average score was 4, 1, 2, and 3 for corneal opacity, iritis, conjunctival redness and conjunctival chemosis, respectively. Irritation was irreversible and the overall severity of irritation increased with time. Therefore, it was concluded that a single instillation of 0.08-0.09 g of the test substance is irritant to the eye. Please refer to Table A6.1.4/2-1.
5.3	**Conclusion**	In accordance with Council Directive 2001/59/EEC (28th ATP), 1,2-Benzisothiazolin-3-one Technical is irritant to the eye and is assigned the symbol “Xi” with the indication of danger “irritant” and the R phrase R41 “Risk of serious damage to eyes” as ocular reactions were demonstrated to be irreversible.
4.2.1	Reliability	1
4.2.2	Deficiencies	One deviation was noted and is outlined under points 2.3 and 5.1. However, it does not compromise the scientific validity of the study.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.4/2 Acute Eye Irritation

Annex Point IIA VI.6.1.4.b/01 **6.1.4 Acute eye irritation (a)**

Date	*August 2008*
Materials and Methods	*Applicant version is adopted.*
Results and discussion	*Applicant version is adopted*
Conclusion	*Applicant version is adopted*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.1.4/2-1 Results of eye irritation study with 1,2-Benzisothiazolin-3-one Technical

Time/rabbit	Corneal Opacity			Iridial Inflammation			Conjunctival Redness			Conjunctival Chemosis		
	1 M	2 F	3 M	1 M	2 F	3 M	1 M	2 F	3 M	1 M	2 F	3 M
1 h	1	1	1	1	1	1	3	3	3	2	2	2
24 h	2	2	2	1	1	1	3	3	3	3	3	3
48 h	2	2	3	1	1	1	3	3	3	3	3	3
72 h	2	3	3	1	1	1	3	3	3	3	3	4
4 days	3	3	3	1	1	1	3	3	3	3	3	4
7 days	4	4	4	1	1	1	2	2	2	3	3	3
Mean score 24-72 h	2	2.33	2.67	1	1	1	3	3	3	3	3	3.33
Mean score 24-72 h	2.33			1			3			3.11		

M: Male
F: Female

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.4/3 Acute Dermal Irritation

Annex Point IIA VI.6.1.4.b/02

6.1.4 Acute dermal irritation

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: June 27 – June 30, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Company with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD Guideline 404.	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazol-3-(2*H*)-one (BIT)	
3.1.1	Lot/Batch number	BT 17301	
3.1.2	Specification	Please refer to Doc. III-A 2/1	
3.1.2.1	Description	Beige to light brown coloured powder	
3.1.2.2	Purity	97.42% (Dry basis) 72.50% (Wet basis)	
3.1.2.3	Stability	Not relevant, single dose only	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.4/3	**Acute Dermal Irritation**
Annex Point IIA VI.6.1.4.b/02	**6.1.4 Acute dermal irritation**

3.2.2	Strain	New Zealand white
3.2.3	Source	Breeding facility, Jai Research Foundation
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Age not documented Weight 2.32-2.48 kg
3.2.6	Number of animals per group	1 group of 3 animals
3.2.7	Control animals	Yes, a contralateral site on each rabbit served as a control.
3.3	**Administration/ Exposure**	Dermal
3.3.1	Application	
3.3.1.1	Preparation of test substance	Test substance was prepared by moistening 500 mg of BIT with distilled water.
3.3.1.2	Test site and Preparation of Test Site	State site: dorso-lumbar area Clipped skin, an area of 6 cm^2 was clipped
3.3.2	Occlusion	Semi-occlusive, using gauze patch and surgical tape
3.3.3	Vehicle	Distilled water
3.3.4	Concentration in vehicle	Not documented
3.3.5	Total volume applied	Not applicable. Test substance was moistened with distilled water
3.3.6	Removal of test substance	Residual test substance removed with cotton soaked in distilled water.
3.3.7	Duration of exposure	4 hours
3.3.8	Postexposure period	72 hours
3.3.9	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Clinical signs	Yes, at 1, 24, 48 and 72 hours after removal of the patches

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.4/3	**Acute Dermal Irritation**
Annex Point IIA VI.6.1.4.b/02	**6.1.4 Acute dermal irritation**

3.4.2 Dermal examination	Yes, at 1, 24, 48 and 72 hours after removal of the patches
4 Scoring system	According to Draize *et al.* method
5 Examination time points	The skin of all animals was observed for signs of irritation at 60 min, 24 hours, 48 hours and 72 hours after removal of the patches
3.4.2 Other examinations	None
3.5 Further remarks	None
	4 RESULTS AND DISCUSSION
4.1 Average score	
4.1.1 Erythema	The mean average score at 24 – 72 hours was about 0.6 for the three rabbits combined. Individual mean scores at 24 – 72 hours were 0.33, 0.33 and 1, respectively, for each of the rabbits used. Please refer to Table A6.1.4/3-1
4.1.2 Edema	The mean average score at 24 – 72 hours was 0 for the three rabbits combined and individual mean scores at 24 – 72 hours was as well 0 for each rabbit. Please refer to Table A6.1.4/3-1
4.2 Reversibility	Yes, erythema was reversible at 72 hours
4.3 Other	No clinical signs related to treatment other than irritation reactions were observed
4.4 Overall result	Mean average score at 24 – 72 hours was 0.6 for erythema, and 0 for oedema.
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	The acute dermal irritation of BIT was investigated by application of a single dose of 500 mg on the skin of 3 New Zealand albino rabbits during four hours of exposure. This test method was conducted according to OECD Guideline 404 and is described under point 3 with no deviations.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.4/3	**Acute Dermal Irritation**
Annex Point IIA VI.6.1.4.b/02	**6.1.4 Acute dermal irritation**

5.2 Results and discussion		Very slight erythema was observed on the treated site of two rabbits and well defined erythema was observed in another rabbit 1 hour after patch removal. The situation remained the same at 24 hours, but at 48 hours only one animal presented very slight erythema, and no skin reaction was observed at 72 hours. The average scores for erythema at 24 – 72 hours were 0.33, 0.33 and 1, respectively, for the individual rabbits and mean average erythema score for all animals (24 – 72 hours) was 0.5533. Oedema effect was not observed. The mean average score for oedema for all animals (24 – 72 hours) was 0. No skin reaction was observed at any time on the control sites. Please refer to Table A6.1.4/3-1. No other clinical signs related to treatment were observed. There were no significant changes on body weight before application.
5.3 Conclusion		Based on the results obtained under the conditions of this study and in accordance with Council Directive 2001/59/EEC (28th ATP), BIT remains unclassified as a skin irritant.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*August 2008*
Materials and Methods	*Applicant version is accepted.*
Results and discussion	*Applicant version is accepted.*
Conclusion	*Applicant's conclusions is adopted.*
Reliability	*1*
Acceptability	*Acceptable*

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.4/3	**Acute Dermal Irritation**
Annex Point IIA VI.6.1.4.b/02	**6.1.4 Acute dermal irritation**

Remarks

Table A6.1.4/3-1: Dermal irritation scores for BIT treated sites on rabbits

	Erythema			Oedema		
Animal no.	1	2	3	1	2	3
After 1 h	1	1	2	0	0	0
After 24 h	1	1	2	0	0	0
After 48 h	0	0	1	0	0	0
After 72 h	0	0	0	0	0	0
Mean score 24-72 h	0.5533			0.0		

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.4/4 Acute Eye Irritation

Annex Point IIA VI.6.1.4.b/02 **6.1.4 Acute eye irritation**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
		Dates of experimental work: June 26 - July 17, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Company with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD 405.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, the following deviation was noted: Body weights after application were not documented, as recommended in the guideline. This deviation is not considered to compromise the scientific validity of this study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazol-3-(2*H*)-one (BIT)	
3.1.1	Lot/Batch number	BT 17301	
3.1.2	Specification	Please refer to Doc. III-A 2/1	
3.1.2.1	Description	Beige to light brown coloured powder	
3.1.2.2	Purity	97.42% (Dry basis) 72.50% (Wet basis)	
3.1.2.3	Stability	Not relevant, single dose only	

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.4/4 Acute Eye Irritation

Annex Point IIA VI.6.1.4.b/02 **6.1.4 Acute eye irritation**

3.2 Test Animals		
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand white
3.2.3	Source	Breeding facility, Jai Research Foundation
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Not documented 2.16-2.63 kg
3.2.6	Number of animals per group	1 group of 3 animals
3.2.7	Control animals	Yes, the contralateral eye of each rabbit served as a control.
3.3 Administration/ Exposure		
3.3.1	Preparation of test substance	Test substance was used as delivered
3.3.2	Amount of active substance instilled	100 mg
3.3.3	Exposure period	24 hours
3.3.4	Postexposure period	21 days
3.4 Examinations		
3.4.1	Ophthalmoscopic examination	Yes
3.4.1.1	Scoring system	OECD guidelines (1987) The eyes of each animal were examined using fluorescein dye staining in order to record loss or damage in corneal epithelium.
3.4.1.2	Examination time points	The eyes of all animals were observed for signs of ocular irritation at 60 min, 24 hours, 48 hours, 72 hours and on day 7, 14 and 21 after instillation. Corneal ophthalmoscopic examination took place after the 24 hours post-treatment period.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.4/4	**Acute Eye Irritation**
Annex Point IIA VI.6.1.4.b/02	**6.1.4 Acute eye irritation**

3.4.2 Other examinations	Individual clinical observations
3.5 Further remarks	None
	4 RESULTS AND DISCUSSION
4.1 Clinical signs	No effects were observed
4.2 Average score	
4.2.1 Cornea	The average score was 1, 1.667 and 2 at 24, 48 and 72 hours, respectively. Please refer to Table A6.1.4/4-1.
4.2.2 Iris	The average score was 0, 0.667 and 1 at 24, 48 and 72 hours, respectively. Please refer to Table A6.1.4/4-1.
4.2.3 Conjunctiva	
4.2.3.1 Redness	The average score was 2 at 24, 48 and 72 hours. Please refer to Table A6.1.4/4-1.
4.2.3.2 Chemosis	The average score was 4, 3.667 and 3.667 at 24, 48, 72 hours respectively. Please refer to Table A6.1.4/4-1.
4.3 Reversibility	No, except for redness, which was reversible from day 14 onwards for all animals, and from day 7 for two of the animals.
4.4 Other	At 24 hours after application, damage of ¾ corneal epithelium was observed in all animals.
4.5 Overall result	Mean scores at 24, 48 and 72 hours for animals 1, 2 and 3 were 1.33, 1.67 and 1.67 for corneal opacity, 0.33, 0.67 and 0.67 for iris lesion, 2 for redness, and 4, 3.33 and 4 for chemosis. The mean scores (24-72 hours) from all animals were 1.56, 0.56, 2 and 3.78 for corneal opacity, iridial inflammation, conjunctival redness and chemosis, respectively. However, observations at day 7, 14 and 21 showed even bigger mean scores: 4, 2.66 and 4 on corneal opacity; 2, 1.67 and 2 on iris lesion.
	5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.4/4 Acute Eye Irritation

Annex Point IIA VI.6.1.4.b/02 **6.1.4 Acute eye irritation**

5.1 Materials and methods

The acute eye irritation of BIT was investigated by instillation of a single dose of 100 mg into the conjunctival sac of one eye of 3 New Zealand albino rabbits.

This test was carried out according to OECD Guidelines 405 and is described under point 3 with the following deviation:

Body weights after application were not documented, as recommended in the guideline.

This deviation is not considered to compromise the scientific validity of this study.

5.2 Results and discussion

The average scores for corneal opacity at 24, 48 and 72 hours were 1, 1.667 and 2, respectively. The mean score (24-72 hours) from all animals was 1.56. After that, damage rises to means of 3.33, 3.67 and 3.67 for days 7, 14 and 21.

The average scores for the iris at 24, 48 and 72 hours were 0, 0.667 and 1, respectively. The mean score (24-72 hours) from all animals was 0.56. They keep on increasing until maximum values (2 at day 14 and 21, "no reaction to light, hemorrhage or gross destruction").

The average scores for conjunctival redness at 24, 48 and 72 hours were always 2. The mean score (24-72 hours) from all animals was 2.0. Mean scores reach 0 at 7-14 days, so redness is the only reversible effect.

The average scores for conjunctival chemosis at 24, 48 and 72 hours were 4, 3.667 and 3.667, respectively. The mean score (24-72 hours) from all animals was 3.78. Mean scores for chemosis diminished over the following days, but the effect remained irreversible (3.67, 2.33 and 1.67 at day 7, 14 and 21, respectively).

At 24 hours, after instillation of the test substance, chemosis, redness and areas of opacity were observed in treated eyes of all animals. Examination with fluorescein revealed damage to ¾ corneal epithelium of the treated eyes of all rabbits.

At 48 hours, chemosis and redness were observed in all treated eyes. Areas of opacity were observed in one animal, and iris damage in two rabbits.

At 72 hours, chemosis, redness, opacity and iris damage were observed in all animals. The following days (7 to 21), chemosis was observed in treated eyes of all rabbits. Slight redness was observed in only one animal on day 7 and in none of them thereafter. Opaque cornea was observed in two animals in addition to the absence of reaction of the iris to light. Translucent areas of opacity with details of iris slightly obscured, along with congestion of iris with slight reaction to light were visible in the other rabbit

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.4/4	**Acute Eye Irritation**
Annex Point IIA VI.6.1.4.b/02	**6.1.4 Acute eye irritation**

		at day 7, but at days 14 and 21 its iris was not reacting to light at all. Please refer to Table A6.1.4/4-1. No other clinical signs related to treatment were observed.
5.3 Conclusion		Since ocular lesions in cornea and iris are severe because they are still present at the end of the observation time, in accordance with Council Directive 2001/59/EEC (28th ATP), BIT is assigned the symbol Xi and the R phrase R41 "Risk of serious damage to eyes".
5.3.1	Reliability	1
5.3.2	Deficiencies	One deviation was noted and is outlined under points 2.3 and 5.1. However, it does not compromise the scientific validity of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*August 2008*
Materials and Methods	*Applicant version is accepted.*
Results and discussion	*Applicant version is accepted. There is a minor mistake in 4th paragraph of section 5.2. The real mean scores for chemosis at day 7, 14 and 21 were 3, 2.33 and 1 respectively.*
Conclusion	*Applicant's conclusions is adopted.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.1.4/4-1: Results of eye irritation study of BIT in rabbits

Time/rabbit	Corneal Opacity			Iridial Inflammation			Conjunctival Redness			Conjunctival Chemosis		
	1 M	2 F	3 M	1 M	2 F	3 M	1 M	2 F	3 M	1 M	2 F	3 M
24 hours	1	1	1	0	0	0	2	2	2	4	4	4
48 hours	1	2	2	0	1	1	2	2	2	4	3	4
72 hours	2	2	2	1	1	1	2	2	2	4	3	4
7 days	4	2	4	2	1	2	0	0	1	3	2	4
14 days	4	3	4	2	2	2	0	0	0	3	1	3
21 days	4	3	4	2	2	2	0	0	0	1	1	1
Mean score 24-72 hours	1.33	1.67	1.67	0.33	0.67	0.67	2	2	2	4	3.33	4
Mean score 24-72 hours	1.56			0.56			2			3.78		

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.5/1 SKIN SENSITISATION

Annex Point IIA VI.6.1.5/01 **Magnusson-Kligman method**

Official use only

1 REFERENCE

1.1 Reference [redacted]

Dates of experimental work: August 14 – September 9, 2001

1.2 Data protection Yes

1.2.1 Data owner: Troy Chemical Company BV

1.2.2 Companies with letter of access: DOW

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.

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study Yes, the study was conducted according to OPPTS 870.2600 which is equivalent to OECD Guideline 406.

2.2 GLP Yes (self-certified)

2.3 Deviations Yes, the following deviations were noted:

1. The temperature range was 16-26 ºC rather than 17-23 ºC as recommended in the guideline
2. The challenge was carried out on the 23rd day rather than the 22nd as indicated in the guideline

These deviations are minor and are not considered to compromise the scientific validity of this study.

3 MATERIALS AND METHODS

3.1 Test material 1,2-Benzisothiazolin-3-one Technical

3.1.1 Lot/Batch number: BT 11600

3.1.2 Specification: Please refer to Doc. III-A 2/2

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.5/1	**SKIN SENSITISATION**
Annex Point IIA VI.6.1.5/01	**Magnusson-Kligman method**

3.1.2.1	Description	Off white solid
3.1.2.2	Purity	98%
3.1.2.3	Stability	The test substance was expected to be stable for the duration of testing.
3.1.2.4	Preparation of test substance for application	Range finding study: Preliminary intradermal injection: 1, 3 and 5% mixture of test substance in distilled water and in a suspension of 50% v/v Complete Freund`s Adjuvant in distilled water. Preliminary topical induction: 80 and 55% w/w mixtures of test substance in distilled water Highest non-irritating concentration: 80, 75, 50 and 25% w/w mixtures of test substance in distilled water Main study: Intradermal induction: 50% v/v Complete Freund`s Adjuvant mixture in distilled water, 5% w/w mixture of test substance in distilled water and 5% w/w mixture of test substance in Complete Freund`s Adjuvant (50% v/v in distilled water). Topical induction: 80% w/w mixture of test substance in distilled water. Challenge: 80% w/w mixture of test substance in distilled water.
3.1.2.5	Pretest performed on irritant effects	Yes

3.2 Test Animals

3.2.1	Species	Guinea pig
3.2.2	Strain	Hartley albino
3.2.3	Source	Elm Hill Breeding Labs, Chelmsford, MA, USA
3.2.4	Sex	Male and Female
3.2.5	Age/weight at study initiation	Primary irritation group: Young adult Test/sham control groups: Young adult/males 331 – 508 g
3.2.6	Number of animals per group	Preliminary irritation testing: 8 Main study: Test substance: 20 Sham control: 10

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.5/1	**SKIN SENSITISATION**
Annex Point IIA VI.6.1.5/01	**Magnusson-Kligman method**

3.2.7	Control animals	Yes
3.3 Administration/ Exposure		Adjuvant
3.3.1	Induction schedule	On the first day of the induction period: three pairs of intradermal induction 7 days later: topical application
3.3.2	Way of induction	Intradermal and topical Occlusive
3.3.3	Concentrations used for induction	Intradermal Induction: 5% w/w mixture of test substance in distilled water Topical induction: 80% w/w mixture of test substance in distilled water
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	50% v/v Complete Freund`s Adjuvant mixture in distilled water
3.3.5	Challenge schedule	23 days after test initiation
3.3.6	Concentrations used for challenge	80% mixture of test substance in distilled water (highest non irritant concentration)
3.3.7	Rechallenge	No
3.3.8	Scoring schedule	Topical application: scoring 1 hour after patch removal Challenge: scoring 24 and 48 hours after patch removal
3.3.9	Removal of the test substance	Topical application: patches were removed after 48 hour exposure and the test sites were wiped gently with water and a clean towel to remove any residual substance Challenge: patches were removed after 24 hour exposure and the test sites were wiped gently with water and a clean towel to remove any residual substance
3.3.10	Positive control substance	75% mixture of HCA (alpha hexylcinnamaldehyde technical) in mineral oil
3.4 Examinations		

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.5/1	**SKIN SENSITISATION**
Annex Point IIA VI.6.1.5/01	**Magnusson-Kligman method**

3.4.1	Pilot study	Yes
3.5 Further remarks		Individual body weights of the animals were recorded just prior to the intradermal induction and again on the day after the primary challenge application. Animals were observed daily for clinical signs of toxicity.
		4 RESULTS AND DISCUSSION
4.1 Results of pilot studies		In the preliminary irritation testing, the application of different concentrations of 1,2-Benzisothiazolin-3-one Technical to guinea pigs resulted in the decision to dose animals in the main study with the following concentrations: Intradermal induction: 5% w/w mixture of test substance in distilled water Topical induction: 80% w/w mixture of test substance in distilled water Challenge phase: 80% w/w mixture of test substance in distilled water
4.2 Results of test		
4.2.1	24 h after challenge	<u>1,2-benzisothiazolin-3-(2*H*)-one (80% w/w mixture of test substance in distilled water):</u> Faint erythema in 6/20 sites and very faint erythema in 12/20 sites. <u>Sham control (80% w/w mixture of the test substance in distilled water):</u> Very faint erythema in 7/10 sites. <u>Historical positive control animals (75% w/w mixture of HCA in mineral oil):</u> Very faint erythema in 1/10 sites, faint erythema in 6/10 sites and moderate erythema in 3/10 sites. Nine positive control animals exhibited signs of sensitisation response. <u>Historical sham control animals (75 % w/w mixture of HCA in mineral oil):</u> Very faint erythema in all sites Please refer to Table A6.1.5-1.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.5/1	**SKIN SENSITISATION**
Annex Point IIA VI.6.1.5/01	**Magnusson-Kligman method**

4.2.2	48 h after challenge	1,2-benzisothiazolin-3-(2*H*)-one (80% w/w mixture of test substance in distilled water): Faint erythema persisted in 3/20 sites, receded to very faint erythema in 3/20 sites and very faint erythema persisted in 9/20 sites. Sham control (80% w/w mixture of the test substance in distilled water): Very faint erythema persisted in 2/10 sites. Historical positive control animals (75% w/w mixture of HCA in mineral oil): Moderate erythema receded to faint erythema in 2/10 sites and to very faint erythema in 1/10 sites. Faint erythema persisted in 5/10 sites, and receded to very faint erythema 1/10 sites. Very faint erythema persisted in 1/10 sites. Historical sham control animals (75 % w/w mixture of HCA in mineral oil): Faint erythema persisted in 4/5 sites. Please refer to Table A6.1.5-1.
4.2.3	Other findings	None
4.3 Overall result		Six of the twenty test animals exhibited a sensitisation response 24 hours after patch removal. Very faint erythema persisted at most sites through 48 hours. The positive response observed in historical positive control validation studies with HCA validated the test system.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The skin sensitisation of 1,2-Benzisothiazoline-3-one Technical was investigated by induction through intradermal injection followed by topical application of 1,2-Benzisothiazoline-3-one Technical. The challenge phase was then carried out 23 days after test initiation.

The study was conducted according to OPPTS 870.2600 which is equivalent to OECD Guideline 406 and is described under point 3. The following deviations were noted:

1. The temperature range was 16-26 ºC rather than 17-23 ºC as recommended in the guideline
2. The challenge was carried out on the 23rd day rather than the 22nd as indicated in the guideline

However, these deviations are minor and are not considered to compromise the scientific validity of this study.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.5/1	**SKIN SENSITISATION**
Annex Point IIA VI.6.1.5/01	**Magnusson-Kligman method**

5.2 Results and discussion

Based on the results obtained in the preliminary irritation testing, the following concentrations were selected for the intradermal induction: 5% w/w mixture of test substance in distilled water, for the topical induction: 80% w/w mixture of test substance in distilled water and for the challenge phase: 80% w/w mixture of test substance in distilled water.

No treatment related effects in bodyweights were noted in test animals, sham control and historical positive control animals.

After the topical induction phase, very faint erythema (0.5) was noted in test animals for 13/20 topical induction test sites one hour after patch removal. Very faint erythema (0.5) was noted in sham controls for 5/10 topical induction sham control sites one hour after patch removal. Moderate to faint erythema (2-1) was noted at all positive control sites following the topical induction phase. No erythema (0) was noted on sham control sites following the topical induction phase.

After challenge phase, very faint erythema (0.5) was noted in 12/20 test sites and faint erythema (1) was noted in 6/20 test sites 24 hours after challenge patch removal. Irritation persisted in 12 and receded in 6 of these sites through 48 hours. Very faint erythema (0.5) was noted for 7/10 sham control sites 24 hours after challenge patch removal. Irritation persisted at 2 of these sites during 48 hours. Nine positive control animals exhibited signs of sensitisation response moderate (1-2) at 24 hours which persisted in 7 sites at 48 hours after challenge. Very faint erythema (0.5) was noted for all five sham control sites 24 hours after challenge. Irritation persisted at four of these sites during 48 hours.

Please refer to Table A6.1.5-1.

5.3 Conclusion

In accordance with Council Directive 2001/59/EEC (28th ATP), 1,2-Benzisothiazolin-3-one Technical is considered to be a contact sensitizer and should be assigned the symbol Xi “irritant” and the R phrase R43 “May cause sensitisation by skin contact”.

5.3.1 Reliability

1

5.3.2 Deficiencies

Two deviations were noted and are outlined under point 2.3 and 5.1. However, they do not compromise the scientific validity of the study.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.5/1 SKIN SENSITISATION

Annex Point IIA VI.6.1.5/01 **Magnusson-Kligman method**

Date	*August 2008*
Materials and Methods	*Applicant version is accepted.*
Results and discussion	*Applicant version is accepted*
Conclusion	*Applicant's conclusion is adopted.*
Reliability	*2 (The study used a historical positive control validation study instead of a positive control simultaneously run with the evaluated substance).*
Acceptability	*Acceptable*
Remarks	

Table A6.1.5-1: Summary of results of skin sensitisation test after challenge with 1,2-Benzisothiazolin-3-one Technical

	Number of animals with signs of allergic reactions / number of animals in group			
	Sham control[a]	**Test group[b]**	**Positive control[c]**	**Sham positive control group[c]**
Scored after 24h	0/10	6/20	9/10	0/5
Scored after 48h	0/10	3/20	7/10	0/5

[a] 80% w/w mixture of the test substance in distilled water
[b] 80% w/w mixture of the test substance in distilled water
[c] 75% w/w mixture of HCA in mineral oil

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.5/2 SKIN SENSITISATION

Annex Point IIA VI.6.1.5/02 **Magnusson-Kligman method**

		Official use only
	1 REFERENCE	
1.1 Reference	[redacted]	
	Dates of experimental work: August 28 - September 21, 2002	
1.2 Data protection	Yes	
1.2.1. Data owner	Dow Benelux BV	
1.2.2. Companies with letter of access	Troy Chemical Company BV	
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.	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes, the study was conducted according to OECD Guideline 406.	
2.2 GLP	Yes	
2.3 Deviations	None	
	3 MATERIALS AND METHODS	
3.1 Test material	1,2-Benzisothiazol-3-(2*H*)-one (BIT)	
3.2.1 Lot/Batch number	BT 17301	
3.2.2 Specification	Please refer to Doc. III-A 2/1	
3.2.2.1 Description	Beige to light brown coloured powder	
3.2.2.2 Purity	97.42% (Dry basis) 72.50% (Wet basis)	
3.2.2.3 Stability	Not relevant, single dose only	
3.2.2.4 Preparation of test substance for application	Range finding study: Intradermal irritancy test: 0.1 mL of BIT at concentrations of 0.5, 1, 2.5 and 5% in propylene glycol.	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.5/2	**SKIN SENSITISATION**
Annex Point IIA VI.6.1.5/02	**Magnusson-Kligman method**

	Topical irritany test: 0.2 mL of BIT at concentrations of 25, 50 and 75% and 100 mg BIT moistened with 80% ethanol. Main study: Intradermal induction: 2.5 % BIT in propylene glycol Topical induction: 100 mg BIT moistened with 80 % ethanol Challenge: 100 mg BIT moistened with acetone
3.2.2.5 Pretest performed on irritant effects	Yes
3.2 Test Animals	
3.2.1 Species	Guinea pig
3.2.2 Strain	Hartley
3.2.3 Source	Mahaveera Enterprises, Hyderabad, India
3.2.4 Sex	Male and female
3.2.5 Age/weight at study initiation	Not documented 282-405 g
3.2.6 Number of animals per group	Intradermal irritancy test: one group of 2 animals/sex Topical Irritancy Test: one group of 2 animals/sex Main study: Test substance group: 10 animals/sex Control group: 5 animals/sex
3.2.7 Control animals	Yes
3.3 Administration/ Exposure	Adjuvant
3.3.1 Induction schedule	Day 0, three pairs of intradermal injections Day 7, topical application
3.3.2 Way of induction	Intradermal and topical Occlusive
3.3.3 Concentrations used for induction	Intradermal injection: 2.5 % BIT in propylene glycol

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.5/2	**SKIN SENSITISATION**
Annex Point IIA VI.6.1.5/02	**Magnusson-Kligman method**

		Topical: 100 mg BIT moistened with 80 % ethanol
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	1:1 mixture (v/v) with distilled water
3.3.5	Challenge schedule	Day 21 after test initiation
3.3.6	Concentrations used for challenge	100 mg BIT moistened with acetone
3.3.7	Rechallenge	No
3.3.8	Scoring schedule	Intradermal injections: 24 hours after intradermal injections (day 1) Topical application: 24 hours after patch removal (day 10) Challenge: 24 hours and 48 hours after removal (days 23 and 24)
3.3.9	Removal of the test substance	Topical application: Day 9. Challenge Phase: Day 22
3.3.10	Positive control substance	2-Mercaptobenzothiazole
3.4 Examinations		
3.4.1	Pilot study	Yes
3.5 Further remarks		Individual body weights of the animals were recorded just prior to the intradermal induction (day 0) and again on the last day of the study (day 24). Animals were observed twice a day for clinical signs of toxicity.
		4 RESULTS AND DISCUSSION
4.1 Overall result		Intradermal: At 24 and 48 hours, well-defined erythema was observed only at 2.5 % and 5 % concentrations, thus 2.5 % dose concentration was selected. Topical: No skin reactions were observed at 25, 50 and 75% and 100 mg BIT moistened with 80 % ethanol dose levels at 24 and 48 hours. 100 mg BIT moistened with 80 % ethanol was selected for topical application during induction exposure and 100 mg BIT moistened with acetone was selected for topical application during challenge exposure.

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Subsection A6.1.5/2	**SKIN SENSITISATION**
Annex Point IIA VI.6.1.5/02	**Magnusson-Kligman method**

4.2 Results of test

4.2.1 24 h after challenge

Treatment group: 4 animals with signs of allergic reactions (discrete or patchy erythema) / 20 animals

Control group: No signs of reactions were observed

Positive control, treatment group: 9 animals with signs of allergic reactions (1 moderate and confluent erythema and 8 discrete or patchy erythema) / 20 animals

Control group of the positive study: No signs of reactions were observed

Please refer to Table A6.1.5/2-1.

4.2.2 48 h after challenge

Treatment group: 2 animals with signs of allergic reactions (discrete or patchy erythema) / 20 animals

Control group: No signs of reactions were observed

Positive control, treatment group: 5 animals with signs of allergic reactions (discrete or patchy erythema) / 20 animals

Control group of the positive study: No signs of reactions were observed

Please refer to Table A6.1.5/2-1.

4.2.3 Other findings

The mean body weight of the treatment group animals was comparable to that of the control group

No clinical signs related to treatment other than irritation/sensitisation were observed.

4.3 Overall result

The percentage of animals that showed positive skin response was 20 % and 10 % at 24 and 48 hours after challenge patch removal, respectively.

The positive response observed in positive control validation studies with 2-Mercaptobenzothiazole validated the test system.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The skin sensitisation of BIT was investigated by induction through intradermal injection followed by topical application of BIT. The challenge phase was then carried out 21 days after test initiation.

The study was conducted according to OECD Guideline 406 and is described under point 3 with no deviations.

5.2 Results and discussion

Based on the results obtained in the preliminary irritation testing, the following concentrations were selected for the intradermal induction: 2.5% mixture of test substance in propylene glycol, for the topical induction: 100 mg test substance moistened with 80% ethanol and for

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.1.5/2	**SKIN SENSITISATION**
Annex Point IIA VI.6.1.5/02	**Magnusson-Kligman method**

		the challenge phase 100 mg of 1,2-benzisothiazoline-3-one moistened with acetone. No treatment related effects in bodyweights were noted in test animals, sham control and positive control animals. After intradermal injections and after the topical induction phase, very slight to well-defined erythema was observed on the treatment group. No erythema was noted on sham control sites following neither the intradermal injections nor the topical induction phase. After challenge phase, faint erythema was noted in 4/20 test sites 24 hours after challenge patch removal. Irritation persisted in 2 and receded in 2 of these sites through 48 hours. No erythema was noted for any of the sham control sites at 24 and 48 hours after challenge patch removal. Nine positive control animals exhibited signs of sensitisation response at 24 hours which persisted in 5 sites at 48 hours after challenge. Please refer to Table A6.1.5/2-1.
5.3 Conclusion		Based on the results of this study and according to 2001/59/EEC (28th ATP) BIT is not considered a skin sensitiser under the conditions of the test. However, based on results obtained in the sensitisation study summarised under IIIA, 6.5.1-1, a sensitisation classification is proposed for BIT which should be assigned the symbol Xi "irritant" and the R phrase R43 "May cause sensitisation by skin contact".
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*August 2008*
Materials and Methods	*Applicant version is accepted.*
Results and discussion	*Applicant version is accepted.*
Conclusion	*Applicant's conclusions is adopted.*
Reliability	*1*

Section A6 Toxicological and Metabolic Studies

Subsection A6.1.5/2 SKIN SENSITISATION

Annex Point IIA VI.6.1.5/02 **Magnusson-Kligman method**

Acceptability	*Acceptable*
Remarks	

Table A6.1.5/2-1: Summary of results of skin sensitisation test after challenge with BIT on guinea pig

	Number of animals with signs of allergic reactions / number of animals in group			
	Control	**Test group**	**Positive control**	**Sham positive control group**
Scored after 24h	0/10	4/20	9/20	0/10
Scored after 48h	0/10	2/20	5/20	0/10

Section A6 Toxicological and Metabolic Studies

Subsection A6.2/1 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2.a/01 **TOXICOKINETIC**

		Official use only
	1 REFERENCE	
1.1 Reference	[redacted] Dates of experimental work: July 10, 2006 – March 26, 2007	
1.2 Data protection	Yes	
1.2.1 Data owner	ROHM & HAAS	
1.2.2 Companies with letter of access	Troy Chemical Company BV	
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.	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes, the study was carried out according to U.S. EPA Guideline 870.7485 and OECD Guideline 417.	
2.2 GLP	Yes (self-certified)	
2.3 Deviations	None	
	3 MATERIALS AND METHODS	
3.1 Test material	1,2-Benzisothiazolin-3-one	
3.1.1 Lot/Batch number	[^{14}C]-1,2-Benzisothiazolin-3-one: 1069.0003 Nonradiolabeled 1,2-Benzisothiazolin-3-one: 060309/1	
3.1.2 Specification	[^{14}C]-1,2-Benzisothiazolin-3-one: Specific activity 53.57 mCi/g	
3.1.2.1 Description	Not documented	
3.1.2.2 Purity	[^{14}C]-1,2-Benzisothiazolin-3-one; Radiochemical purity 99.6%; Radiochemical purity in dose solution: 97.98%	
3.1.2.3 Stability	The radiolabeled test substance was stored at approximately -20 ºC in a tightly closed container, protected from light. The non-radiolabeled 1,2-	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.2/1	**Absorption, distribution, metabolism and excretion**
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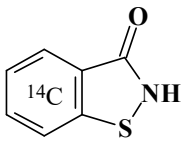
		Benzisothiazolin-3-one was stored at room temperature. Standard solutions were stored refrigerated. The stability of the test substance was confirmed by HPLC over the dosing time frame. Additional data also indicated that ^{14}C-BIT in dose solution was stable at least 5 days, however this data is not documented.
3.1.2.4	Radiolabeling	[^{14}C]-1,2-Benzisothiazolin-3-one  * labelled with ^{14}C at the benzene ring

3.2 Test animals

3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley rats
3.2.3	Source	Charles River Laboratories Inc., Raleigh, NC, Kingston, NY and Portage MI, USA
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	8-13 weeks old Approximately 210 – 306 g
3.2.6	Number of animals	Group 1: Single low dose group for urine, faeces, tissue, blood and plasma collection: 4 rats/sex Group 2: Single low dose group for blood and plasma collection: 3 rats/sex Group 3: Single high dose group for urine, faeces, tissue, blood and plasma collection: 4 rats/sex Group 4: Single high dose group for blood and plasma collection: 3 rats/sex Group 5: Single low dose for urine, faeces, tissue, blood and plasma: 3 rats/sex Group 6: Repeated low dose for urine, faeces, tissue, blood and plasma: 4 rats/sex
3.2.7	Control animals	1 rat

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3.3	**Administration/ Exposure**	Oral
3.3.1	Type	Oral gavage, approximately 10 mL/kg
3.3.2	Concentration	Group 1- Single low-dose for urine, faeces, tissue, blood and plasma collection: 10 mg/kg bw ^{14}C-BIT Group 2 - Single low-dose for blood and plasma collection: 10 mg/kg bw ^{14}C-BIT Group 3 - Single high-dose for urine, faeces, tissue, blood and plasma collection: 100 mg/kg bw ^{14}C-BIT Group 4 - Single high-dose for blood and plasma collection: 100 mg/kg bw ^{14}C-BIT Group 5- Single low-dose for urine, faeces, tissue, blood and plasma collection: 10 mg/kg bw ^{14}C-BIT Group 6- Repeated low-dose for urine, faeces, tissue, blood and plasma collection: 10 mg/kg bw/day ^{14}C-BIT for 5 days
3.3.3	Vehicle	0.5% methylcellulose aqueous solution
3.4	**Examinations**	
3.4.1	Blood samples	Blood samples (~ 0.4 ml) were collected at 1, 3, 6, 24, 48 and 72 hours post dose from the tail veins of Group 2 and from the jugular vein cannula or tail vein from Group 4. At the time of euthanisia, blood (~ 5ml) was collected by cardiac puncture. Duplicate aliquots of blood were combusted for total radioactivity; remaining blood was centrifugated at 4°C and 2500 rpm or 10000 rpm for 10 min to obtain plasma. Duplicate aliquots of plasma were assayed directly by LSC for radioactivity concentration.
3.4.2	Urine, faeces and cage rinses	Urine was collected at pre-dose, 0-6, 6-12, 12-24, 24-48, 48-72 and 72-96 hours post dose. Samples were collected into tared sample cups and freeze-trapped using dry ice to avoid atmospheric oxidation, evaporation, and bacterial degradation. Cages were rinsed with NANOPure® water at 24, 48 and 72 hours post-dose; at the end of the study cages were thoroughly washed with IPA/water (1:1). All cages rinse/wash samples were collected in tared sample containers The faeces samples were collected at the same time points as the cage point at room temperature. Urine, cage rinse, and faeces were collected at the time of sacrifice from animals in groups 5 and 7, for group 6, all excreta samples were collected daily until 96 hour post 5th dose.

Section A6 Toxicological and Metabolic Studies

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3.5 Sacrifice and pathology

3.5.1 Euthanasia

Animals in Groups 1-4 were sacrificed at 96 hours post dose. Group 5 animals were sacrificed at 1 hour post-dose (3/sex). Group 6 animals were sacrificed at 96 hours post dose 5th dose. Rats in Group 7 were not dosed and were used to obtain control samples.

3.5.2 Tissues and organs

At the time of euthanasia, the following tissues and organs were harvested from Groups 1, 3, 5, 6 and 7: liver, fat, kidneys, bone marrow (femur bone), heart, lungs, brain, testes (males), ovaries (females), muscle (both hind legs), spleen, adrenals, thyroids, and remaining carcass.

3.5.3 Analytical methods

Test samples were analysed using the following methods:

(a) Sample Combustion

(b) Liquid Scintillation Counting (LSC)

(c) HPLC-PDA

(d) HPLC-UV

(e) LC/MS positive ion electrospray

(f) LC/MS positive turbulon (ESI)

4 RESULTS AND DISCUSSION

4.1 Toxic effects, clinical signs

In the low dose group, the mean T_{max} for both sexes was 1.7 and 1 hours for blood and plasma concentration of total radioactivity, respectively.

In the high dose group, the mean T_{max} for both blood and plasma was 3 hours in males and 2.3 hours in females.

Blood/plasma exposure was similar in male and female in low dose groups (mean blood AUC_{0-t} in hour*µg equiv./g was 30.06 in females and 24.47 in males; mean plasma AUC_{0-t} was 21.74 in females and 23.93 in males).

In high dose groups, the blood/exposure was approximately two times higher in female rats (mean blood AUC_{0-t} was 401.80 and 216.81 in female and male rats, respectively; mean plasma AUC_{0-t} was 483.37 and 231.87 in female and male rats, respectively).

Please refer to Table A6.2/1-1.

4.2 Recovery of labelled compound

Most of the radioactivity was recovered in urine and cage rinse with a lesser amount recovered in faeces within 24 hours post dose. In the low dose groups, the percentages of dosed radioactivities recovered in urine, cage rinse and faeces were 87.00%, 7.35% and 4.03% in males and 74.90%, 22.29% and 1.87% in females.

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In the high dose groups, the percentages recovered in urine, cage rinse and faeces were 83.98%, 12.09% and 4.18% in males whereas 81.30%, 12.87% and 2.55% were recovered in females.

In the repeated dose groups, the percentages recovered in urine, cage rinse and faeces were 82.94%, 7.98% and 5.90% in male rats and 80.05%, 11.45% and 4.96% in females. Please refer to Table A6.2/1-2.

The tissue radioactivity was negligible, less than 0.01% was found in all groups with the exception of Group 5 (approximately 7.9%) at 1 hour (Tmax) post-dose.

Female tissue contained higher radioactivity than male tissue. Adrenals, bone marrow, and thyroids are the prominent tissues with high radioactivity concentration in high dose groups. In low dose groups, only thyroids contained radioactivity above the lower limit of quantification (LLOQ). In multiple dose groups, bone marrow in females and thyroids in both sexes contained significantly radioactivity.

The mean percent of dose recovered from rat tissues at 96 hours post last dose at single dose of test substance (~0.01%) was not different from rat tissues at multiple doses. This indicated that test substance and its related metabolites do not bioaccumulate in tissues. Please refer to Table A6.2/1-3.

4.3 Metabolism study

The major excretion route was through urine. No test substance was detected in rat urine. Three major metabolites were observed. For the single oral low dose group, M1, M2 and M3 accounted for 14.87%, 6.12% and 63.24% of the dose in the 0-24 hour male rat urine, and 8.58%, 4.92% and 57.34% of the dose in the 0-24 hour female rat urine. The metabolite profile of male and female rat urine from multiple oral low dose groups were similar to those of the single dose group. For the single oral high dose group, M1, M2 and M3 accounted for 21.64%, 3.69% and 54.46% of the dose in the 0-24 hour male rat urine, and 19.17%, 2.77%, and 54.80% of the dose in the 0-24 hour female rat urine. Please refer to Table A6.2/1-4.

Minor radioactivity was recovered in faeces. No test substance was detected in rat faeces. Four minor radio-components, M1 to M4, were observed, each accounting for < 1.32% of the administered dose.

The metabolites were analysed and identified by LC/MS and the test substance was analysed by LC/MS and MS/MS. The proposed chemical formulae are $C_{13}H_{16}NO_7S$, $C_8H_{10}NO_3S$, $C_8H_{10}NO_2S$ and $C_8H_{10}NOS$, respectively for M1, M2, M3 and M4.

It was proposed that a thiazolin ring-opening (between sulphur and nitrogens atoms) precursor (not detected) was initially formed, followed by glucuronyl (M1) or methyl (M4) conjugations. Mono or di-oxidation of the formed thioanisole (M4) resulted in M3 (sulfoxide) and M2 (sulfone), respectively. The proposed pathway for the metabolism of the test substance is outlined in Figure A6.2/1-1.

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6 Toxicological and Metabolic Studies

Subsection A6.2/1 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2.a/01 **TOXICOKINETIC**

5.1 Materials and methods

^{14}C-1,2-Benzisothiazolin-3-one was administered to male and female Sprague-Dawley rats at single low dose, single high dose and repeated low dose and its absorption, distribution, excretion and metabolism was examined.

This study was conducted according to OECD Guideline 417 and U.S. EPA Guideline 870.7485 and is described under point 3 with no deviations.

5.2 Results and discussion

No behavioural changes, ill-health or reaction to treatment were observed for the duration of the study in all of the rats.

In the low dose group, the mean T_{max} for both sexes was 1.7 and 1 hours for blood and plasma concentration of total radioactivity, respectively. In the high dose group, the mean T_{max} for both blood and plasma was 3 hours in males and 2.3 hours in females.

Blood/plasma exposure was similar in male and female in low dose groups. In high dose groups, the blood/exposure was approximately two times higher in female rats.

Most of the radioactivity was recovered in urine and cage rinse with a lesser amount un faeces. More than 91% was absorbed following oral administration at both concentrations. Radioactivity was mostly excreted in urine between 82.94 and 87.00% in male treated groups and between 74.90 to 81.30% in female treated groups. Radioactivity was also found in cage rinses between 7.35 to 12.09% in male treated groups and between 11.45 to 22.29% in female treated groups. No apparent gender difference was observed in excretion pattern.

The tissue radioactivity was negligible, less than 0.01% was found in all groups except Group 5 (~7.9%) at 1 hour (T_{max}) post dose.

The mean percent of dose recovered from rat tissues at 96 hours post last dose at single dose of test substance (~0.01%) was not different from rat tissues at multiple doses. This indicated that the test substance and its related metabolites do not bioaccumulate in tissues.

Sex differences in tissue residue were observed predominantly at the high dose. Female tissue contained higher radioactivity than male tissues. Adrenals, bone marrow, and thyroids are the prominent tissues with high radioactivity concentration in high dose groups. In low dose groups, only thyroids contained radioactivity above the lower limit of quantification (LLOQ). In multiple dose groups, bone marrow in females and thyroids in both sexes contained significantly radioactivity.

The test substance is extensively metabolised and excreted primarily in urine following single or repeated doses to rats. No test substance was recovered in urine or faeces.

In urine, three major metabolites (M1, M2 and M3) were identified. M3 was the dominant metabolite, accounting for > 54.46% of the dose of the single dose groups (low and high). M2 and M1 accounted for 2.77-6.12% and 8.58-21.64% of the dose in 0-24 hour rat urine from the single dose group (low and high).

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		Based on the above, it was proposed that a thiazolin ring-opening (between sulphur and nitrogens atoms) precursor (not detected) was initially formed, followed by glucuronyl (M1) or methyl (M4) conjugations. Mono or di-oxidation of the formed thioanisole (M4) resulted in M3 (sulfoxide) and M2 (sulfone), respectively.
5.3 Conclusion		Most of the radioactivity was recovered in urine and cage rinse with a lesser amount in faeces. More than 91% was absorbed following oral administration at 10 and 100 mg/kg dose. The toxicokinetic part of the study indicated that at high dose, female had higher exposure to ^{14}C-1,2-Benzisothiazolin-3-one derived radioactivity than males. At the high dose, the highest concentrations were measured in adrenals, thyroids and bone marrow. 1,2-Benzisothiazolin-3-one is extensively metabolised and excreted primarily in urine following single or repeated doses to rats. No ^{14}C-1,2-Benzisothiazolin-3-one was recovered in urine or faeces. It was proposed that a thiazolin ring-opening (between sulphur and nitrogen atoms) precursor (not detected) was initially formed, followed by glucuronyl (M1) or methyl (M4) conjugations. Mono or di-oxidation of the formed thioanisole (M4) resulted in M3 (sulfoxide) and M2 (sulfone), respectively.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*August 2008*
Materials and Methods	*Applicant version is accepted.*
Results and discussion	*Applicant version is accepted.*
Conclusion	*LO(A)EL: Not applicable* *NO(A)EL: Not applicable* *Applicant's conclusion is adopted.*
Reliability	*1*
Acceptability	*Acceptable*

Section A6 Toxicological and Metabolic Studies

Subsection A6.2/1 Absorption, distribution, metabolism and excretion

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Remarks

Table A6.2/1-1: Pharmacokinetic parameters of ^{14}C-1,2-Benzisothiazolin-3-one in rat blood and plasma

Blood	**Group 2 (10 mg/kg)**		**Group 4 (100 mg/kg)**	
PK Parameter	**Female**	**Male**	**Female**	**Male**
T_{max} (h)	1.7	1.7	2.3	3.0
C_{max} (µg/g)	6.11	5.43	55.87	36.03
AUC_{0-t} (h*µg/g)	30.06	24.47	401.80	216.81
Plasma	**Group 2 (10 mg/kg)**		**Group 4 (100 mg/kg)**	
PK Parameter	**Female**	**Male**	**Female**	**Male**
T_{max} (h)	1.0	1.0	2.3	3.0
C_{max} (µg/g)	6.32	6.54	72.27	38.66
AUC_{0-t} (h*µg/g)	21.74	23.93	483.37	231.87

PK parameters were mean of individual PK parameters from individual animals.

Table A6.2/1-2: Mean percent of dose recovered from urine, cage rinse, faeces and tissues from low, high and multiple dose groups

Group ID	Sex		% of Dose Recovered				
			Urine	Faeces	Cage Rinse	Tissues	Total
Group 1	M	Mean	87.00	4.03	7.35	0.01	98.39
		SD	5.52	2.89	1.34	0.00	3.30
Group 1	F	Mean	74.90	1.87	22.29	0.01	99.07
		SD	8.28	1.32	5.68	0.00	3.13
Group 3	M	Mean	83.98	4.18	12.09	0.01	100.26
		SD	2.93	2.16	4.28	0.01	3.04
Group 3	F	Mean	81.30	2.55	12.87	0.01	96.73
		SD	8.75	0.77	6.14	0.01	3.86
Group 6	M	Mean	82.94	5.90	7.98	0.01	96.83
		SD	3.08	0.51	1.53	0.01	2.05
Group 6	F	Mean	80.05	4.96	11.45	0.01	96.47
		SD	3.20	1.64	0.63	0.01	1.26

Table A6.2/1-3: Mean percent of dose recovered from rat tissues at 96 h post last dose

	Group 1 (Single oral low dose) Male		Group 1 (Single oral low dose) Female		Group 3 (Single oral high dose) Male		Group 3 (Single oral high dose) Female		Group 6 (Repeated oral low dose) Male		Group 6 (Repeated oral low dose) Female	
Tissue	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Adrenals	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blood	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bone Marrow	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brain	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fat	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heart	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kidneys	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Liver	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.00
Lungs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Muscle	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ovaries	NA	NA	0.00	0.00	NA	NA	0.00	0.00	NA	NA	0.00	0.00
Plasma	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Spleen	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Tissue	Group 1 (Single oral low dose) Male		Group 1 (Single oral low dose) Female		Group 3 (Single oral high dose) Male		Group 3 (Single oral high dose) Female		Group 6 (Repeated oral low dose) Male		Group 6 (Repeated oral low dose) Female	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Testes	0.00	0.00	NA	NA	0.00	0.00	NA	NA	0.00	0.00	NA	NA
Thyroid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total:	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.00

NA: not applicable

Table A6.2/1-4: Percent Distribution (%Dose) of 1,2-Benzisothiazolin-3-one metabolites in rat urine and faeces

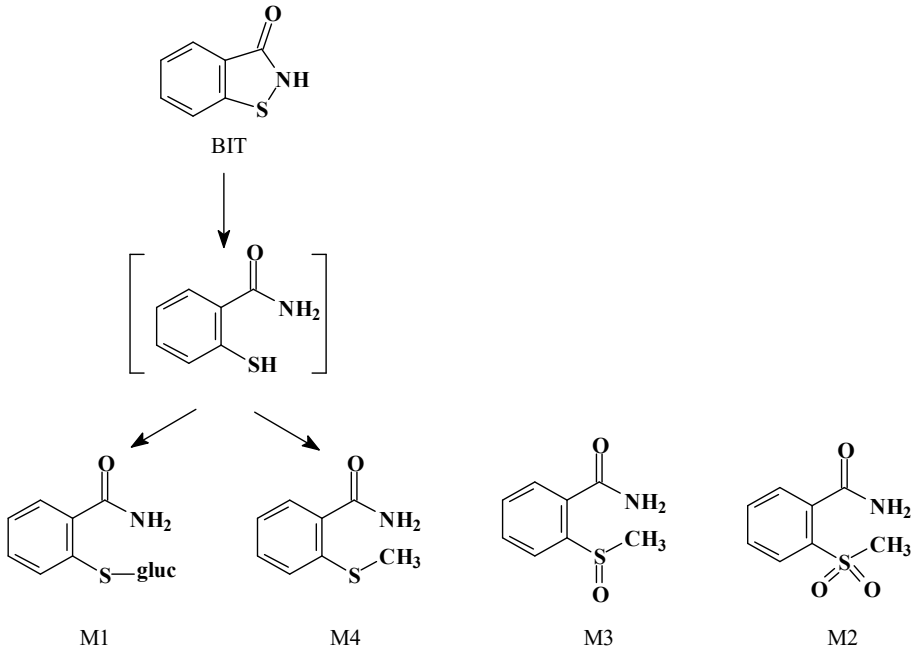
Group	Gender	Met	Urine			Faeces	Urine and Faeces
			0-6 h	6-24 h	0-24 h	0-24 h	0-24 h
1 (Single oral low dose)	Male	M1	14.07	0.80	14.87	0.30	15.17
		M2	1.25	4.87	6.12	0.32	6.44
		M3	35.34	27.90	63.24	1.32	64.56
		M4	ND	ND	ND	0.44	0.44
	Female	M1	6.60	1.98	8.58	0.04	8.62
		M2	0.93	3.99	4.92	0.04	4.96
		M3	24.62	32.72	57.34	0.27	57.61
		M4	ND	ND	ND	0.02	0.02
3 (Single oral high dose)	Male	M1	17.67	3.97	21.64	1.18	22.82
		M2	0.67	3.02	3.69	0.13	3.82
		M3	21.11	32.35	54.46	1.02	55.48
		M4	ND	ND	ND	0.05	0.05
	Female	M1	15.38	3.79	19.17	0.17	19.34
		M2	0.57	2.20	2.77	0.07	2.84

Group	Gender	Met	Urine			Faeces	Urine and Faeces
			0-6 h	6-24 h	0-24 h	0-24 h	0-24 h
		M3	22.04	32.76	54.80	0.87	55.67
		M4	ND	ND	ND	0.04	0.04

			0-24 hr	24-96 hr
6 (Repeated oral low dose)	Male	M1	2.80	5.44
		M2	1.28	1.09
		M3	12.30	10.09
	Female	M1	2.64	4.45
		M2	0.71	0.60
		M3	12.43	10.26

ND: Not detected.
Met: Metabolite

Figure A6.2/1-1: Proposed metabolic pathways of 1,2-Benzisothiazolin-3-one (BIT) in rats



Section A6 Toxicological and Metabolic Studies

Subsection A6.2/2 Percutaneous absorption (*in-vitro* test)

Annex Point IIA
VI.6.2.b/01

		Official use only
	1 REFERENCE	
1.1 Reference	[redacted] Dates of experimental work: April 13 - April 27, 2007	
1.2 Data protection	Yes	
1.2.1 Data owner	ROHM & HAAS	
1.2.2 Company with letter of access	Troy Chemical Company BV	
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.	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes, the study was conducted according to the OECD Guideline 428.	
2.2 GLP / GCP	Yes	
2.3 Deviations	Yes, the following deviation was noted: The humidity was not documented as recommended in the guideline This deviation is minor and is not considered to compromise the scientific validity of this study.	
	3 MATERIALS AND METHODS	
3.1 Test material	[^{14}C]-1,2-Benzisothiazolin-3-one	
3.1.1 Lot/Batch number	Sub Lot Number 1069.0006	
3.1.2 Specification	Specific activity: 53.57 mCi/g	
3.1.2.1 Description	Non-radiolabelled: White to pale yellow solid powder	
3.1.2.2 Purity	Radiochemical purity was determined as 98.3%.	

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3.1.2.3 Stability	Not relevant, single dose use only
3.1.2.4 Radiolabelling	[^{14}C]-1,2-Benzisothiazolin-3-one
3.2 Test animals	
3.2.1 Species	Human
3.2.2 Source	3 donors, TCS CellWorks Ltd, Buckingham, UK
3.2.3 Sex	Not documented
3.2.4 Skin type	Abdominal skin membranes
3.2.5 Skin preparation	Dermatomed human abdominal skin membranes were deep frozen at approximately -74°C until use. The frozen skin samples were thawed to room temperature and the skin examined prior to use. Acceptable thickness of the skin membranes was confirmed using a Micrometer and assessed for skin integrity.
3.2.6 Membrane integrity	After visual inspection, the integrity of each prepared membrane once it was in the diffusion cell was evaluated by electrical resistance measured via electrodes integral to the diffusion cell prior to dosing. Membrane integrity was deemed acceptable if the electrical resistance across each membrane was ≥ 12.8 kΩ (20 kΩ/cm^2).
3.2.7 Diffusion cells	Human skin membranes of adequate size were clamped in flow-through diffusion cells with a dose exposure area of 0.64 cm^2. The cells were positioned within a temperature controlled heater block in order to maintain the skin membranes at a constant temperature of 32°C ± 1°C. The receptor fluid was pumped at a rate of between 1.5 and 2.0 ml.h^{-1} and consisted of 10mM phosphate buffered saline, pH 7.4.
3.3 Administration/ Exposure	Dermal (*in vitro*)
3.3.1 Cell selection	Only skin membranes with an electrical resistance of greater than or equal to 20 kΩ/cm^2 were used.
3.3.2 Number of skin samples per group	12 in total, 6 per dose level
3.3.3 Controls	No

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3.3.4	Dose	Low dose: 30 ppm High dose: 300 ppm
3.3.5	Volume applied	6.4 µL
3.3.6	Size of test site	0.64 cm²
3.3.7	Occlusion	None
3.4	**Absorption**	
3.4.1	Exposure period	8 hours
3.4.2	Sampling time	Receptor fluid was collected at 1 hour intervals for 24 hours post dosing. After 8 hours of exposure, the membranes were swabbed with 1% Tween 80 solution in water using natural sponge swabs.
3.5	**Mass balance**	
3.5.1	Receptor fluid	6 replicates per dose of the individual fractions of the receptor fluid collected post dosing (including the pre-dose sample), the samples were collected in vials for subsequent analysis by liquid scintillation counting (LSC)
3.5.2	Skin wash	The natural sponge used to swab the skin was solubilised by digestion in 5 mL Soluene 350.
3.5.3	Donor compartment	The donor compartment was soaked over night in 30 mL acetonitrile.
3.5.4	Receptor fluid	The receptor compartment was soaked over night in 30 mL acetonitrile.
3.5.5	Outlet tubing	The contents of the receptor compartment outlet tubing were collected in vials for subsequent analysis by LSC.
3.5.6	Skin membranes	Skin membranes were solubilised by digestion in 5 mL Soluene 350.

Section A6 Toxicological and Metabolic Studies

Subsection A6.2/2 Percutaneous absorption (*in-vitro* test)

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3.6 Sample analysis	Dose formulations: Aliquots were taken prior, during and immediately after dosing. 10 mL Ready Safe scintillation fluid was added prior to analysis by LSC. Receptor fluid: samples were added directly to 10 mL Ready Safe scintillation fluid and analysed by LSC. Skin wash: duplicate 100 µL aliquots of the digested natural sponges (5 mL) were added to 10 mL of Hionic Fluor™ scintillation fluid and analysed by LSC. Skin membrane: duplicate 100 µL aliquots of the digested skin membranes (5 mL) were added to 10 mL of Hionic Fluor™ scintillation fluid and analysed by LSC.	
	4 RESULTS AND DISCUSSION	
4.1 Absorption	The dermal absorption of 1,2-benzisothiazolin-3-one is approximately 30%.	
4.2 Mass balance	The mean percentage recovery of the applied test substance was 109.43%. The mean percentage found in the receptor fluid was 0.74%, the mean percentage found remaining in the skin was 28.63% and the mean percentage found remaining unabsorbed was 80.06%. Please refer to Table A6.2/2-1.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	[^{14}C]-1,2-Benzisothiazolin-3-one was applied to 0.64 cm² dermatomed human abdominal skin membranes at 30 and 300 ppm to evaluate the dermal absorption. The study was conducted according to OECD Guideline 428 and is described under point 3 with the following deviation. The humidity was not documented as recommended in the guideline. This deviation is minor and is not considered to compromise the scientific validity of this study.	
5.2 Results and discussion	The dermal absorption of the test substance is approximately 30%. The mean percentage recovery of the applied test substance was 109.43%. The mean percentage found in the receptor fluid was 0.74%, the mean percentage found remaining in the skin was 28.63% and the mean percentage found remaining unabsorbed was 80.06%. Please refer to Table A6.2/2-1.	
5.3 Conclusion	The distribution of dose was very similar in both the low and high dose groups. The majority of dose was removed with the skin swabs at 8 hours for both doses. Very little dose penetrated the skin and was detected in the receptor fluid. The absorbed dose is considered to be	**X**

Section A6 **Toxicological and Metabolic Studies**

Subsection A6.2/2 **Percutaneous absorption (*in-vitro* test)**

Annex Point IIA
VI.6.2.b/01

	the % receptor dose and the % dose retained in the skin. Therefore, it could be considered that the dermal absorption of 1,2-benzisothiazolin-3-one is approximately 30%.
5.3.1 Reliability	1
5.3.2 Deficiencies	One deviation was noted and is out lined under points 2.3 and 5.1. However, it does not compromise the scientific validity of the study.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *July 2021*

Materials and Methods *Applican'st version is accepted.*

Results and discussion *Applicant's version is accepted.*

Conclusion *X According to the 2017 EFSA Guidance on dermal absorption this must be calculated as: Absorption (mean value) + ks, where k is a correction factor depending on the number of replicates (for 6 replicates k = 1.0) and s is the sample standard deviation:*

Dose (ppm)	*% Mean value*	*k*	*s*	*Result (%)*
300	*28.85*	*1.0*	*14.112*	*43*
30	*29.87*	*1.0*	*16.275*	*46*

Reliability *1*

Acceptability *Acceptable*

Remarks

Table A6.2/2-1: Percentage of [^{14}C]-1,2-Benzisothiazolin-3-one recovered

	% Dose actual recoveries (±SD)*		% Dose normalised recoveries (±SD)	
	Low dose	High dose	Low dose	High dose
Unabsorbed dose**	80.51 ± 7.62	79.61 ± 7.20	72.93 ± 6.12	73.33 ± 5..45

Remaining in skin	29.87 ± 6.90	27.40 ± 5.74	27.05 ± 6.12	25.33 ± 5.74
Absorbed dose*** (excluding skin)	0.00 ± 0.00	1.45 ± 1.06	0.00 ± 0.00	1.33 ± 0.97
Total recovery	110.38 ± 4.71	108.46 ± 2.64	100.00 ± 0.00	100.00 ± 0.00

* All results were mean and standard deviation of six individual cells.
** Unabsorbed dose is the residual dose recovered from the donor chamber and skin swabs.
*** Absorbed dose is the hourly receptor fluid fraction up to 24 hours, the receptor tubing and the receptor chamber washings

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.3.1	**Short-term repeated-dose toxicity test**	
Annex Point IIA VI.6.3.1	**SHORT-TERM REPEATED DOSE ORAL TOXICITY**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [X]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	28 days studies are not a mandatory requirement. Therefore, no such studies are presented in this submission.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2008*	
Evaluation of applicant's justification	*Arguments are reasonable.*	
Conclusion	*Applicant is exempted of the short-term repeated dose oral toxicity study.*	
Remarks		

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.3.2	**Short-term repeated-dose toxicity test**	
Annex Point IIA VI.6.3.2	**SHORT-TERM REPEATED DOSE DERMAL TOXICITY**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [X]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	28 days studies are not a mandatory requirement. Therefore, no such studies are presented in this submission.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2008*	
Evaluation of applicant's justification	*Arguments are reasonable.*	
Conclusion	*Applicant is exempted of the short-term repeated dose oral toxicity study.*	
Remarks		

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.3.3	**Short-term repeated-dose toxicity test**	
Annex Point IIA VI.6.3.3	**SHORT-TERM REPEATED DOSE INHALATION TOXICITY**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [X]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	28 days studies are not a mandatory requirement. Therefore no such studies are presented in this submission.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2008*	
Evaluation of applicant's justification	*Arguments are reasonable.*	
Conclusion	*Applicant is exempted of the short-term repeated dose oral toxicity study.*	
Remarks		

Section A6 Toxicological and Metabolic Studies

Subsection A6.4.1/1 Repeated dose toxicity

Annex Point IIA VI.6.4.1.a/01 **SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
		Dates of experimental work: June 19 – November 5, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Company with letter of access	Troy Chemical Company BV	
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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was carried out in accordance with OECD guideline 408.	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	Yes, the following deviations were noted: 1. Water consumption was not measured 2. Bone marrow samples were not taken for histopathology These deviations are minor and are not considered to compromise the scientific validity of this study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one	
3.1.1	Lot/Batch number	BT 17301	
3.1.2	Specification	Please refer to Doc. III-A 2/1	
3.4.1.1	Description	Beige to light brown coloured powder	
3.1.2.2	Purity	97.42% w/w (dry basis)	
3.1.2.3	Stability	Shown to be stable throughout the experiment	
3.2	**Test Animals**		

Section A6 Toxicological and Metabolic Studies

Subsection A6.4.1/1 Repeated dose toxicity

Annex Point IIA VI.6.4.1.a/01 **SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

3.2.1	Species	Rat
3.2.2	Strain	Wistar
3.2.3	Source	Breeding Facility, Jai Research Foundation, Valvada – 396108, Dist. Valsad, Gujarat, India
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	6-7 weeks Males: 113 – 152 g Females: 102 – 138 g
3.2.6	Number of animals per group	Range finding study: 5 animals/sex/group Main test: 10 animals/sex/group Recovery groups: 10 animals/sex/group
3.2.7	Control animals	Yes, 10 animals/sex
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	Range finding study: 14 days Main test: 90 days Recovery group: 90 days
3.3.2	Frequency of exposure	Daily (7 days per week)
3.3.3	Post-exposure period	28 days for the recovery groups
3.3.4	**Oral**	
3.3.4.1	Type	Gavage
3.3.4.2	Concentration	Range finding study: 0, 75, 175, and 350 mg/kg bw Main test: 0, 10, 27.5 and 75 mg/kg bw Recovery groups: 0 and 75 mg/kg bw
3.3.4.3	Controls	0.5% Carboxymethyl Cellulose Sodium Salt LR

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/1	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.a/01	**SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

3.3.4.4	Concentration in vehicle	Range finding study: 0, 7.5, 17.5 and 35.0 mg/mL Main test: 0, 1.0, 2.75 and 7.5 mg/mL Recovery groups: 0 and 7.5 mg/mL
3.3.4.5	Total volume applied	Calculated depending on the weight of the individual rat.
3.3.4.6	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, once daily, all visible signs and symptoms such as skin and fur changes, eye and mucous membrane changes, respiratory, circulatory, autonomic, and central nervous system, somatomotor activity, behavioural pattern and general changes. Home cage observations and observations during removal and handling were made such as posture, convulsions, ease of removal, handling reactivity, palpebral closure, lacrimation, eye examination, piloerection, skin examination, salivation, open field observation, gait, mobility, arousal, vocalisations, rearing, respiration, clonic or tonic movements, urination and defecation, stereotypy and bizarre behaviour. Sensory motor measurements were also made such as approach response, touch response, click response, tail-pinch response, pupil response, air righting reflex, landing hind limb foot splay, grip strength and motor activity.
3.4.1.2	Mortality	Yes, twice daily
3.4.2	Body weight	Yes, weekly
3.4.3	Food consumption	Yes, weekly
3.4.4	Water consumption	Not measured
3.4.5	Ophthalmoscopic examination	Yes, once before the commencement of treatment and prior to terminal and recovery sacrifice.
3.4.6	Haematology	Yes, Number of animals: All animals Time points: At the end of treatment and recovery periods Parameters: Leukocyte count (WBC), erythrocyte count (RBC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/1	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.a/01	**SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

		haemoglobin concentration (MCHC), platelet count (PLT), clotting time and differential leukocyte count.
3.4.7	Clinical chemistry	Yes, Number of animals: All animals Time points: At the end of treatment and recovery periods Parameters: Alanine aminotransferase (ALT), albumin (Alb.), aspartate aminotrasferase (AST), calcium (Ca^{++}), chloride (Cl^{-}), cholesterol (Chol.), creatinine (Creat.), gammaglutamyltranspeptidase (GGT), glucose, phosphorous (Phos.), potassium (K^{+}), sodium (Na^{+}), total bilirubin (T. Bil), total proteins (Tot. Prot.), urea and blood urea nitrogen (BUN)
3.4.8	Urinalysis	Not performed
3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes Organs: adrenals, brain, uterus, ovaries, testes, epididymides, heart, kidneys, liver, spleen and thymus Paired organs were weighed together and relative weights were calculated.
3.5.2	Gross and histopathology	Yes All animals Organs: adrenals, aorta, brain (medulla/pons, cerebellum and cerebrum), caecum, cervix, colon, coagulation gland, duodenum, epididymides, oesophagus, eyes, female mammary gland, gonads, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes (mesenteric and prescapular), nose, pancreas, peripheral nerve (sciatic nerve), pituitary, prostate, pharynx, rectum, salivary glands, seminal vesicles, skin, spleen, spinal cord (cervical, mid-thoracic and lumbar), sternum, stomach, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus, vagina and all gross lesions and masses.
3.5.3	Other examinations	None

Section A6 Toxicological and Metabolic Studies

Subsection A6.4.1/1 Repeated dose toxicity

Annex Point IIA VI.6.4.1.a/01 **SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

3.5.4	Statistics	Raw data and treated groups were processed using in-house developed statistical software in order to give group means and standard deviations with significance between controls. All parameters characterised by continuous data such as rearing count, urination count, defecation, body weight, feed consumption, organ weight, relative organ weight, haematological and clinical chemistry data were subjected to Bartlett's test to meet the homogeneity of variance before conducting Analysis of Variance (ANOVA) and Dunnett's t-test. Where data did not meet the homogeneity of variance, Student's t-test was performed to calculate significance.
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No treatment related clinical symptoms were observed in any of the treatment groups. One female animal from the 75 mg/kg bw group displayed signs of gasping during weeks 8 and 9 of exposure. Irrespective of treatment wry-neck, nasal irritation, snuffles, diarrhoea, and microphthalmos were observed in few animals. All animals revealed normal postures and none exhibited convulsions. Neurobehavioural observations did not reveal any treatment related abnormalities. During open field observation, no consistent alterations were revealed in any of the observed parameters. A few incidental changes were observed in the rearing count. A significantly increased mean rearing count in 27.5 and the 75 mg/kg bw group males during week 11 and decreased mean rearing count in 75 mg/kg bw recovery females during week 14 were observed. No treatment related sensory reactivity observations were made at any dose level. Some incidences of lack of click responses and changes in tail pinch reaction were noted across the groups but not deemed to be significant. No changes in hind limb foot splay, motor activity or grip strength were noted at any dose level. Pupil response to light stimulus was found normal in all rats except one male rat in the high dose recovery group which had microphthalmos of the left eye
4.1.2	Mortality	There were no mortalities at any dose level during the study.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/1	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.a/01	**SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

4.2	**Body weight gain**	10 mg/kg bw males: No significant treatment related differences were found 10 mg/kg bw females: No significant treatment related differences were found 27.5 mg/kg bw males: No significant treatment related differences were found 27.5 mg/kg bw females: No significant treatment related differences were found 75 mg/kg bw males: No statistically significant decrease was observed in the body weight though a decreasing trend was evident in the mean body weight when compared to control group. The percent decrease in mean body weight ranged from 1.4% to 8.2% during the treatment period. The recovery group weeks 2, 6-11 and 13 of exposure period showed a significantly decreased mean body weight as compared to control recovery group. The percent decrease in mean body weight ranged from 6.3% to 11.2% during the treatment period. This is considered to be treatment related and withdrawal of the test substance for 28 days led to recovery in the body weight. 75 mg/kg bw females: No significant treatment related differences were found Please refer to Table A6.4.1/1-1.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/1	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.a/01	**SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

4.3	**Food consumption and compound intake**	10 mg/kg bw males: No significant treatment related differences were found 10 mg/kg bw females: No significant treatment related differences were found 27.5 mg/kg bw males: No significant treatment related differences were found 27.5 mg/kg bw females: No significant treatment related differences were found 75 mg/kg bw males: A significant decrease was observed during weeks 1, 2, 6, 7 and 13 in the recovery group 75 mg/kg bw females: No significant treatment related differences were found. An incidental decrease in mean food consumption was observed in the recovery group animals during week 2.
4.4	**Ophtalmoscopic examination**	No ocular abnormalities were noted except for one male in the 75 mg/kg bw recovery group who had microphthalmos of the left eye during the pre-sacrifice examination.
4.5	**Blood analysis**	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/1	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.a/01	**SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

4.5.1	Haematology	10 mg/kg bw males: No significant treatment related differences were found. Differential leukocyte count analysis revealed a significantly decreased mean lymphocyte count and a significantly increased mean eosinophil count. These alterations were considered to be due to random biological variation. 10 mg/kg bw females: No significant treatment related differences were found. There were incidental increases in platelet count. 27.5 mg/kg bw males: A significant increase of 3.8% in mean values of MCH was noted. However, no treatment related changes in mean HB values or total RBC count were observed. 27.5 mg/kg bw females: No significant treatment related differences were found. Significantly decreased HB of 3.2% and HCT of 3.5% were detected. This could not be considered treatment related. 75 mg/kg bw males: A significant increase of 3.8% in mean values of MCH was noted. However, no treatment related changes in mean HB values or total RBC count were observed. There was a significant increase of 8.8% in mean values of platelet count. However, this was not deemed to be of biological significance. The recovery group revealed a significantly decreased RBC count and platelet count and a significantly increased MCH values. These alterations are not thought to be due to treatment. Differential leukocyte count analysis revealed a significantly decreased lymphocyte and a significantly increased neutrophil count as compared to the control recovery group. 75 mg/kg bw females: RBC count decreased significantly 5.5% in high dose group. A treatment related cause was not ruled out. Please refer to Table A6.4.1/1-2.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/1	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.a/01	**SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

4.5.2	Clinical chemistry	10 mg/kg bw males: No significant treatment related differences were found 10 mg/kg bw females: No significant treatment related differences were found 27.5 mg/kg bw males: No significant treatment related differences were found 27.5 mg/kg bw females: No significant treatment related differences were found. Incidental increases in levels of ALT were detected. 75 mg/kg bw males: Cholesterol values significantly increased 32% as compared to the control group. Values returned to normal after withdrawal of treatment for 28 days. 75 mg/kg bw females: A significantly decreased level of calcium was observed. There were no corresponding changes in phosphorous values as is usually seen with decreased calcium levels. After 28 days recovery period both calcium at 3.9% and phosphorous at 20.4% were significantly increased in the recovery group as compared to control recovery group. An incidental decrease in mean value of phosphorous was observed in the control recovery group. Please refer to Table A6.4.1/1-3.
4.5.3	Urinalysis	Not performed
4.6	**Sacrifice and pathology**	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/1	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.a/01	**SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

4.6.1	Organ weights	10 mg/kg bw males: No significant treatment related differences were found 10 mg/kg bw females: No significant treatment related differences were found There was an incidental increase in the absolute weight of thymus was observed as compared to control group. There was an incidental decrease in relative weight of the spleen as compared to control group. 27.5 mg/kg bw males: No significant treatment related differences were found 27.5 mg/kg bw females: No significant treatment related differences were found 75 mg/kg bw males: No significant treatment related differences were found There was an incidental decrease in the absolute weight of the thymus observed in the recovery group as compared to the control recovery group. 75 mg/kg bw females: No significant treatment related differences were found	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/1	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.a/01	**SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

4.6.2	Gross and histopathology	External examination did not reveal any lesions of pathological significance except microphthalmos in one male from high dose recovery group. Internal examination in males belonging to control and different treatment groups showed varying degree of gross lesions in the lungs, liver, spleen and kidneys. In females, gross lesions were comparable to those observed in males with the exception of bilateral hydrometra. Microscopic examination revealed varying degree of pathological changes in different organs belonging to both control and treatment groups: Lungs: perivascular / peribronchiolar mono nuclear cell (MNC) infiltration / alveolar consolidation with histiocytosis / fibrous connective tissue proliferation, emphysema Kidneys: congestion, cystic space contain eosinophilic material, nephrosis Spleen: congestion, fibrosis / lymphoid depletion Liver: congestion / haemorrhage Lymph node: lymphoid hyperplasia Thymus: lymphoid hyperplasia, starry sky appearance Trachea: dilatation of submucosal gland / MNC infiltration submucosa Adrenal: medullary congestion Intestine: lymphoid hyperplasia / hyperplastic intestinal gland Optic nerve: vacuolation Additional lesions were recorded in females: Liver: degenerative / necrotic changes / vacuolation in hepatocytes Uterus: luminal dilatation It was inferred that the changes were spontaneous and incidental and were not related to treatment.
4.7	**Other**	None
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The subchronic oral toxicity of 1,2-Benzisothiazolin-3-one was investigated by orally dosing four groups of 10 rats/sex once daily for 90 days at the concentrations of 0, 10, 27.5 and 75 mg/kg bw/day. Two additional groups of 10 animals/sex/group were treated at the doses of 0 and 75 mg/kg bw for 90 days and were retained for a 28-day post-treatment recovery period.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/1	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.a/01	**SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

		The study was conducted according to OECD guideline 408 and is described under point 3. The following deviations were noted: 1. Water consumption was not measured 2. Bone marrow samples were not taken for histopathology However, these deviations are minor and are not considered to compromise the scientific validity of this study.
5.2	**Results and discussion**	No treatment related clinical symptoms were observed in any of the treatment groups. There were no mortalities at any dose level during the study. There was a decreasing trend evident in the mean body weight of the 75 mg/kg bw/day males group compared to the control group that was considered to be treatment related. There was a significant decrease in food consumption observed in the 75 mg/kg bw males group. There were no treatment related ocular abnormalities. A significant increase in the haematology parameter MCH was noted in the 27.5 and 75 mg/kg bw/day males group. A significant decrease in HB and HCT were detected in the 27.5 mg/kg bw/day females group and a significant decrease in RBC count was detected in the 75 mg/kg bw/day females group A significant increase in the clinical chemistry parameter cholesterol was detected in the 75 mg/kg bw/day males group. A significant increase in calcium was detected in the 75 mg/kg bw/day females group. There were no treatment related changes found in organ weights at necropsy. Gross and histopathology did not reveal any treatment related changes. Please refer to Tables A6.4.1/1-1, A6.4.1/1-2 and A6.4.1/1-3
5.3	**Conclusion**	Based on the results of the study, it is concluded that the NOAEL of 1,2-Benzisothiazolin-3-one in Wistar rats exposed over a period of 90 days is 27.5 mg/kg bw.
5.3.1	LO(A)EL	75 mg/kg bw
5.3.2	NO(A)EL	27.5 mg/kg bw
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	Two deviations were noted and are outlined under point 5.1. However they will not compromise the scientific validity of this study.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/1	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.a/01	**SUBCHRONIC ORAL TOXICITY TEST IN RATS (90 DAYS)**

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2008*
Materials and Methods	*Applicant version is accepted.*
Results and discussion	*Applicant version is accepted*
Conclusion	*LO(A)EL: 75 mg BIT/kg bw/day* *NO(A)EL: 27.5 mg BIT/kg bw/day* *Other conclusions: Other applicant's conclusions are adopted.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.4.1/1-1: Summary of body weight gain in males and females

Week of Study	Male						Female					
	0 mg/kg bw Weight (g) ± SD	0 mg/kg bw recovery Weight (g) ± SD	10 mg/kg bw Weight (g) ± SD	27.5 mg/kg bw Weight (g) ± SD	75 mg/kg bw Weight (g) ± SD	75 mg/kg bw recovery Weight (g) ± SD	0 mg/kg bw Weight (g) ± SD	0 mg/kg bw recovery Weight (g) ± SD	10 mg/kg bw Weight (g) ± SD	27.5 mg/kg bw Weight (g) ± SD	75 mg/kg bw Weight (g) ± SD	75 mg/kg bw recovery Weight (g) ± SD
0	148 ± 10.76	153 ± 12.95	153 ± 13.04	152 ± 9.13	149 ± 13.48	150 ± 9.60	134 ± 9.29	138 ± 9.84	136 ± 9.76	134 ± 10.21	134 ± 11.12	137 ± 6.38
1	191 ± 20.16	199 ± 16.36	198 ± 17.30	185 ± 17.16	180 ± 23.06	183 ± 19.88	156 ± 9.30	161 ± 12.14	160 ± 13.98	159 ± 13.64	157 ± 11.59	154 ± 7.84
2	228 ± 25.96	237 ± 21.53	238 ± 18.28	224 ± 22.83	218 ± 29.68	217* ± 20.90	176 ± 11.57	182 ± 13.01	182 ± 14.84	182 ± 15.64	175 ± 12.83	172 ± 9.45
3	264 ± 29.27	268 ± 29.69	276 ± 22.68	255 ± 23.66	253 ± 27.49	251 ± 21.69	189 ± 13.62	198 ± 14.05	195 ± 14.74	196 ± 16.99	191 ± 12.19	188 ± 10.37
4	293 ± 33.47	292 ± 40.38	303 ± 25.30	287 ± 27.20	278 ± 29.85	273 ± 21.09	201 ± 15.59	209 ± 15.44	211 ± 16.79	207 ± 18.40	201 ± 15.02	203 ± 10.57
5	313 ± 37.25	320 ± 35.16	326 ± 29.76	307 ± 32.36	295 ± 32.08	293 ± 25.28	208 ± 14.40	217 ± 15.61	222 ± 17.68	213 ± 19.17	211 ± 15.42	213 ± 13.84
6	329 ± 42.41	342 ± 33.45	344 ± 32.99	325 ± 33.88	316 ± 32.63	313* ± 21.77	217 ± 17.26	230 ± 19.78	229 ± 18.45	221 ± 20.40	217 ± 15.95	220 ± 18.35
7	339 ± 45.12	359 ± 33.92	357 ± 33.59	336 ± 37.77	329 ± 32.70	327* ± 20.43	221 ± 16.69	235 ± 22.83	232 ± 17.25	227 ± 20.56	223 ± 16.46	226 ± 15.86

8	354 ± 43.58	375 ± 33.20	375 ± 35.84	352 ± 42.66	349 ± 35.02	338* ± 26.14	227 ± 18.58	241 ± 23.07	241 ± 16.76	231 ± 19.32	229 ± 19.33	232 ± 19.89
9	362 ± 43.25	383 ± 36.39	388 ± 35.37	364 ± 47.53	347 ± 37.50	349* ± 26.76	231 ± 16.45	245 ± 21.57	245 ± 15.09	239 ± 19.76	235 ± 22.09	236 ± 21.01
10	378 ± 47.17	397 ± 39.15	395 ± 36.57	374 ± 50.36	353 ± 38.14	360* ± 26.20	236 ± 18.77	249 ± 17.83	248 ± 15.93	243 ± 22.56	237 ± 24.94	241 ± 20.76
11	388 ± 47.70	409 ± 45.67	405 ± 36.98	379 ± 51.37	362 ± 38.60	367* ± 25.29	241 ± 19.97	255 ± 20.62	252 ± 14.24	243 ± 19.60	242 ± 26.45	245 ± 22.91
12	393 ± 47.38	417 ± 49.24	415 ± 42.32	392 ± 55.57	361 ± 36.96	382 ± 24.51	241 ± 20.59	261 ± 22.87	251 ± 12.35	246 ± 18.07	242 ± 23.38	250 ± 23.82
13	393 ± 51.64	419 ± 51.22	416 ± 41.31	394 ± 56.57	365 ± 36.63	379* ± 22.51	241 ± 20.79	262 ± 22.62	255 ± 15.51	246 ± 18.08	246 ± 27.68	251 ± 23.72
14	-	427 ± 54.82	-	-	-	390 ± 23.02	-	263 ± 25.14	-	-	-	253 ± 26.92
15	-	427 ± 57.91	-	-	-	399 ± 23.05	-	261 ± 26.83	-	-	-	253 ± 27.31
16	-	434 ± 61.48	-	-	-	404 ± 20.71	-	263 ± 23.01	-	-	-	259 ± 30.54
17	-	434 ± 65.87	-	-	-	410 ± 22.98	-	264 ± 26.33	-	-	-	259 ± 28.69

* Significantly lower than control recovery ($p \leq 0.05$)

Table A6.4.1/1-2: Haematology parameters in male and female rats

Sex	Dose (mg/kg)	Number of animals	Parameters mean ± SD
			MCH (pg)
Male	0	10	18.4 ± 0.30
	0 (recovery)	10	18.4 ± 0.69
	10	10	18.5 ± 0.97
	27.5	10	19.1* ± 0.83
	75	10	19.1** ± 0.68
	75 (recovery)	10	19.0* ± 0.51
Female	0	10	19.2 ± 0.58
	0 (recovery)	10	19.5 ± 0.99
	10	10	19.1 ± 0.70
	27.5	10	19.4 ± 0.54
	75	10	19.8 ± 0.79
	75 (recovery)	10	20.1 ± 0.66

* Significantly higher than control recovery ($p \leq 0.05$)
** Significantly higher than control recovery ($p \leq 0.01$)

Table A6.4.1/1-3: Clinical chemistry parameters in male and female rats

Sex	Dose mg/kg	Number of animals	Parameter mean ± SD		
			Cholesterol (mg/dL)	Calcium (mg/dL)	Phosphorous (mg/dL)
Male	0	10	52.9 ± 17.17	9.7 ± 0.46	5.96 ± 0.41
	0 (recovery)	10	48.8 ± 8.63	10.2 ± 0.29	5.15 ± 0.36
	10	10	53.4 ± 8.72	9.9 ± 0.38	6.09 ± 0.65
	27.5	10	52.6 ± 12.12	9.8 ± 0.23	5.86 ± 0.61
	75	10	69.8* ± 10.43	9.9 ± 0.29	5.87 ± 0.35
	75 (recovery)	10	51.2 ± 5.81	10.4 ± 0.27	5.17 ± 0.24
Female	0	10	75.0 ± 13.28	10.6 ± 0.45	5.12 ± 0.77
	0 (recovery)	10	71.2 ± 11.8	10.4 ± 0.34	3.78 ± 0.64
	10	10	75.5 ± 16.51	10.4 ± 0.31	5.43 ± 1.02
	27.5	10	72.5 ± 5.78	10.4 ± 0.35	4.73 ± 1.09
	75	10	77.2 ± 6.12	10.1** ± 0.32	4.91 ± 0.83
	75 (recovery)	10	77.7 ± 11.15	10.8* ± 0.39	4.55* ± 0.68

* Significantly higher than control recovery ($p \leq 0.05$)
** Significantly lower than control recovery ($p \leq 0.05$)

Section A6 Toxicological and Metabolic Studies

Subsection A6.4.1/2 Repeated dose toxicity

Annex Point IIA VI.6.4.1.b/01 **SUBCHRONIC ORAL TOXICITY TEST IN DOGS (90 DAYS)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: July 20, 2006 – January 2, 2007	
1.2	**Data protection**	Yes	
1.2.4	Data owner	ROHM & HAAS	
1.2.5	Company with letter of access	Troy Chemical Company BV	
1.2.6	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purposeof its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was carried out in accordance with OECD Guideline 409	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one	
3.1.1	Lot/Batch number	Lot No. 2005-051	
3.1.2	Specification	As given under point 3.1.2.2	
3.1.2.1	Description	Off-white powder containing lumps	
3.1.2.2	Purity	89.8%	
3.1.2.3	Stability	Pre-study test diet formulations were prepared and samples for stability were collected from the middle strata of the 200, 1000 and 4000 ppm test diet formulations. These samples were analyzed after	

		room temperature storage for 6 and 24 hours and 3, 7, 10 and 15 days and after frozen storage for 1, 3, 7, 10 and 15 days. With the exception of the formulations prepared on November 1, 2006 for administration to groups 2 and 3 animals (83.2 and 83.0% of target, respectively), the analyzed dietary formulations were found to contain the amount of test article prescribed in the protocol within 85-115% of the target concentration, were homogeneous and were stable when stored frozen for 15 days and at room temperature for 6 hours.
3.2	**Test Animals**	
3.2.1	Species	Dog
3.2.2	Strain	Beagle
3.2.3	Source	Ridglan Farms, Mt. Horeb, Wisconsin, USA
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	5 months Males: 6.4 – 8.2 kg Females: 5.1 – 7.3 kg
3.2.6	Number of animals per group	Four groups of 4 animals/sex/group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	90, 91 or 92 days
3.3.2	Frequency of exposure	Daily (7 days per week)
3.3.3	Post-exposure period	Not applicable
3.3.4	**Oral**	
3.3.4.1	Type	Diet
3.3.4.2	Concentration	Males: 0, 11, 37 and 106 mg/kg bw/day Females: 0, 11, 38 and 89 mg/kg bw/day

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/2	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.b/01	**SUBCHRONIC ORAL TOXICITY TEST IN DOGS (90 DAYS)**

3.3.4.3	Controls	PMI Nutrition International, LLC Certified Canine LabDiet® 5007 (meal)
3.3.4.4	Concentration in vehicle	0, 300, 1000 and 3000 ppm
3.3.4.5	Total volume applied	Not applicable
3.3.4.6	Controls	Vehicle (basal diet)
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, clinical examinations were performed daily. Detailed physical examinations were conducted on all animals one week prior to dosing and weekly thereafter.
3.4.1.2	Mortality	Yes, twice daily
3.4.2	Body weight	Yes, weekly, beginning at two weeks prior to dosing
3.4.3	Food consumption	Yes, weekly, beginning at two weeks prior to dosing
3.4.4	Water consumption	Not measured
3.4.5	Ophthalmoscopic examination	Yes, once two weeks before dosing and at week 12 of the study. Ocular examinations were conducted using as indirect ophthalmoscope and slit lamp biomicroscope preceded by pupillary dilatation with mydriatic agent.
3.4.6	Haematology	Yes, Number of animals: All animals Time points: prior to dosing by one week and during the study in weeks 6 and 12. Parameters: Total leukocyte count (WBC), erythrocyte count (RBC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT), prothrombin time (Pro Time), activated partial thromboplastin time (APTT), Reticulocyte count, differential leukocyte count (neutrophil, lymphocyte, monocyte, eosinophil, basophil, large unstained cell) platelet estimate, red cell morphology (RBC morphology)

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/2	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.b/01	**SUBCHRONIC ORAL TOXICITY TEST IN DOGS (90 DAYS)**

3.4.7	Clinical chemistry	Yes, Number of animals: All animals Time points: prior to dosing by one week and during the study in weeks 6 and 12. Parameters: Albumin (Alb.), total proteins (Tot. Prot.), globulin, albumin/globulin ratio (A/G ratio), total bilirubin (Total. Bili), urea nitrogen, creatinine (Creat.), alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotrasferase (AST), gamma glutamyltransferase (GGT), glucose, cholesterol (Chol.), calcium (Ca^{++}), chloride (Cl^{-}), phosphorous (Phos.), potassium (K^{+}), sodium (Na^{+}), triglycerides and sorbitol dehydrogenase
3.4.8	Urinalysis	Yes, Number of animals: All animals Time points: prior to dosing by one week and during the study in weeks 6 and 12. Parameters: Specific gravity (SG), pH, urobilinogen (URO), total volume (TVOL), colour (COL), clarity (CLA), protein (PRO), glucose (GLU), ketones (KET), bilirubin (BIL), occult blood (BLD), leukocytes (LEU), nitrites (NIT) and microscopy of sediment
3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes Organs: adrenals, brain, epididymides, heart, kidneys, liver with gall bladder, ovaries, spleen, testes, thymus, thyroid with parathyroid and uterus. Paired organs were weighed together and relative weights were calculated.

Section A6 Toxicological and Metabolic Studies

Subsection A6.4.1/2 Repeated dose toxicity

Annex Point IIA VI.6.4.1.b/01 **SUBCHRONIC ORAL TOXICITY TEST IN DOGS (90 DAYS)**

3.5.2	Gross and histopathology	Yes All animals Examination of the external surface, all orifices, and the cranial, thoracic, abdominal and pelvic cavities including contents. Organs: adrenals (2), aorta, bone marrow (femur and sternum), bone marrow smear, brain (medulla/pons, cerebellum and cerebrum level 1 and 2), epididymides (2), eyes with optic nerve (2), gall bladder, gastrointestinal tract (oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum), heart, kidneys (2), larynx, liver (sections of 2 lobes), lungs (including bronchi) (2), lymph nodes (mesenteric and mandibular (2)), nose, ovaries (2), oviducts (2), pancreas, peripheral nerve (sciatic nerve), pharynx, pituitary, prostate, salivary glands (mandibular (2)), skeletal muscle (rectus femoris), skin (with mammary gland), spinal cord (cervical, mid-thoracic and lumbar), spleen, testes (2), thymus, thyroid/parathyroid (2), tongue, trachea, ureters (2), urinary bladder, uterus with cervix, vagina and gross lesions.
3.5.3	Other examinations	None
3.5.4	Statistics	Analysis was conducted using two-tails tests for minimum significance levels of 5%, comparing each test article-treated group to the control group by sex. Each mean was presented with the standard deviation and the number of animal used to calculate the mean. Percent change form control is presented for body weights, clinical pathology parameters and organ weights. Statistical analyses were not conducted if the number of animals was two or less. Due to the different rounding conventions inherent in the types of software used, the means and standard deviations on the summary and individual tables may differ by ± 1 in the last significant figure. Body weight, body weight change, food consumption, clinical pathology and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p < 0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group.
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No treatment related clinical symptoms were observed in any of the treatment groups.
4.1.2	Mortality	There were no mortalities at any dose level during the study.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/2	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.b/01	**SUBCHRONIC ORAL TOXICITY TEST IN DOGS (90 DAYS)**

4.2	**Body weight gain**	11 mg/kg bw/day males: No significant treatment related differences were found. 37 mg/kg bw/day males: No significant treatment related differences were found. 106 mg/kg bw/day males: Mean body weight losses in weeks 0-1 and 1-2 were observed. At week 13 the mean body weights were 6.6% lower than the control. Although these differences were not statistically significant, the effect was considered adverse. 11 mg/kg bw/day females: No significant treatment related differences were found. 38 mg/kg bw/day females: No significant treatment related differences were found. 89 mg/kg bw/day females: Mean body weight losses in weeks 0-1, 1-2, 2-3 and 3-4 and throughout the study were observed. At 13 weeks, the mean body weights were 18.7% lower than the control. Although these differences were not statistically significant, the effect was considered adverse. Please refer to Table A6.4.1/2-1.
4.3	**Food consumption and compound intake**	11 mg/kg bw/day males: No significant treatment related differences were found. 37 mg/kg bw/day males: No significant treatment related differences were found. 106 mg/kg bw/day males: Statistically significant decreases in food consumption were observed during the first 3 weeks and were consistent with lower body weights. 11 mg/kg bw females: No significant treatment related differences were found. 38 mg/kg bw/day females: No significant treatment related differences were found. 89 mg/kg bw/day females: Statistically significant decreases in food consumption were observed throughout the study. Please refer to Table A6.4.1/2-2.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/2	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.b/01	**SUBCHRONIC ORAL TOXICITY TEST IN DOGS (90 DAYS)**

4.4	**Ophtalmoscopic examination**	No significant treatment related ophthalmic lesions were found.
4.5	**Blood analysis**	
4.5.1	Haematology	No significant treatment related differences were found.
4.5.2	Clinical chemistry	11 mg/kg bw/day males: No significant treatment related differences were found. 37 mg/kg bw/day males: No significant treatment related differences were found. 106 mg/kg bw/day males: No significant treatment related differences were found. 11 mg/kg bw/day females: No significant treatment related differences were found. 38 mg/kg bw/day females: No significant treatment related differences were found. 89 mg/kg bw/day females: A statistically significant decrease in calcium was observed at week 6. Please refer to Table A6.4.1/2-3.
4.5.3	Urinalysis	No significant treatment related differences were found.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No treatment related differences were found at any of the dose levels in both sexes.
4.6.2	Gross and histopathology	There were no test article related macroscopic or microscopic findings.
4.7	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The subchronic oral toxicity of 1,2-Benzisothiazolin-3-one was investigated by orally dosing four groups of 4 dogs/sex once daily for 90 days at the concentrations of 0, 11, 37 and 106 mg/kg bw/day for males and 0, 11, 38 and 89 mg/kg bw/day for females. The study was conducted according to OECD Guideline 409 and is described under point 3 with no deviations.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/2	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.b/01	**SUBCHRONIC ORAL TOXICITY TEST IN DOGS (90 DAYS)**

5.2	**Results and discussion**	No treatment related clinical symptoms were observed in any of the treatment groups. There were no mortalities at any dose level during the study. In the 106 mg/kg bw/day males, mean body weight losses in weeks 0-1 and 1-2 were observed. At week 13 the mean body weights were 6.6% lower than the control. In the 89 mg/kg bw/day females, mean body weight losses in weeks 0-1, 1-2, 2-3 and 3-4 and throughout the study were observed. At 13 weeks, the mean body weights were 18.7% lower than the control. Although these differences were not statistically significant, the effects were considered adverse. In the 106 mg/kg bw/day males, lower food consumption was observed during the first 3 weeks and was consistent with lower body weights. In the 89 mg/kg bw/day females, lower food consumption was observed throughout the study. These differences were statistically significant. No significant treatment related ophthalmic lesions were found. No significant treatment related differences were found in haematology and urine analysis. In the 89 mg/kg bw/day females, a statistically significant decrease in calcium was observed at week 6. No treatment related differences in organ weights were found at any of the dose levels in both sexes. There were no test substance related macroscopic or microscopic findings at necropsy. Please refer to Tables A6.4.1/2-1, A6.4.1/2-2 and A6.4.1/2-3
5.3	**Conclusion**	Based on the effects observed in body weights and food consumption, it is concluded that the NOAEL for dietary administration of 1,2-Benzisothiazolin-3-one to beagle dogs exposed over a period of 90 days is 37 mg/kg bw/day in males and 38 mg/kg bw/day in females.
5.3.1	LO(A)EL	Males: 106 mg/kg bw/day Females: 89 mg/kg bw/day
5.3.2	NO(A)EL	Males: 37 mg/kg bw/day Females: 38 mg/kg bw/day
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	None

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.1/2	**Repeated dose toxicity**
Annex Point IIA VI.6.4.1.b/01	**SUBCHRONIC ORAL TOXICITY TEST IN DOGS (90 DAYS)**

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2008*
Materials and Methods	*Applicant version is adopted.*
Results and discussion	*Applicant version is adopted.*
Conclusion	*LO(A)EL: males: 106 mg BIT/kg bw/day; females: 89 mg BIT/kg bw/day* *NO(A)EL: males: 37 mg BIT/kg bw/day; females: 38 mg BIT/kg bw/day* *Other conclusions: Other applicant's conclusions are adopted.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.4.1/2-1: Summary of body weight in males and females in a 90-day oral study in dogs with 1,2-benzisothiazolin-3-one

Week of Study	Males				Females			
	0 mg/kg bw/day Weight (kg) ± SD	11 mg/kg bw/day Weight (kg) ± SD	37 mg/kg bw/day Weight (kg) ± SD	106 mg/kg bw/day Weight (kg) ± SD	0 mg/kg bw/day Weight (kg) ± SD	11 mg/kg bw/day Weight (kg) ± SD	38 mg/kg bw/day Weight (kg) ± SD	89 mg/kg bw/day Weight (kg) ± SD
-2	7.2 ± 0.76	7.3 ± 0.79	7.1 ± 0.49	7.2 ± 0.67	6.0 ± 0.82	6.2 ± 0.72	6.5 ± 0.72	6.2 ± 0.78
-1	7.2 ± 0.76	7.2 ± 0.82	7.1 ± 0.49	7.2 ± 0.57	6.0 ± 0.91	6.2 ± 0.69	6.3 ± 0.81	6.2 ± 0.76
0	7.2 ± 0.73	7.3 ± 0.72	7.1 ± 0.59	7.3 ± 0.58	6.0 ± 0.89	6.3 ± 0.72	6.3 ±0.81	6.2 ± 0.81
1	7.5 ± 0.81	7.7 ± 0.71	7.5 ± 0.63	7.1 ± 0.59	6.3 ± 0.98	6.5 ± 0.63	6.7 ± 0.94	6.0 ± 0.81
2	7.5 ± 0.78	7.8 ± 0.67	7.6 ± 0.66	6.8 ± 0.46	6.4 ± 0.97	6.6 ± 0.67	6.8 ± 0.93	5.7 ± 0.55
3	8.2 ± 0.88	8.5 ± 0.70	8.3 ± 0.72	7.4 ± 0.80	7.0 ± 1.01	7.1 ± 0.75	7.4 ± 1.06	5.9 ± 0.55
4	8.4 ± 0.85	8.8 ± 0.75	8.5 ± 0.70	7.7 ± 0.84	7.2 ± 1.10	7.2 ± 0.82	7.6 ± 1.10	5.9 ± 0.61
5	8.8 ± 0.84	9.2 ± 0.76	8.8 ± 0.74	8.1 ± 0.94	7.6 ± 1.14	7.4 ± 0.90	8.0 ± 1.11	6.1 ± 0.67
6	9.1 ± 0.98	9.8 ± 0.90	9.3 ± 0.78	8.5 ± 0.97	7.9 ± 1.24	7.7 ± 0.97	8.5 ± 1.25	6.2 ± 0.74
7	9.5 ± 0.97	70.0 ± 0.94	9.5 ± 0.74	8.7 ± 0.99	8.1 ± 1.26	8.0 ± 0.94	8.6 ± 1.30	6.4 ± 0.75
8	9.6 ± 1.01	10.4 ± 0.95	9.7 ± 0.82	8.9 ± 1.01	8.3 ± 1.25	8.1 ± 1.00	8.9 ± 1.39	6.6 ± 0.65
9	9.8 ± 1.13	10.7 ± 0.93	10.0 ± 0.83	9.2 ± 1.03	8.6 ± 1.38	8.4 ± 1.08	9.3 ± 1.43	6.9 ± 0.66
10	10.0 ± 1.09	10.7 ± 1.12	10.1 ± 0.90	9.4 ± 1.19	8.6 ± 1.48	8.3. ± 1.25	9.4 ± 1.50	7.0 ± 0.67
11	10.2 ± 1.16	11.1 ± 1.14	10.3 ± 0.80	9.7 ± 1.20	8.8 ± 1.63	8.5 ± 1.20	9.7 ± 1.59	7.2 ± 0.68

12	10.5 ± 1.24	11.5 ± 1.04	10.6 ± 0.99	9.9 ± 1.32	9.0 ± 1.51	8.8 ± 1.24	9.9 ± 1.60	7.3 ± 0.63
13	10.6 ± 1.36	11.7 ± 1.17	10.6 ± 1.05	9.9 ± 1.23	9.1 ± 1.50	8.9 ± 1.22	10.1 ± 1.66	7.4 ± 0.68

Table A6.4.1/2-2: Summary of food consumption in males and females in a 90-day oral study in dogs with 1,2-benzisothiazolin-3-one

Week of Study	Males				Females			
	0 mg/kg bw/day Food (g/animal/day) ± SD	11 mg/kg bw/day Food (g/animal/day) ± SD	37 mg/kg bw/day Food (g/animal/day) ± SD	106 mg/kg bw/day Food (g/animal/day) ± SD	0 mg/kg bw/day Food (g/animal/day) ± SD	11 mg/kg bw/day Food (g/animal/day) ± SD	38 mg/kg bw/day Food (g/animal/day) ± SD	89 mg/kg bw/day Food (g/animal/day) ± SD
-2 – -1	192 ± 38.7	195 ± 31.4	223 ± 39.3	214 ± 40.4	209 ± 41.4	169 ± 34.0	195 ± 47.7	183 ± 27.4
-1 – 0	241 ± 35.4	252 ± 40.3	268 ± 46.5	271 ± 7.0	235 ± 57.2	208 ± 40.2	232 ± 45.0	217 ± 38.0
0 – 1	273 ± 44.0	267 ± 31.3	258 ± 49.4	184* ± 9.0	256 ± 73.2	220 ± 33.7	236 ± 35.0	133* ± 16.2
1 – 2	265 ± 41.4	276 ± 27.1	280 ± 48.0	181* ± 45.6	278 ± 71.4	240 ± 31.4	262 ± 45.6	112* ± 42.0
2 – 3	347 ± 48.1	356 ± 29.1	334 ± 49.1	268* ± 32.8	336 ± 55.2	291 ± 35.6	312 ± 48.2	164* ± 31.8
3 – 4	332 ± 33.6	355 ± 31.7	325 ± 42.5	311 ± 41.6	323 ± 62.9	277 ± 29.5	299 ± 38.9	169* ± 16.5
4 – 5	364 ± 36.1	376 ± 24.7	352 ± 39.1	326 ± 32.0	339 ± 50.0	295 ± 42.3	330 ± 48.8	201* ± 24.3
5 – 6	350 ± 38.1	372 ± 28.0	349 ± 37.6	327 ± 25.8	337 ± 49.8	295 ± 42.7	335 ± 48.2	198* ± 39.5
6 – 7	354 ± 30.9	358 ± 27.4	349 ± 42.1	311 ± 22.8	321 ± 67.0	301 ± 31.1	331 ± 47.9	212* ± 30.6
7 – 8	357 ± 43.2	379 ± 23.9	367 ± 33.4	326 ± 30.8	338 ± 42.9	299 ±30.7	339 ± 47.0	225* ± 34.4
8 – 9	343 ± 65.0	370 ± 22.3	361 ± 36.2	335 ± 14.1	341 ± 39.3	307 ± 33.3	345 ± 39.6	221* ± 28.4
9 – 10	315 ± 32.3	326 ± 53.2	341 ± 50.6	305 ± 14.6	305 ± 65.8	243 ± 59.5	320 ± 53.3	217 ± 37.3
10 – 11	320 ± 33.1	365 ± 26.8	341 ± 43.1	320 ± 20.3	312 ± 71.3	256 ± 41.9	320± 56.5	208* ± 27.7

11 – 12	368 ± 26.6	386 ± 15.6	373 ± 31.0	335 ± 26.1	333 ± 45.7	310 ± 42.4	346 ± 38.6	223* ± 14.6
12 – 13	363 ± 34.0	369 ± 40.1	368 ± 47.6	331 ± 18.3	328 ± 48.4	288 ± 25.2	335 ± 45.4	213* ± 42.0

* Significantly different from the control group at 0.05 using Dunnett´s test

Table A6.4.1/2-3: Clinical chemistry parameters in male and female in a 90-day study in dogs with 1,2-benzisothiazolin-3-one

Sex	Dose (mg/kg bw/day)	Number of animals	Calcium (mg/dl) mean		
			Prior to dosing	Week 6	Week 12
Males	0	4	11.4 ± 0.19	11.3 ± 0.26	11.0 ± 0.05
	11	4	11.7 ± 0.13	11.6 ± 0.29	11.4 ± 0.48
	37	4	11.3 ± 0.22	11.2 ± 0.12	11.1 ± 0.08
	106	4	11.5 ± 0.06	11.3 ± 0.17	11.0 ± 0.19
Females	0	4	11.4 ± 0.22	11.4 ± 0.37	11.1 ± 0.18
	11	4	11.3 ± 0.34	11.5 ± 0.47	11.4 ± 0.13
	38	4	11.6 ± 0.21	11.6 ± 0.26	11.4 ± 0.15
	89	4	11.3 ± 0.08	10.7* ± 0.22	10.9 ± 0.35

* Significantly different at 0.05 using Dunnett's test

Section A6 Toxicological and Metabolic Studies

Subsection A6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 SUBCHRONIC DERMAL TOXICITY TEST IN RATS (90 DAYS)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: January 5 – April 6, 2000	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Company with letter of access	Not applicable	
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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to US EPA OPPTS 870.3250 which is equivalent to OECD Guideline 411.	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazol-3-(2*H*)-one ([redacted])	
3.1.3	Lot/Batch number	579	
3.1.4	Specification	Please refer to Doc. III-A 2/2	
3.1.2.1	Description	Tan powder	
3.1.2.4	Purity	98.71%	
3.1.2.5	Stability	Not relevant, fresh substance was prepared daily prior to application	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar albino	

Section A6 Toxicological and Metabolic Studies

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Annex Point IIA VI.6.4.2 SUBCHRONIC DERMAL TOXICITY TEST IN RATS (90 DAYS)

3.2.3	Source	Ace animals, Boyertown, PA, USA
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Approximately 6 weeks prior to acclimatisation period Males: 220 – 277 g Females: 162 – 196 g
3.2.6	Number of animals per group	4 groups of 10 animals/sex/group
3.2.7	Control animals	Yes, 10 animals/sex
3.3	**Administration/ Exposure**	Dermal
3.3.1	Duration of treatment	90 days
3.3.2	Frequency of exposure	5 days per week
3.3.3	Post-exposure period	Not applicable
3.3.4	**Oral**	
3.3.4.1	Area covered	10 % of body surface
3.3.4.2	Occlusion	Occlusive
3.3.4.3	Vehicle	Administered as supplied at 0, 100, 300 and 1000 mg/kg bw/day moistened with sufficient distilled water
3.3.4.4	Concentration in vehicle	Not applicable
3.3.4.5	Total volume applied	0.05 mL
3.3.4.6	Duration of exposure	6 hours
3.3.4.7	Removal of test substance	Wrappings were removed and the residual test article was wiped
3.3.4.8	Controls	Deionised water

Section A6 Toxicological and Metabolic Studies

Subsection A6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 SUBCHRONIC DERMAL TOXICITY TEST IN RATS (90 DAYS)

3.4	**Examinations**	
3.4.1	Observations	The sites were scored for dermal irritation prior to the study and once per week
3.4.1.1	Clinical signs	Yes, once daily for toxicity and pharmacological effects and once per week a detailed clinical observation was made including changes in skin, fur, eyes, mucous membranes, occurrence of secretions or excretions and autonomic activities. Additionally, changes in level of activity, gait, posture, strength, response to handling, the presence of tonic or clonic movements, stereotypical behaviour and bizarre behaviour were noted.
3.4.1.2	Mortality	Yes, twice daily
3.4.2	Body weight	Yes, at 0 (prior to study initiation), weekly and at termination
3.4.3	Food consumption	Yes, was calculated weekly
3.4.4	Water consumption	Not specified
3.4.5	Ophthalmoscopic examination	Yes, prior to start and within one week of termination
3.4.6	Haematology	Yes Number of animals: all animals Time points: after anesthetization with ether at the time of sacrifice. Parameters: erythrocyte count (RBC), haemoglobin concentration (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), total and differential leukocyte count (WBC), platelet counts (PLT) and prothrombin time (PT).
3.4.7	Clinical chemistry	Yes Number of animals: all animals Time points: after anesthetization with ether at the time of sacrifice. Parameters: alanine aminotransferase (ALT), albumin (Alb.), alkaline phosphatase (ALKP), aspartate aminotransferase (AST), calcium (Ca^{++}), chloride (Cl^{-}), creatinine (Creat.), gamma glutamyl transpeptidase (GGT), glucose (fasting), phosphorous (Phos.), potassium (K^{+}), sodium (Na^{+}), total bilirubin (T. Bil.), total cholesterol (Chol.), total protein (Tot. Prot.), blood urea nitrogen (BUN), magnesium (Mg^{++}), sorbitol dehydrogenase, globulin, and triglycerides.
3.4.8	Urinalysis	No

Section A6 Toxicological and Metabolic Studies

Subsection A6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 SUBCHRONIC DERMAL TOXICITY TEST IN RATS (90 DAYS)

3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes All animals Organs: liver, brain, kidneys, spleen, adrenal glands, testes, epididymides, ovaries, uterus, thymus and heart.
3.5.2	Gross and histopathology	Yes All animals Examination of external surfaces of the body, all orifices, the external and cut surfaces of the viscera, the cervical tissues and all organs and their contents. The following tissues and organs from each animal were preserved: salivary glands, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, liver, pancreas, brain, peripheral nerve, spinal cord, eyes, pituitary, adrenals, thyroids, parathyroid, trachea, lung, pharynx, larynx, nose, aorta, heart, bone marrow, spleen, thymus, lymph nodes, kidneys, urinary bladder, prostate, testes, epididymides, seminal vesicle, uterus, ovaries, female mammary glands, all gross legions and masses, skin and ear tag.
3.5.3	Other examinations	All preserved organs and tissues from animals in the control and 1000 mg/kg bw/day groups were examined microscopically.
3.5.4	Statistics	All non-discrete data for clinical chemistry, haematology, organ weights, body weights, body weight gains, organ/body weight ratios and food consumption were tabulated with means and standard deviations and Analysis of Variance (ANOVA) was performed. Parametric data was analysed using ANOVA techniques with the Turkey-Kramer post hoc test. Non-parametric data was analysed using Kruskal-Wallis ANOVA with Dunn's post hoc test. Instat® Version 2.0 software was used for statistical analyses
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Observations**	

Section A6 Toxicological and Metabolic Studies

Subsection A6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 SUBCHRONIC DERMAL TOXICITY TEST IN RATS (90 DAYS)

4.1.1	Clinical signs	Most animals in all groups appeared normal throughout the observation period. 0 mg/kg bw/day group: Some animals had self-inflicted wounds. Instances of dermal irritation around the abdomen were noted on some of the animals. In one male, diarrhoea, lethargy, few faeces, emaciation, sagging eyelids, chromodacryorrhea, piloerection, soiling and wetness of the anogenital area, hunched posture and red staining of the nose/mouth area were noted later during the study. 100 mg/kg bw/day group: One instance of emaciation was noted in one animal for one day. One instance of diarrhoea was noted for one animal for one day. A swollen eye was noted for another animal. A self inflicted wound to the left hip was noted in one animal. Treated skin abnormalities were noted. 300 mg/kg bw/day group: Instances of diarrhoea, soiling of the anogenital area, chromorhinorrea, lethargy and treated skin abnormalities were noted. 1000 mg/kg bw/day group: Instances of diarrhoea, soiling of the anogenital area, sagging eyelids, treated skin abnormalities and a swollen eye were noted in one animal. Later during the study tremors, sagging eyelids, chromodacryorrhea, lethargy, piloerection, flaccid muscle tone, wetness of the anogenital area, red staining of the nose/mouth area and twitching of the front and hind limbs was noted in one male.
4.1.2	Mortality	All animals survived.
4.2	**Body weight gain**	No statistically significant treatment related differences in mean body weight or body weight gain at any dose level. Please refer to Table A6.4.2-1.
4.3	**Food consumption and compound intake**	No treatment related changes in food consumption.
4.4	**Ophtalmoscopic examination**	No evidence of treatment related ocular diseases in any animal.
4.5	**Blood analysis**	

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Annex Point IIA VI.6.4.2 SUBCHRONIC DERMAL TOXICITY TEST IN RATS (90 DAYS)

4.5.1	Haematology	Males: No statistically significant treatment related differences were found in any of the treated groups. Females: The mean white blood cell count was significantly higher in the 100 mg/kg bw/day group. No other significant differences were found in the treated groups.
4.5.2	Clinical chemistry	Males: No statistically significant treatment related differences were found in any of the treated groups. Females: The mean triglycerides were significantly greater in the 300 mg/kg bw/day group when compared to the control. In the 1000 mg/kg bw/day group, the mean albumin was significantly less than the control and the mean total protein was significantly less when compared to the control and the 100 mg/kg bw/day groups. Please refer to Table A6.4.2-2.
4.5.3	Urinalysis	Not applicable
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Males: In the 1000 mg/kg bw/day group, the mean liver/body ratio was significantly larger than the mean of the control. No other statistically significant treatment related differences were found. Females: No statistically significant treatment related differences were found. Please refer to Table A6.4.2-3.
4.6.2	Gross and histopathology	In the control group, most animals appeared normal at necropsy. Skin lesions were noted on the right hind leg of one female and a flaccid and smaller that normal right testis and epididymides were noted in one male. The male that showed physical signs during the in-life phase revealed abnormalities of the gastrointestinal tract, pancreas, adrenals

and thymus and soiling of the anogenital area and emaciation. A dark area was noted on the kidney of one female.

100 mg/kg bw/day group:

Abnormalities of the treated skin, flaking and eschar were noted in most animals. Kidney, spleen and urinary bladder abnormalities were noted in one male and a herniated liver was noted in another male.

300 mg/kg bw/day group:

Abnormalities of the treated skin, flaking and eschar were noted in most animals. A liver abnormality was noted in one animal and fluid filled uteri was noted in two animals, one of which also had a smaller than normal right adrenal.

1000 mg/kg bw/day group:

Abnormalities of the treated skin, flaking and eschar were noted in most animals. For two or fewer animals, chromodacryorrhea, stomach lesions, herniated liver, fluid-filled uterus and kidney abnormalities were noted.

Compound related microscopic changes were seen in the treated skin of male and female rats of all compound treated groups, most commonly thickening of the epidermis, sebaceous gland hyperplasia, dermal fibrosis, dermal inflammation, necrosis of the superficial epidermis. In general these occurred in a dose-related manner.

Several of the control rats had minimal or mild hyperplasia, hyperkeratosis of the epidermis and minimal sebaceous gland hyperplasia. This is likely to be the result of the repeated shaving and sham application procedure.

On untreated sites of skin in a few of the treated rats there was epidermal hyperplasia, hyperkeratosis, sebaceous gland hyperplasia and dermal inflammation. This may have been due to wrapping procedures or migration of the test substance onto the adjacent skin.

Microscopic examination of the stomach revealed thickening of the nonglandular mucosa due to hyperplasia and hyperkeratosis. Male rats in the 300 and 1000 mg/kg bw/day group and females in the 1000 mg/kg bw/day group had varying incidences of erosions of the glandular or nonglandular mucosa. The males in the 1000 mg/kg bw/day group had submucosal oedema and inflammation in the glandular and nonglandular areas and ulcers of the nonglandular mucosa. These changes are considered to be the result of local superficial irritation of the gastric mucosa and not a systemic effect, therefore, may be a result of ingestion.

Two neoplasms were observed and were considered to be spontaneous and not treatment related.

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Subsection A6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 SUBCHRONIC DERMAL TOXICITY TEST IN RATS (90 DAYS)

4.7	**Other**	There was no erythema or oedema noted on any sham-treated site during the observation period in the control group. Dermal responses were similar in intensity in all treated groups with evidence of eschar noted in at least some animals. Instances of poor hair re-growth and shiny areas of skin, indicative of injuries in depth were noted in some animals in each group.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The subchronic dermal toxicity of [redacted] was investigated by topically dosing 4 groups of 10 Wistar albino rats/sex/group at the concentrations of 0, 100, 300 and 1000 mg/kg bw/day for 90 days. The test was conducted according to US EPA OPPTS 870.3250 which is equivalent to OECD Guideline 411 and is described under point 3 with no deviations.
5.2	**Results and discussion**	Most animals in all groups appeared normal throughout the observation period. The clinical observations noted in some animals were self-inflicted wounds, diarrhoea, lethargy, sagging eyelids, chromodacryorrhea, soiling of the anogenital area and treated skin abnormalities. All animals survived. No treatment related statistical differences were observed in the mean body weights or body weight gains. Please refer to Table A6.4.2-1. No treatment related changes in food consumption. No evidence of treatment related ocular diseases in any animal. There were no statistically significant differences in mean clinical chemistry parameters in males. The mean white blood cell count in females of the 100 mg/kg bw/day group was significantly higher than the mean of females in the control group. There were no statistically significant differences in mean clinical parameters in males. The mean triglycerides in the 300 mg/kg group on females were significantly greater that the mean in the control group. In the 1000 mg/kg bw/day female group, the mean albumin was significantly less than the control and the mean total protein was significantly less that the mean of the control and the mean of the 100 mg/kg bw/day group. Please refer to Table A6.4.2-2. There were no statistically significant differences in mean organ weights between groups with the exception of the mean liver/body ratio of the 1000 mg/kg group of males which was significantly larger than the mean of the control. Please refer to Table A6.4.2-3. In the control group, most animals appeared normal at necropsy. Skin lesions were noted on the right hind leg of one female and a flaccid and smaller that normal right testis and epididymides were noted in

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.4.2	**Repeated dose toxicity**
Annex Point IIA VI.6.4.2	**SUBCHRONIC DERMAL TOXICITY TEST IN RATS (90 DAYS)**

		one male. The male that showed physical signs during the in-life phase revealed abnormalities of the gastrointestinal tract, pancreas, adrenals and thymus and soiling of the anogenital area and emaciation. A dark area was noted on the kidney of one female. Abnormalities of the treated skin, flaking and eschar were noted in most animals in the 100, 300 and 1000 mg/kg bw/day groups. Compound related microscopic changes were seen in the treated skin of male and female rats of all compound treated groups, most commonly thickening of the epidermis, sebaceous gland hyperplasia, dermal fibrosis, dermal inflammation, necrosis of the superficial epidermis. In general these occurred in a dose-related manner. Some control rats showed hyperplasia, hyperkeratosis of the epidermis and minimal sebaceous gland hyperplasia which were considered to be due to the repeated shaving and sham application procedure. On untreated sites in some treated rats, epidermal hyperplasia, hyperkeratosis, sebaceous gland hyperplasia and dermal inflammation were observed and considered to be due to wrapping procedures or migration of the test substance. Microscopic examination of the stomach revealed thickening of the nonglandular mucosa due to hyperplasia and hyperkeratosis. These changes were considered to be the result of local superficial irritation of the gastric mucosa and not a systemic effect. Two neoplasms were observed and considered to be spontaneous and not treatment related.
5.3	**Conclusion**	Macroscopic and microscopic skin abnormalities are considered to be due to local irritation and not due to a systemic effect. Based on the significant differences noted in kidney and liver clinical chemistry (triglycerides, albumin and protein) and the lack of relevant microscopic changes between the animals dosed with 1000 mg/kg bw/day and the controls, the No Observed Adverse Effect Level (NOAEL) was considered to be 300 mg/kg bw/day.
5.3.1	LO(A)EL	1000 mg/kg bw/day
5.3.2	NO(A)EL	300 mg/kg bw/day
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Section A6 Toxicological and Metabolic Studies

Subsection A6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 **SUBCHRONIC DERMAL TOXICITY TEST IN RATS (90 DAYS)**

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2008*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted.*
Conclusion	*LO(A)EL: males: 1000 mg BIT/kg bw/day;* *NO(A)EL: males: 300 mg BIT/kg bw/day;* *Other conclusions: Other applicant's conclusions are adopted.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.4.2-1: Summary of mean body weights in males and females in a 90-day dermal study with 1,2-benzisothiazolin-3-one

Day of Study	Male				Female			
	0 mg/kg bw/day Weight (g) ± SD	100 mg/kg bw/day Weight (g) ± SD	300 mg/kg bw/day Weight (g) ± SD	1000 mg/kg bw/day Weight (g) ± SD	0 mg/kg bw/day Weight (g) ± SD	100 mg/kg bw/day Weight (g) ± SD	300 mg/kg bw/day Weight (g) ± SD	1000 mg/kg bw/day Weight (g) ± SD
1	253 ± 11.0	246 ± 11.3	247 ± 16.3	245 ± 15.2	177 ± 8.0	177 ± 8.0	178 ± 8.7	179 ± 11.8
8	294 ± 15.1	291 ± 10.4	294 ± 15.7	283 ± 16.3	194 ± 8.3	191 ± 9.7	194 ± 11.8	197 ± 8.2
15	335 ± 20.8	329 ± 14.5	329 ± 22.1	322 ± 25.4	209 ± 7.2	209 ± 11.9	212 ± 13.1	215 ± 11.3
22	368 ± 23.9	359 ± 21.3	360 ± 25.1	349 ± 25.8	222 ± 11.8	222 ± 16.1	219 ± 19.8	229 ± 14.5
29	388 ± 28.4	377 ± 24.1	377 ± 24.6	372 ± 30.8	230 ± 12.7	235 ± 17.7	228 ± 18.2	238 ± 19.7
36	403 ± 32.4	388 ± 27.0	395 ± 24.3	391 ± 38.6	235 ± 15.5	239 ± 19.0	235 ± 17.2	252 ± 25.8
43	411 ± 36.8	394 ± 38.8	401 ± 25.3	403 ± 39.0	240 ± 17.1	242 ± 18.3	242 ± 15.8	254 ± 18.6
50	426 ± 37.3	415 ± 29.2	417 ± 29.0	417 ± 41.1	247 ± 14.7	248 ± 20.7	245 ± 18.0	263 ± 19.5
57	437 ± 41.4	426 ± 31.7	421 ± 28.6	431 ± 42.0	255 ± 16.2	254 ± 23.2	252 ± 18.4	271 ± 23.0
64	441 ± 36.7	432 ± 30.5	436 ± 30.0	441 ± 44.6	259 ± 18.0	263 ± 24.8	258 ± 18.5	277 ± 25.1
71	457 ± 43.0	444 ± 38.8	450 ± 30.4	449 ± 48.4	262 ± 18.0	265 ± 24.7	264 ± 17.2	278 ± 21.7
78	464 ± 46.5	453 ± 35.2	458 ± 30.4	458 ± 49.8	266 ± 17.6	267 ± 25.1	272 ± 28.7	282 ± 22.1
85	459 ± 73.9	457 ± 34.5	465 ± 28.4	463 ± 48.7	271 ± 15.0	272 ± 24.7	272 ± 25.6	287 ± 25.6
91	438 ± 68.8	434 ± 33.2	441 ± 27.7	437 ± 51.1	271 ± 15.0	272 ± 24.7	272 ± 25.6	287 ± 25.6

Term	463 ± 71.0	460 ± 37.0	469 ± 28.3	466 ± 55.6	274 ± 18.6	271 ± 26.6	272 ± 24.2	297 ± 40.0

Table A6.4.2-2: Clinical chemistry parameters in male and female rats in a 90-day dermal study with 1,2-benzisothiazolin-3-(2*H*)-one

Sex	Dose mg/kg bw/day	Number of animals	Parameter mean ± SD		
			Triglycerides (mg/dL)	Albumin (g/dL)	Total protein (g/dL)
Male	0	10	36 ± 12	4.0 ± 0.2	5.9 ± 0.3
	100	10	47 ± 15	4.1 ± 0.2	6.1 ± 0.2
	300	10	38 ± 10	4.1 ± 0.2	6.1 ± 0.1
	1000	10	42 ± 14	4.0 ± 0.2	6.0 ± 0.2
Female	0	10	24 ± 3	4.2 ± 0.2	6.1 ± 0.2
	100	10	25 ± 4	4.3 ± 0.2	6.0 ± 0.2
	300	10	30** ± 7	4.2 ± 0.2	5.9 ± 0.3
	1000	10	28 ± 4	3.9* ± 0.2	5.7* ± 0.3

* Statistically significantly ($p \leq 0.05$) less than controls
**Statistically significantly ($p \leq 0.05$) greater than controls

Table A6.4.2-3: Summary of terminal liver weights (g) and liver/body weight ratios in a 90-day dermal study with 1,2-benzisothiazolin-3-(2*H*)-one

Data collection	Sex	Dose mg/kg bw/day	Liver weight (g)	Liver/ body weight
Terminal	Male	0	11.52 ± 1.82	2.5 ± 0.24
		100	12.02 ± 1.17	2.616 ± 0.174
		300	12.71 ± 1.23	2.704 ± 0.141
		1000	12.87 ± 1.84	2.76** ± 0.165
Terminal	Female	0	7.01 ± 0.86	2.556 ± 0.22
		100	6.94 ± 0.78	2.562 ± 0.16
		300	7.10 ± 0.62	2.614 ± 0.089
		1000	7.66 ± 0.85	2.612 ± 0.342

** Statistically significantly ($p \leq 0.05$) greater than controls

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.4.3	**Subchronic toxicity test**	
Annex Point IIA VI.6.4.3	**SUBCHRONIC REPEATED INHALATION DOSE TOXICITY**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	1,2-benzisothiazol-3(2*H*)-one (BIT) has a vapour pressure of 3.02 x 10^{-3} Pa at 20 ºC ([redacted], 2003) IIIA 3.2. Therefore, it is not considered to be volatile and significant levels in the air are unlikely. Furthermore, the biocidal product contains a very low level of BIT (up to 0.05% w/w). For most of the use patterns of BIT, the inhalation route is not expected to be the major route of exposure. Therefore, conducting a 90-day inhalation study which would not supply additional toxicological knowledge on BIT is not deemed to be required. In conclusion, there are no ethical grounds (that would not contravene the requirements of Directive 86/609/EC which advises against unnecessary testing using animals) for performing further studies on animals. It is therefore proposed that no additional investigations are required to address this point.	
Undertaking of intended data submission [ ]	Not applicable	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*September 2008*
Evaluation of applicant's justification	*Arguments are reasonable.*
Conclusion	*Applicant is exempted of the subchronic repeated dermal dose toxicity study.*
Remarks	

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.5	**Chronic Toxicity**	
Annex Point IIA VI.6.5		
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	In order to avoid unnecessary vertebrate testing, Troy Chemical Company BV propose to waive chronic toxicity studies, based on the following arguments: In a 90-day oral study in the rat summarised under point IIIA, 6.4.1/1, 1,2-benzisothiazol-3(2H)-one (BIT) was administered by gavage to 10 rats/sex/group at 0, 10, 27.5 and 75 mg/kg bw/day for 90 days. In addition, a high dose recovery (75 mg/kg bw/day) and a control recovery group (0 mg/kg bw/day) were included in the study and observed for a further period of 28 days to investigate the persistence, recovery or delayed effect of the test substance, if any. No treatment related mortalities and clinical signs were recorded. A slight reduction in body weight was noted at 75 mg/kg bw/day in males and a statistically significant decrease was reported in the high dose recovery male group (75 mg/kg bw/day). No changes in body weight were noted in females. The only change in feed consumption was observed in the high dose recovery male group during 1st, 2nd, 6th, 7th and 13th weeks of treatment period. No treatment related changes in haematological parameters were reported with the exception of a statistically significant decrease in RBC (5.5%) count. The only change in clinical chemistry parameters was an increase in cholesterol levels (32%) at 75 mg/kg bw/day in males. No treatment related changes were observed in absolute and relative organ weights in any of the organs in any animals. No treatment related findings were noted at gross pathology and histopathology. Based on the above results, an NOAEL of 27.5 mg/kg bw/day was established. In an oral 90-day study in the dog summarised under point IIIA, 6.4.1/2, 1,2-benzisothiazol-3(2H)-one (BIT) was administered in the diet to 4 beagle dogs/sex/group at 300, 1000 and 3000 ppm (which is equivalent to 11, 37 and 106 mg/kg bw/day in male and 11, 38 and 89 mg/kg bw/day in females) for a minimum of 90 days. All animals survived throughout the study. There were no treatment related clinical observations or changes in haematology or urinalysis parameters or organ weights. There were no ophthalmic, macroscopic or microscopic findings. The only changes noted were lower body weights at 3000 ppm in males and females and lower food consumption at 3000 ppm in females when compared to control animals. Based on the above, an NOAEL of 1000 ppm (which is equivalent to 37 mg/kg bw/day in males and 38 mg/kg bw/day in females) was established. It is concluded that these studies do not show any particular substance related organ toxicity that would require further investigation.	

A 90-day dermal study was also carried out in rats which is summarised under point IIIA, 6.4.2. BIT was applied dermally to the intact skin of Wistar albino rats (10 rats/sex/group) at 100, 300 and 1000 mg/kg bw/day. All animals survived and a few abnormal systemic signs were reported. Treated skin abnormalities were noted in some animals in each treated group. No treatment related changes in bodyweights were recorded. The only change in haematological parameters was the mean white blood cell count of females at 100 mg/kg bw/day. Mean triglycerides at 300 mg/kg bw/day, albumin and protein levels at 1000 mg/kg bw/day in females were statistically significant from the control. The mean liver/body weight at 1000 mg/kg bw/day in males was significantly greater than the control males. No other absolute or relative organ weight change was noted. At necropsy, a few abnormalities such as skin lesions were noted in the control group. At necropsy, abnormalities of the treated skin, flaking and eschar were reported in most animals in all treated groups. At histopathology, microscopic changes in the treated skin of male and female rats were recorded. No other systemic effect of the test substance in any other tissue examined. Based on the significant differences noted in kidney and liver clinical chemistry (triglycerides, albumin and protein) and the lack of relevant microscopic changes between the animals dosed with 1000 mg/kg bw/day and the controls, the No Observed Adverse Effect Level (NOAEL) was considered to be 300 mg/kg bw/day.

BIT is manufactured outside the EU and is used as a preservative. The product [redacted] and the product type PT13 “Metalworking fluids” are intended to be supported by the dossier. Preservatives are added to metalworking fluids to preserve them in their action of cooling, lubricating and carrying cuttings from mechanical cutting operations. Metal is shaped by moving past a cutting tool, or the cutting tool moves around or past metal. Metalworking fluid is supplied at the cutting tool for cooling, lubrication and swarf removal. The end-use products containing BIT are intended for professionals. There are three different population groups that may be exposed to BIT: industrial and professional users and the general public via indirect exposure as a result of use.

Preservative [redacted] containing 20% w/w BIT is incorporated by industrial users into the different products by simple dilution into them. Industrial users manufacture metalworking fluid concentrates adding the preservative [redacted] to the preparation to obtain a concentration up to 0.4% w/w BIT. Professionals dilute the metalworking fluid concentrate to obtain the metalworking fluid emulsion containing up to 0.02% w/w of the preservative BIT and use the metalworking emulsion in different metalworking activities.

A study on dermal absorption has been summarised under point IIIA, 6.2/2. The dermal absorption value was established to be approximately 28.86% for the high dose and can therefore be rounded to 30%.

The NOAEL to be compared with the estimated exposures must be the lowest NOAEL value obtained in the most sensitive species. Therefore, the most appropriate NOAEL is obtained from a teratogenicity study in the rabbit which was summarised under point IIIA, 6.8.1/1. The NOAEL for maternal toxicity was determined to be 6 mg/kg bw/day.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.5	**Chronic Toxicity**
Annex Point IIA VI.6.5	
	The exposure to BIT was calculated based in the selected models and default values from the User Guidance (2002). Metalworking fluids is the only use which is taken into account for PT13. The primary exposures have been calculated for industrial users when they dilute the product [redacted] to manufacture the end-use products and for professionals when diluting and using the metalworking fluids containing BIT. The secondary exposures for adults, children and infants have been calculated for the post-application period of the end-use products. For metalworking fluids, the estimated primary exposures when taking into account industrial and professional users range from 0.0001 to 0.0443 with margins of safety (MOS) ranging from 60000 to 135 indicating an acceptable risk. The estimated secondary exposures for adults, children and infants range from 0.0005 to 0.0014 (MOS ranging from 12000 to 4286 indicating an acceptable risk). These values have been obtained taking into account the worst-case scenario. However, they are well above the MOS of 1000. It is important to outline that the total exposure when taking into account the equilibrium vapour concentration of BIT is much lower than the estimated total exposure when taking into account the default values stated in the guidelines and therefore, the MOS are higher. It is concluded that the risk is acceptable for both primary and secondary exposures when taking into account the end-use products containing BIT. Based on the low human exposure expected from use of the product and the absence of related organ toxicity in any of the 90 day studies, the requirement to investigate the chronic of BIT is further negated. In conclusion, there are no ethical grounds (that would not contravene the requirements of Directive 86/609/EC which advises against unnecessary testing using animals) for performing further studies on animals. It is therefore proposed that no additional investigations are required to address this point.
Undertaking of intended data submission [ ]	Not applicable

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*September 2008*
Evaluation of applicant's justification	*Applicant's justification is accepted.*

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.5	**Chronic Toxicity**
Annex Point IIA VI.6.5	
Conclusion	*Applicant is exempted of the chronic toxicity study.*
Remarks	

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.1/1 *In vitro* gene mutation study in bacteria

Annex Point IIA VI.6.6.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: September 18 – October 26, 2001	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Company with letter of access	DOW	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD guideline 471	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, the following deviation was noted: The number of cells per culture was not specified at the beginning of the study. This deviation is minor and is not considered to compromise the scientific validity of the study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-(2*H*)-one	
3.2.1	Lot/Batch number	BT 12000	
3.2.2	Specification	Please refer to Doc. III-A 2/2	
3.4.1.1	Description	Brown solid	
3.4.1.2	Purity	98%	
3.4.1.3	Stability	Not documented (expiry date September 5, 2002)	
3.2	**Study Type**	Bacterial reverse mutation test	

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.1/1 *In vitro* gene mutation study in bacteria

Annex Point IIA VI.6.6.1

3.2.1	Organism/cell type	*Salmonella. typhimurium:* TA 1535, TA 1537, TA 98 and TA 100 Provided by Dr. B. N. Ames, University of California Berkley, USA *Escherichia coli*: WP2 *uvrA* Provided by Dr. C. Voogd, National of Public Health and Environmental Protection, Bilthoven, the Netherlands
3.2.2	Deficiencies / Proficiencies	TA 1535 was histidine deficient in G46. TA 1537 was histidine deficient on C3076. TA 98 was histidine deficient on D3052. TA 100 was histidine deficient on G46.

Strain	Amino Acid mutation	LPS mutation	UV-repair mutation	R-factor mutation
TA 1535	His G46	rfa^-	$uvrB^-$	-R
TA 1537	His C3076	rfa^-	$uvrB^-$	-R
TA 98	His D3052	rfa^-	$uvrB^-$	+R
TA 100	His G46	rfa^-	$uvrB^-$	+R
WP2 *uvrA*	Trp	rfa^+	$uvrA^-$	-R

Rfa: the mutation causes partial loss of the lipopolysaccharide (LPS) barrier that coats the surface of the bacteria; it increases the permeability to large molecules, e.g. crystal violet

uvrB/A: these mutations comprise a deletion of a gene coding for the DNA excision repair system, which result in greatly increased sensitivity in detecting many mutagens including UV radiation

R-factor: the R-factor strains contain the plasmid pKM 101, which increases chemical and spontaneous mutagenesis by enhancing an error-prone DNA-repair system normally present in *S. typhimurium*. It carries an ampicillin resistance gene.

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.1/1 *In vitro* gene mutation study in bacteria

Annex Point IIA VI.6.6.1

3.2.3 Metabolic activation system

S9 mix

Male Wistar rats were injected intraperitonally with a single dose (500 mg/kg bw) of Aroclor 1254. Five days later, the rats were sacrificed, their livers removed and a microsomal enzyme fraction was prepared. The contents of the S9 fraction were determined as follows:

Protein	31.0 g/L
Cytochrome P-450	21.7 µmol/L

The sterility check resulted in 2 colonies per 100 µL of S9.

The components of the S9 mix were as follows:

Components	Concentration
$MgCl_2$	8 mM
KCl	33 mM
G-6-P	5 mM
NADP	4 mM
Sodium phosphate (pH 7.4)	100 mM
NaCl	46 mM
S9	10%

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.1/1 *In vitro* gene mutation study in bacteria

Annex Point IIA VI.6.6.1

3.2.4 Positive control

Strain	S9 mix	Positive controls	µg/plate
TA1535	-	Sodium azide	1.0
TA1535	+	2-aminoanthracene	2.0
TA1537	-	9-aminoacridine	80
TA1537	+	Benzo(a)pyrene	4.0
TA98	-	2-nitrofluorene	2.0
TA98	+	2-aminoanthracene	2.0
TA100	-	Sodium azide	1.0
TA100	+	2-aminoanthracene	2.0
WP2 uvrA	-	*N*-ethyl-*N*-nitrosourea	100
WP2 uvrA	+	2-aminoanthracene	80

Vehicle controls (DMSO) were plated for all tester strains with and without microsomal enzymes.

3.3 Application of test substance

3.3.1 Concentrations

First assay: 0, 62, 185, 556, 1677 and 5000 µg/plate

Second assay: 0, 2.47, 7.41, 22.2, 66.7 and 200 µg/plate

The test substance was toxic to all strains in the first assay. This was evidenced by a decrease in the mean number of revertant colonies. Therefore, a second assay was carried o0ut at lower concentrations.

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.1/1 *In vitro* gene mutation study in bacteria

Annex Point IIA VI.6.6.1

3.3.2	Way of application	The test substance was serially diluted immediately prior to its use (vehicle: DMSO). To 2 mL molten top agar, maintained at 46ºC, 0.1 mL of a fully grown culture, 0.1 mL of the test substance solution or of the negative or positive control solution, and 0.5 mL S9- mix (with metabolic activation) or 0.5 mL sodium phosphate 100 mM (without metabolic activation) were added. The ingredients were thoroughly mixed and the mix was poured onto minimal glucose agar plates in triplicate. The plates were incubated at 37ºC for three days. The his^+ and trp^+ revertants were counted and the background lawn of bacterial growth was examined microscopically to determine any growth inhibiting or growth enhancing effects (a 2-fold or greater increase in the mean number of his^+ or trp^+ revertants)
3.3.3	Pre-incubation time	None
3.3.4	Other modifications	None
3.4	**Examinations**	Not documented
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No, please refer to Tables A6.6.1/1-1, A6.6.1/1-2, A6.6.1/1-3 and A6.6.1/1-4
4.1.2	with metabolic activation	No, please refer to Tables A6.6.1/1-1, A6.6.1/1-2, A6.6.1/1-3 and A6.6.1/1-4
4.2	**Cytotoxicity**	No
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	1,2-Benzisothiazolin-3-one was tested *in-vitro* for gene mutation using the following bacterial strains; TA 1535, TA 1537, TA 98, TA 100 and WP2 *uvrA*. The assay was carried out in the presence and in the absence of an S9 activation system. This study was conducted according to OECD guideline 471 and is described under point 3. The following deviation was noted: The number of cells per culture is not specified at the beginning of the study. However, this deviation is minor and is not considered to compromise the scientific validity of the study.

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.1/1 *In vitro* gene mutation study in bacteria

Annex Point IIA VI.6.6.1

5.2	**Results and discussion**	No positive responses were observed with any of the tester strains in the presence or absence of metabolic activation. Please refer to Tables A6.6.1/1-1, A6.6.1/1-2, A6.6.1/1-3 and A6.6.1/1-4
5.3	**Conclusion**	1,2-Benzisothiazolin-3-one is not mutagenic under the conditions employed in this study.
5.3.1	Reliability	1
5.3.2	Deficiencies	One deviation was noted and is outlined under point 2.3 and 5.1. However, it does not compromise the scientific validity of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted.*
Conclusion	*Applicant's version is adopted.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.6.1/1-1: Mutagenicity assay in the presence of metabolic activation (first assay)

Average Revertants Per Plate ± Standard Deviation					
Liver Microsomes: Rat liver S9					
Dose (µg/plate)	**TA1535**	**TA1537**	**TA98**	**TA100**	**WP2 *uvrA***
0.0	14 ± 2	12 ± 1	40 ± 5	114 ± 9	24 ± 1
62	13 ± 2	13 ± 2	49 ± 8	124 ± 5	20 ± 8
185	2 ± 3	8 ± 3	22 ± 7	45 ± 21	27 ± 4
556	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1667	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Positive control	389 ± 31	258 ± 15	870 ± 184	1395 ± 69	674 ± 37

Table A6.6.1/1-2: Mutagenicity assay in the absence of metabolic activation (first assay)

Average Revertants Per Plate ± Standard Deviation					
Liver Microsomes: None					
Dose (µg/plate)	**TA1535**	**TA1537**	**TA98**	**TA100**	**WP2 *uvrA***
0.0	19 ± 3	10 ± 3	29 ± 6	118 ± 4	25 ± 2
62	15 ± 1	12 ± 4	26 ± 2	130 ± 19	23 ± 1
185	0 ± 1	0 ± 0	0 ± 0	0 ± 0	3 ± 4
556	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1667	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Positive control	432 ± 3	1082 ± 50	1411 ± 63	440 ± 33	165 ± 11

Table A6.6.1/1-3: Mutagenicity assay in the presence of metabolic activation (second assay)

Average Revertants Per Plate ± Standard Deviation					
Liver Microsomes: Rat liver S9					
Dose (µg/plate)	**TA1535**	**TA1537**	**TA98**	**TA100**	**WP2 *uvrA***
0.0	15 ± 3	18 ± 2	39 ± 2	146 ± 13	33 ± 7
2.47	16 ± 1	15 ± 6	34 ± 4	145 ± 7	22 ± 3
7.41	16 ± 2	18 ± 1	35 ± 9	158 ± 15	29 ± 3
22.22	11 ± 3	17 ± 3	38 ± 9	151 ± 9	28 ± 7
66.67	11 ± 4	14 ± 8	41 ± 12	138 ± 4	29 ± 9
200	1 ± 1	1 ± 1	0 ± 1	0 ± 0	32 ± 11
Positive control	340 ± 19	339 ± 27	1106 ± 97	1368 ± 19	694 ± 92

Table A6.6.1/1-4: Mutagenicity assay in the absence of metabolic activation (second assay)

Average Revertants Per Plate ± Standard Deviation					
Liver Microsomes: None					
Dose (µg/plate)	**TA1535**	**TA1537**	**TA98**	**TA100**	**WP2 *uvrA***
0.0	18 ± 3	10 ± 4	27 ± 7	140 ± 16	31 ± 6
2.47	17 ± 5	8 ± 4	23 ± 6	156 ± 15	22 ± 5
7.41	18 ± 5	7 ± 2	31 ± 6	126 ± 7	30 ± 5
22.22	16 ± 3	8 ± 3	25 ± 1	143 ± 17	27 ± 3
66.67	6 ± 5	7 ± 2	25 ± 2	218 ± 33	25 ± 6
200	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1
Positive control	358 ± 14	613 ± 181	1223 ± 17	445 ± 5	161 ± 23

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.1/2 *In vitro* gene mutation study in bacteria

Annex Point IIA VI.6.6.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: July 2 – July 5, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Company with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD guideline 471.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, the following deviation was noted: Duplicate rather than triplicate plates were assessed without justification This deviation is minor and is not considered to compromise the scientific validity of the study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazol-3-(2*H*)-one (BIT)	
3.2.1	Lot/Batch number	BT 17301	
3.2.2	Specification	Please refer to Doc. III-A 2/1	
3.4.1.1	Description	Beige to light brown coloured powder	
3.4.1.2	Purity	97.42% (dry basis)	
3.4.1.3	Stability	The test substance was stored in the original container at room temperature (expiry date March 16, 2005)	
3.2	**Study Type**	Bacterial reverse mutation test	

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.1/2 *In vitro* gene mutation study in bacteria

Annex Point IIA VI.6.6.1

3.2.1 Organism/cell type

Salmonella typhimurium: TA 1537, TA 1535, TA 98, TA 100 and TA 102

Provided by Bruce Ames Laboratory, Molecular and Cell Biology, University of California, 401, Barker Hall, Berkeley, CA 94720 – 3202, USA

3.2.2 Deficiencies / Proficiencies

TA 1537 was histidine deficient in C3076. TA 1557 was histidine deficient on C46. TA 98 was histidine deficient on D3052. TA 100 was histidine deficient on G46. TA 102 was histidine deficient in G428.

Strain	His$^-$ mutation locus	LPS or rfa mutation	uvrB	R-factor plasmid pKM101	R-factor plasmid pAQ1
TA 1537	C3076	Yes	Yes	No	No
TA 1535	C46	Yes	Yes	No	No
TA 98	D3052	Yes	Yes	Yes	No
TA 100	G46	Yes	Yes	Yes	No
TA 102	G428	Yes	No	Yes	Yes

Rfa: the mutation creates a partial loss of the lipopolysaccharide (LPS) barrier. This deficiency increases the permeability of the cells to higher molecular weight compounds.

uvrB: increases the susceptibility to several classes of mutagens by decreasing their ability for DNA excision repair. It also causes deletion of the biotin gene.

R- factor: the R-factor plasmid , pKM101, increases the sensitivity by enhancing error-prone repair and confirms ampicillin resistance. The R-factor plasmid, pAQ1 confirms tetracycline resistance.

3.2.3 Metabolic activation system

S9 fraction and S9 mix

S9 fraction was provided by the Division of Microbiology, Defence Research and Development Establishment, Gwalior, India. S9 mix

was prepared by buffering and supplementing the S9 fraction with the essential co-factors NADP and Glucose-6-phosphate.

0.1 mL of S9 mix was transferred aseptically to 2mL of top agar tube, mixed thoroughly and this mixture was poured on to minimal glucose agar plates.

The components of the S9 mix were as follows:

Components	Volume in 10 mL o S9 mix (mL)
Sterile distilled water	3.8
Phosphate buffer (pH 7.4;0.2M)	5.0
Salt solution (0.4M MgCl2 + 1.65M KCl)	0.2
Glucose-6-phosphate (1M)	0.1
NADP (0.1M)	0.4
S9 fraction	0.5

S9 mix was prepared on the day of use and placed on an ice bath.

3.2.4 Positive control

Strain	S9 mix	Positive controls	µg/plate
TA1537	-	9-aminoacridine	50
TA1537	+	2-aminofluorene	20
TA1537	-	2-aminofluorene	20
TA1535	-	Sodium azide	0.5
TA1535	+	2-aminofluorene	20
TA1535	-	2-aminofluorene	20
TA98	-	2-nitrofluorene	5
TA98	+	2-aminofluorene	20
TA98	-	2-aminofluorene	20

Section A6 **Toxicological and Metabolic Studies**

Subsection A6.6.1/2 ***In vitro* gene mutation study in bacteria**

Annex Point IIA VI.6.6.1

TA100	-	Sodium azide	5
TA100	+	2-aminofluorene	20
TA100	-	2-aminofluorene	20
TA 102	-	Cumene hydroperoxide	50
TA 102	+	2-aminofluorene	20
TA 102	-	2-aminofluorene	20

Vehicle controls of dimethyl sulphoxide (DMSO) were plated for all tester strains with and without microsomal enzymes.

3.3 Application of test substance

3.3.1 Concentrations

Cytotoxicity test: 0, 39.06, 78.12, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate

Mutagenicity test: 0, 3.125, 6.25, 12.5, 25 and 50 µg/plate

3.3.2 Way of application

Cytotoxicity test: A stock solution of test substance was prepared in DMSO. This was further diluted using DMSO to the desired concentrations.

A quantity of 2 mL of top agar was added to two sets of sterile test tubes. Then 500 µL of 5% v/v S9 mix was added to one of the sets and 500 µL of 0.2 M phosphate buffer was added to the second set. 100 µL of appropriately diluted test substance or solvent control was then added to the tubes. Finally 100 µL of standard bacterial suspension was added to both tubes and mixed. This top agar was added to minimal glucose agar plates and allowed to solidify. Duplicate sets were used for each concentration.

The petriplates were incubate at 37 ± 1 °C for 48-72 hours and then examined to assess the state of the background bacterial growth. Cytogenicity was assessed by clearance of the background lawn.

Mutagenicity test: A stock solution of test substance was prepared in DMSO. This was further diluted using DMSO to the desired concentrations

A quantity of 2 mL of top agar was added to two sets of sterile test tubes. Then 500 µL of 5% v/v S9 mix was added to one of the sets and 500 µL of 0.2 M phosphate buffer was added to the second set. 100 µL of appropriately diluted test substance or solvent control was then added to the tubes. Finally 100 µL of standard bacterial suspension was added to both tubes and mixed. This top agar was

		added to minimal glucose agar plates and allowed to solidify. Duplicate sets were used for each concentration. Positive controls were also separately included. The petriplates were incubate at 37 ± 1 °C for 48-72 hours and then examined to assess the state of the background bacterial growth. Positive and negative controls were also included.
3.3.3	Pre-incubation time	None
3.3.4	Other modifications	None
3.4	**Examinations**	
3.2.1	Number of cells evaluated	10^8-10^9 cells/mL
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No, please refer to Tables A6.6.1/2-1 and A6.6.1/2-2.
4.1.2	with metabolic activation	No, please refer to Tables A6.6.1/2-1 and A6.6.1/2-2.
4.2	**Cytotoxicity**	Yes, at dose levels of 156.25 to 5000 μg/plate in both the absence and presence of metabolic activation.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	BIT was tested *in vitro* for gene mutation using the following *Salmonella typhimurium* strains: TA 1537, TA 1535, TA 98, TA 100 and TA 102. The assay was carried out in the presence and in the absence of an S9 activation system. This study was conducted according to OECD guideline 471 and is described under point 3. The following deviation was noted: Duplicate rather than triplicate plates were assessed without justification However, this deviation is minor and is not considered to compromise the scientific validity of the study.
5.2	**Results and discussion**	The test substance was found to be cytotoxic at the dose levels of 156.25 to 5000 μg/plate in both the absence and presence of metabolic activation. The revertant frequencies in all five strains exposed to the test substance showed no positive responses in the presence or absence of metabolic activation.

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.1/2 *In vitro* gene mutation study in bacteria

Annex Point IIA VI.6.6.1

		Please refer to Tables A6.6.1/2-1 and A6.6.1/2-2.
5.3	**Conclusion**	BIT is not mutagenic under the conditions employed in this study.
5.3.1	Reliability	1
5.3.2	Deficiencies	One deviation was noted and is outlined under point 2.3 and 5.1. However, it does not compromise the scientific validity of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted.*
Conclusion	*Applicant's version is adopted.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.6.1/2-1: Mutagenicity assay in *Salmonella typhimurium* with BIT in the absence of metabolic activation

Average Revertants Per Plate ± Standard Deviation*					
Liver Microsomes: None					
Dose (µg/plate)	**TA1537**	**TA1535**	**TA98**	**TA100**	**TA 102**
0.0	6.00 ± 2.83	10.00 ± 2.83	19.50 ± 6.36	47.50 ± 3.54	132.50 ± 17.68
3.125	7.00 ± 2.83	9.00 ± 0.00	24.00 ± 5.66	64.00 ± 8.49	89.5 ± 61.52
6.25	6.50 ± 2.12	3.00 ± 0.00	20.00 ± 1.41	63.50 ± 2.12	145.50 ± 14.85
12.5	6.50 ± 2.12	6.50 ± 0.71	15.00 ± 5.66	51.50 ± 2.12	141.00 ± 25.46
25	3.00 ± 0.00	9.50 ± 2.12	20.50 ± 0.71	61.00 ± 1.41	133.50 ± 7.78
50	2.50 ± 0.71	7.00 ± 2.83	28.50 ± 3.54	42.00± 5.66	131.00 ± 22.63
Positive control**	5.50 ± 0.71	13.0 ± 1.41	26.50 ± 0.71	32.00 ± 5.66	108.50 ± 72.83
Positive control***	399.00 ± 140.01	129.0 ± 18.38	492.50 ± 48.79	361.00 ± 5.66	373.0 ± 11.31

* Mean of two replicates
** For all strains positive control is 2-Aminofluorene
***For TA 1537 positive control is 9-Aminoacridine
For TA 1535 positive control is Sodium azide
For TA 98 positive control is 2-Nitrofluorene
For TA 100 positive control is Sodium azide
For TA 102 positive control is Cumene hyperoxide

Table A6.6.1/2-2: Mutagenicity assay in *Salmonella typhimurium* with BIT in the presence of metabolic activation

Average Revertants Per Plate ± Standard Deviation*					
Liver Microsomes: Rat liver S9					
Dose (µg/plate)	**TA1537**	**TA1535**	**TA98**	**TA100**	**TA 102**
0.0	8.00 ± 2.83	15.00 ± 2.83	36.0 ± 0.00	76.50 ± 4.95	108.50 ± 62.93
3.125	7.50 ± 0.71	9.50 ± 2.12	33.0 ± 1.41	79.50 ± 3.54	167.50 ± 3.54
6.25	7.50 ± 0.71	10.00 ± 1.41	30.50 ± 3.54	65.50 ± 7.78	141.00 ± 1.41
12.5	9.50 ± 0.71	16.0 ± 4.24	31.00 ±0.00	76.50 ± 9.19	139.00 ± 36.77
25	2.50 ± 3.54	8.50 ± 3.54	23.50 ± 0.71	74.50 ± 13.44	145.00 ± 1.41
50	3.50 ± 3.54	7.00 ± 2.83	38.50 ± 0.71	69.00 ± 5.66	129.00 ± 43.84
Positive control**	95.0 ± 1.41	92.50 ± 10.61	250.1 ± 181.02	799.50 ± 54.45	458.50 ± 2.12

* Mean of two replicates
** For all strains positive control is 2-Aminofluorene

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.2/1 *In vitro* cytogenicity study in mammalian cells

Annex Point IIA VI.6.6.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: October 9 – October 19, 2001	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access	DOW	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD Guideline 473.	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-one	
3.1.1	Lot/Batch number	BT 12000	
3.1.2	Specification	Please refer to Doc. III-A 2/2	
3.1.2.1	Description	Brown solid	
3.1.2.2	Purity	98%	
3.1.2.3	Stability	Not documented	
3.2	**Study Type**	*In vitro* mammalian chromosome aberration test.	
3.2.1	Organism/cell type	Chinese hamster ovary (CHO-K_1) cells Provided by Dr. A. T. Natarajan, University of Leiden, the Netherlands	

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.2/1 *In vitro* cytogenicity study in mammalian cells

Annex Point IIA VI.6.6.2

3.2.2	Deficiencies / Proficiencies	Not applicable
3.2.3	Metabolic activation system	S9 mix Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. Male Wistar rats received a single intraperitoneal injection of Aroclor 1254, 500 mg/kg in soya bean oil, five days prior to sacrifice. The livers were removed aseptically, placed, washed and stored in 0.15 M KCl solution. The livers were cut, homogenized, and centrifuged. The supernatant, S9, was taken and stored at < -60 ºC until used. The S9 was checked for sterility, which was found to be 2 colonies per 10 µl S9. The contents of the S9 fraction were determined as follows:

Protein	31.0 g/L
Cytochrome P-450	21.7 µmol/L

Prior to use, the S9 was thawed and mixed with a NADPH-generating system.

The components of the S9 mix were as follows:

Components	Concentration
$MgCl_2$	8 mM
KCl	33 mM
G-6-P	5 mM
NADP	4 mM
Sodium phosphate (pH 7.4)	100 mM
S9	40%

3.2.4	Positive control	Without metabolic activation: Mitomycin C (MMC) With metabolic activation: Cyclophosphamide (CP)
3.3	**Application of test substance**	

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.2/1 *In vitro* cytogenicity study in mammalian cells

Annex Point IIA VI.6.6.2

3.3.1	Concentrations	0, 0.37, 0.7, 1.5, 2.9, 5.9, 11.8, 23.6, 47.2, 94.4, 189, 378, 755 and 1510 µg/mL
3.3.2	Way of application	Exponentially growing cells were seeded in sterile, vented screw-capped tissue culture flasks containing 5 ml culture medium and then incubated at 37ºC in humidified air containing 5% CO_2. The next day, the cells were exposed to the test substance in the absence and presence of S9 mix. Just before use, stock dilutions of the test substance were prepared in culture medium. Treatment time was 4 hours and the harvest time was 18 hours after the onset of treatment. In the absence of the S9-mix, 50 µL of the test substance dilutions in dimethyl sulfoxide (DMSO), was added directly to 4.95 mL freshly prepared tissue culture medium and the culture medium was checked visually. In the presence of S9-mix, the culture medium was replaced by 4.45 mL Ham's F-12 medium with penicillin and streptomycin but without foetal calf serum. To each culture, 50 µL of the test substance dilutions in DMSO, was added to the cell cultures and the culture medium was checked visually. 0.5 mL of the S9-mix was added to each culture. All cultures were incubated at 37ºC in humidified air containing 5% CO_2. After the 4 hours period, the cells and culture medium were checked visually. The medium with the test substance was removed, cells washed twice with phosphate-buffered saline and supplied with 5 mL freshly prepared culture medium. The cultures were incubated for an additional 14 hours at 37ºC in humidified air containing 5% CO_2. Two hours before the end of the culture period, the cells and culture medium of all treatment groups were checked again.
3.3.3	Pre-incubation time	Yes, 24 hours
3.3.4	Other modifications	None
3.4	**Examinations**	
3.4.1	Number of cells evaluated	200 metaphase cells (100 per duplicate flask) were examined and scored for structural chromosome aberrations and other anomalies. The mitotic index was recorded as the percentage of cells in mitosis per 1000 cells counted.
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.2/1 *In vitro* cytogenicity study in mammalian cells

Annex Point IIA VI.6.6.2

4.1.1	without metabolic activation	Yes The number of cells with structural aberrations in the 5.9 µg/mL and 2.9 µg/mL treated groups was significantly increased above that of the vehicle. Please refer to Table A6.6.2/1-1. The test substance induced a statistically significant increase in the number of cells with the chromosomal aberrations in the absence of a metabolic activation system. Therefore, it was decided not to carry out a second test.
4.1.2	with metabolic activation	No The number of cells with structural aberrations in the treated groups was not significantly increased above that of the solvent control when cells were harvested. Please refer to Table A6.6.2/1-1.
4.2	**Cytotoxicity**	Yes Without S9-mix the mitotic index of the 5.9 µg/mL treated group was reduced to 31% of that for the vehicle control With the S9-mix the mitotic index of the 11.8 and 5.9 µg/mL treated groups was reduced to 39% and 58%, respectively of that for the vehicle control Please refer to Table A6.6.2/1-1.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	An *in vitro* mammalian chromosome aberration test was carried out on Chinese hamster ovary (CHO-K_1) cells with the test substance 1,2-benzisothiazolin-3-one. This study was conducted according to OECD Guideline 475 and is described under section 3 with no deviations.
5.2	**Results and discussion**	In the absence of metabolic activation, the number of cells with structural aberrations in the 5.9 µg/mL (the highest concentration analysed) and 2.9 µg/mL treated groups was significantly increased above that of the vehicle. A second independent chromosomal aberration test was not conducted on this basis. The mitotic index of the 5.9 µg/mL treated group was reduced to 31% of that for the vehicle control In the presence of metabolic activation, the number of cells with structural aberrations in the treated groups was not significantly increased above that of the solvent control when cells were harvested. The mitotic index of the 11.8 and 5.9 µg/mL treated

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.6.2/1	***In vitro* cytogenicity study in mammalian cells**
Annex Point IIA VI.6.6.2	

		groups (the two highest groups analysed) was reduced to 39% and 58%, respectively of that for the vehicle control Please refer to Table A6.6.2/1-1.
5.3	**Conclusion**	Under the conditions of this assay, 1,2-benzisothiazolin-3-one was concluded to be positive in the chromosome aberration assay using Chinese hamster ovary (CHO) cells.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted*
Conclusion	*Applicant version is adopted*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.6.2/1-1: Summary of structural chromosome aberrations in CHO cells with and without S-9 activation

Treatment (µg/mL)	S9 activation	Mitotic index*	Cells with aberrations
DMSO	+	100	4
2.9	+	77	6
5.9	+	58	3
11.8	+	39	8
CP 3.0	+	47	51
DMSO	-	100	1
1.5	-	137	0
2.9	-	114	29
5.9	-	31	28
MMC	-	150	56

*: Percentage of treated versus negative control value
Note: Harvest time was at 18 hours.
200 cells were scored in each sample

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.2/2 *In vitro* cytogenicity study in mammalian cells

Annex Point IIA VI.6.6.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: November 21 – December 14, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD Guideline 473	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, the following deviation was noted: The cells were treated with colchicine for 4-5 hours rather than 1-3 hours as recommended by the guideline. This deviation is minor and is not considered to compromise the scientific validity of the study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazol-3-(2*H*)-one (BIT)	
3.1.1	Lot/Batch number	BT 17301	
3.1.2	Specification	Please refer to Doc. III-A 2/1	
3.1.2.1	Description	Beige to light brown coloured powder	
3.1.2.2	Purity	97.42% (dry basis)	
3.1.2.3	Stability	Stock solutions were prepared freshly prior to treatment.	
3.2	**Study Type**	*In vitro* mammalian chromosome aberration test.	

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.2/2 *In vitro* cytogenicity study in mammalian cells

Annex Point IIA VI.6.6.2

3.2.1	Organism/cell type	Human blood lymphocytes Blood was collected from healthy female and male human subjects.
3.2.2	Deficiencies / Proficiencies	Not applicable
3.2.3	Metabolic activation system	S9 mix preparation The post mitochondrial fraction (S9) was obtained for the Division of Microbiology, Defence Research and Development Establishment (DRDE), Gwalior, India. A volume of 250 µL S9 mix (5% v/v in phase I, 10% v/v in phase III) supplemented with co-factors was added to cultures with S9. The composition of S9 mix is given below:

Components	Volume (mL)	
	5%	10%
Sterile distilled water	1.9	1.65
Phosphate buffer (pH 7.4;0.2M)	2.5	2.5
Salt solution ($MgCl_2$ + KCl)	0.1	0.1
Glucose-6-phosphate (1M)	0.05	0.05
NADP (0.1M)	0.20	0.2
S9 fraction	0.25	0.5
Final volume	5.0	5.0

3.2.4	Positive control	Without metabolic activation: Mitomycin C (MMC) at 9 x 10^{-7} M With metabolic activation: Cyclophosphamide (CP) at 2 x 10^{-3} M
3.3	**Application of test substance**	
3.3.1	Concentrations	Cytotoxicity test: 0.00125, 0.0025, 0.005, 0.01, 0.078, 0.1563, 0.3125, 0.625, 1.25, 2.5 and 5.0 mg/mL Main test: 0, 0.0015, 0.003, 0.006 mg/mL

3.3.2	Way of application	The culture medium was prepared by dissolving 10.3 g of RPMI-1640 in 1000 mL of sterile distilled water followed by addition of 34.4 mg penicillin and 34.4 mg streptomycin and 2000 mg of sodium bicarbonate. This was filtered and stored in the refrigerator. Prior to planting the cultures the culture medium was supplemented with 10% of foetal bovine serum. Before treatment with the test substance, the peripheral lymphocytes were allowed to proliferate in a culture medium. The blood was transferred aseptically to a culture flask of 250 mL capacity containing culture medium and mitogen. The ratio of blood:culture medium:mitogen were 1.0:14.0:0.2 mL. The culture flask was incubated for 48 hours at 37 ± 1 ºC. The required volume of culture was dispensed to screw capped culture tubes before treatment. Phase I: The proliferating cells were exposed to the test substance for 3 hours both with and without metabolic activation system 5% v/v. Duplicate culture tubes were maintained for each concentration. The cells were harvested at 1.5 normal cell cycle length after the beginning of treatment. Phase II: The proliferating cells were exposed to the test substance for a 30 hour continuous period of time without metabolic activation system. Duplicate culture tubes were maintained for each concentration. The cells were harvested at 1.5 normal cell cycle length. Phase III: The results in phase I with metabolic activation system were confirmed by a modification in the concentration of metabolic activation system (10% v/v). The cells were treated with metaphase arresting substance 5 hours prior to harvesting. The experimental procedure was similar to phase I. Duplicate culture tubes were maintained for each concentration. The cultures were transferred into a clean centrifuge tube and centrifuged. The supernatant was discarded and the cells were suspended in 7 ml of freshly prepared 0.075 M potassium chloride. The cells were incubated at 37 ± 1 ºC for 30 minutes. The tubes were centrifuged and the supernatant was discarded. Freshly prepared chilled Carnoy's fixative (3:1 methanol: glacial acetic acid) was added to the cell pellet to fix the cells. Tubes were centrifuged and the same procedure was repeated three times and the tubes were refrigerated overnight. The following day the tubes were centrifuged, the supernatant was discarded and cell pellet was resuspended in freshly prepared Carnoy's fixative. Tubes were centrifuged and the supernatant was discarded leaving 0.5 mL of fixative with cell pellet. The cell pellet was flushed thoroughly and suspended in 0.5 mL fixative. Two slides were prepared from each culture tube by dropping about 0.5 mL of the fixed cell suspension, drop by drop on pre-cleaned, ice-chilled slides. The slides were dried over a slide warmer and labelled. The slides were stained with 5% Giemsa in phosphate buffer. Out of these two slides, one was used for scoring and the other served as a stand by. The slides were made permanent by mounting a coverslip with DPX mountant.
3.3.3	Pre-incubation time	Yes, 48 hours

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.2/2 *In vitro* cytogenicity study in mammalian cells

Annex Point IIA VI.6.6.2

3.3.4	Other modifications	None
3.4	**Examinations**	
3.4.1	Number of cells evaluated	200 metaphase cells (100 per duplicate flask) were examined and scored for structural chromosome aberrations and other anomalies. The mitotic index was recorded as the percentage of cells in mitosis per 1000 cells counted.
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No No significant effect was observed in the percent of aberrant cells at the different dose levels when compared with the vehicle and negative controls. Please refer to Table A6.6.2/2-1.
4.1.2	with metabolic activation	No No significant effect was observed in the percent of aberrant cells at the different dose levels when compared with the vehicle and negative controls. Please refer to Table A6.6.2/2-1.
4.2	**Cytotoxicity**	No No significant effect was observed in the mitotic index at the different dose levels when compared with the vehicle and negative control in both the absence and presence of metabolic activation.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	An *in vitro* mammalian chromosome aberration test was carried out on Human blood lymphocytes with the test substance BIT. This study was conducted according to OECD Guideline 473 and is described under section 3 with the following deviation: The cells were treated with colchicine for 4-5 hours rather than 1-3 hours as recommended by the guideline. However, this deviation is minor and is not considered to compromise the scientific validity of the study.

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.2/2 *In vitro* cytogenicity study in mammalian cells

Annex Point IIA VI.6.6.2

5.2	**Results and discussion**	No significant effect was observed in the percent of aberrant cells at the different dose levels when compared with the vehicle and negative controls in both the absence and presence of metabolic activation. No cytotoxicity was observed at any of the doses in the presence or absence of metabolic activation system. Please refer to Table A6.6.2/2-1.
5.3	**Conclusion**	Under the conditions of this assay, it is concluded that BIT did not induce chromosomal aberrations in human lymphocytes both in the absence and presence of metabolic activation.
5.3.1	Reliability	1
5.3.2	Deficiencies	One deviation was noted and is outlined under point 2.3 and 5.1. However, it does not compromise the scientific validity of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted*
Conclusion	*Applicant's version is adopted*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.6.2/2-1: Summary of mitotic index and structural chromosome aberrations in Human blood lymphocytes exposed to 1,2-benzisothialin-3-one with and without S-9 activation

Dose levels	Phase I (Without S9)		Phase I (With S9)		Phase II (Without S9)		Phase III (With S9)	
	Mitotic Index	Aberrated Cells %	Mitotic Index	Aberrated Cells %	Mitotic Index	Aberrated Cells %	Mitotic Index	Aberrated Cells %
Negative control	5.051 ± 1.887	0.500 ± 0.707	4.012 ± 0.493	0.500 ± 0.707	3.627 ± 0.406	1.000 ± 0.000	4.732 ± 0.639	0.500 ± 0.707
Vehicle control	3.234 ± 0.016	0.500 ± 0.707	4.530 ± 1.511	0.500 ± 0.707	3.037 ± 0.217	0.500 ± 0.707	6.060 ± 0.878	2.000 ± 1.414
0.0015 mg/mL	5.932 ± 0.783	0.500 ± 0.707	3.359 ± 0.440	0.000 ± 0.000	3.639 ± 0.324	1.500 ± 0.707	5.446 ± 0.552	0.500 ± 0.707
0.003 mg/mL	4.086 ± 0.682	0.000 ± 0.000	5.025 ± 1.636	1.000 ± 1.414	3.585 ± 1.426	0.500 ± 0.707	5.791 ± 0.590	1.000 ± 0.000
0.006 mg/mL	3.849 ± 0.665	1.500 ± 0.707	3.564 ± 0.566	2.000 ± 1.414	2.271 ± 0.059	1.000 ± 1.414	6.255 ± 1.043	3.000 ± 2.828
Mitomycin-C	6.180 ± 1.422	14.500* ± 0.707	-	-	1.709 ± 0.383	25.000** ± 4.243	-	-
Cyclophosphamide	-	-	4.298 ± 0.754	12.500* ± 0.707	-	-	6.941 ± 0.334	12.000** ± 2.828

* Significantly higher than the control at 1% level ($p \leq 0.01$)
** Significantly higher than the control at 5% level ($p \leq 0.05$)

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.3/1 *IN VITRO* GENE MUTATION ASSAY IN MAMMALIAN CELLS (L5178Y MOUSE LYMPHOMA CELLS)

Annex Point IIA VI.6.6.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: October 9 – October 26, 2001	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Company with letter of access	DOW	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD guideline 476 and EU guideline B.17	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-one	
3.1.1	Lot/Batch number	BT 12000	
3.1.2	Specification	Please refer to Doc. III-A 2/2	
3.1.2.1	Description	Brown powder	
3.1.2.2	Purity	98%	
3.1.2.3	Stability	The test substance solutions were prepared just before use.	
3.2	**Study Type**	*In vitro* mammalian cell gene mutation test	

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.3/1 *IN VITRO* GENE MUTATION ASSAY IN MAMMALIAN CELLS (L5178Y MOUSE LYMPHOMA CELLS)

Annex Point IIA VI.6.6.3

3.2.1	Organism/cell type	Mammalian cell lines: Mouse lymphoma L5178Y cells (L5178Y tk +/- 3.7.2C line) obtained from Dr J. Cole, MRC Cell Mutation Unit, University of Sussex, UK
3.2.2	Deficiencies / Proficiencies	Thymidine Kinase proficient
3.2.3	Metabolic activation system	S9 mix Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. Male Wistar rats received a single intraperitoneal injection of Aroclor 1254, 500 mg/kg bw in soya bean oil, five days prior to sacrifice. The livers were removed aseptically, placed, washed and stored in 0.15 M KCl solution. The livers were cut, homogenized, and centrifuged. The supernatant, S9, was taken and stored at < -60 ºC until used. The S9 was checked for sterility, which was found to be 2 colonies per 10 µL S9. The contents of the S9 fraction were determined as follows:

Protein	31.0 g/L
Cytochrome P-450	21.7 µmol/L

Prior to use, the S9 was thawed and mixed with a NADPH-generating system.

The components of the S9 mix were as follows:

Components	Concentration
$MgCl_2$	8 mM
KCl	33 mM
G-6-P	5 mM
NADP	4 mM
RPMI 1640 medium	40 % (v/v)
S9	20 % (v/v)

3.2.4	Positive control	Without metabolic activation: methyl methanesulfonate (MMS) With metabolic activation: 3-methylcholanthrene (MCA)

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.3/1 *IN VITRO* GENE MUTATION ASSAY IN MAMMALIAN CELLS (L5178Y MOUSE LYMPHOMA CELLS)

Annex Point IIA VI.6.6.3

3.3	**Application of test substance**	
3.3.1	Concentrations	Cell treatment without metabolic activation: 1,2-benzisothiazolin-3-one: 0.37, 0.74, 1.5, 2.9, 5.9, 12, 24, 47, 94, 189, 378, 755, and 1510 µg/mL Negative control: DMSO Positive control: MMS 0.1mMol/L Cell treatment with metabolic activation: 1,2-benzisothiazolin-3-one: 0.37, 0.74, 1.5, 2.9, 5.9, 12, 24, 47, 94, 189, 378, 755, and 1510 µg/mL Negative control: DMSO Positive control: MCA 10 µg/mL
3.3.2	Way of application	The L5178Y cells were grown in culture medium consisting of RPMI 1640 medium supplemented with heat-inactive horse serum, sodium pyruvate and penicillin/streptomycin. The cells were cultured in a humidified incubator at 37°C in air containing 5% CO_2. 5-7 days before treatment, the cells were generated from a frozen stock culture by seeding them in sterile, screw-capped tissue culture flasks containing 50 mL culture medium. Fresh cultures of L5178Y cells were harvested from a number of culture flasks and suspended in a culture medium, and the number of cells was counted. For cytotoxicity and gene mutation tests portions of approximately 5 x 10^6 L5178Y cells were used per culture. On the day of exposure, the growth rate and viability of the cells were checked. For cell treatment without metabolic activation, 100 µL test substance, negative control or positive control and 4.9 mL culture medium were added to the cells in 5 mL culture medium to a final volume of 10 mL. The cells were exposed for 24 hours at 37°C and 5% CO_2 in a humidified incubator. For cell treatment with metabolic activation, 100 µL test substance, negative control or positive control, 3.9 mL culture medium and 1 mL 20% (v/v) S9-mix were added to the cells in 5 mL culture medium to a final volume of 10 mL. The cells were exposed for 4 hours at 37°C and 5% CO_2 in a humidified incubator. <u>Assessment of cytotoxicity</u> After the treatment period, the medium containing the test substance, negative control or positive control was removed and the cells were washed twice with culture medium. The cells were resuspended in culture medium and the number of cells was counted. The cell suspensions were diluted to 200,000 cells per mL and the cultures were incubated for 44-48 hours at 37°C and 5% CO_2 in a humidified

		incubator. Some dose levels were discarded after treatment or after 24 hours culture period. After 20-24 hour the cells were counted and suspensions were diluted to 200,000 cells per cells per mL and incubated at 37°C and 5% CO_2 as described above. After 44-48 hours the cells were counted and a portion of the cells was diluted to 10 cells per mL for determining cloning efficiency. The remaining cultures were used for determining the frequency of TFT-resistant mutants. Portions of each dilution at 10 cells per mL were transferred to each well of two 96-well microtiter plates, and the plates were incubated for 10-14 days at 37°C and 5% CO_2 in a humidified incubator. After this period the number of wells without growth of cells was counted and the cloning efficiency was determined. <u>Assessment of mutagenicity</u> To determine the frequency of TFT-resistant mutants, the cell suspensions were diluted to a density of 10,000 cells per mL in culture medium containing 4 µg TFT per mL. 200 µL portions of each dilution were transferred to each well of two 96-wells microtiter plats and the plates were incubated for 10-14 days at 37°C and 5% CO_2 in a humidified incubator. After this period the number of wells without growth of cells were counted and the cloning efficiency in the TFT plates were calculated as before. The TK mutant frequency per 1,000,000 clonable cells was finally calculated.
3.3.3	Pre-incubation time	None
3.3.4	Other modifications	None
3.4	**Examinations**	
3.4.1	Number of cells evaluated	1 x 10^6 cells
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	Yes The mutant frequency, which was dose related, increased above 0.10 and 0.20 mmol/L test substance. At concentrations of 0.020 and 0.039 mmol/L test substance, the mutant frequencies were increased by 56 and 257 mutants per 1,000,000 clonable cells, respectively compared to the negative control. Please refer to Table A6.6.3/1-1.

Section A6 **Toxicological and Metabolic Studies**

Subsection A6.6.3/1 ***IN VITRO* GENE MUTATION ASSAY IN MAMMALIAN CELLS (L5178Y MOUSE LYMPHOMA CELLS)**

Annex Point IIA VI.6.6.3

4.1.2	with metabolic activation	Yes The mutant frequency, which was dose related, increased above 0.10 and 0.20 mmol/L test substance. At concentrations of 0.039, 0.078 and 0.16 mmol/L test substance, the mutant frequencies were increased by 114, 419 and 875 mutants per 1,000,000 clonable cells, respectively compared to the negative control. Please refer to Table A6.6.3/1-1.
4.2	**Cytotoxicity**	Yes In the absence of the S9-mix, the initial cell yield was dose related decreased above the concentration of 0.0049 mmol/L (0.74 µg/mL) test substance. The relative suspension growth and the relative total growth were decreased above a concentration of 0.020 mmol/L (3.0 µg/mL). In the presence of the S9-mix, the initial cell yield, the relative suspension growth and the relative total growth were decreased above a concentration of 0.020 mmol/L (3.0 µg/mL). Please refer to Table A6.6.3/1-1.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The genotoxicity of 1,2-benzisothiazolin-3-one was tested *in vitro* using L5178Y mouse lymphoma cells in both the absence and the presence of a metabolic activation system to examine the ability to induce gene mutations at the TK-locus. The study was conducted according to the OECD guideline 476 and EU guideline B.17 and is described under point 3 with no deviations.
5.2	**Results and discussion**	In the absence as well as in the presence of S9-mix, the mutant frequency was dose related increased above 0.10 and 0.20 mmol/L test substance. In the absence of the S9-mix, at concentrations of 0.020 and 0.039 mmol/L test substance, the mutant frequencies were increased by 56 and 257 mutants per 1,000,000 clonable cells, respectively compared to the negative control. In the presence of the S9-mix, at concentrations of 0.039, 0.078 and 0.16 mmol/L test substance, the mutant frequencies were increased by 114, 419 and 875 mutants per 1,000,000 clonable cells, respectively compared to the negative control. In the absence of the S9-mix, the initial cell yield was dose related decreased above the concentration of 0.0049 mmol/L (0.74 µg/mL) test substance. The relative suspension growth and the relative total growth were decreased above a concentration of 0.020 mmol/L (3.0 µg/mL). In the presence of the S9-mix, the initial cell yield, the relative suspension growth and the relative total growth were decreased above a concentration of 0.020 mmol/L (3.0 µg/mL). Please refer to Table A6.6.3/1-1.

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.6.3/1 **Annex Point IIA VI.6.6.3**	***IN VITRO* GENE MUTATION ASSAY IN MAMMALIAN CELLS (L5178Y MOUSE LYMPHOMA CELLS)**

5.3	**Conclusion**	It was concluded that under the test conditions used in this study, 1,2-benzisothiazolin-3-one is cytotoxic and mutagenic at the TK-locus of mouse lymphoma L5178Y cells.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted.*
Conclusion	*Applicant's version is adopted.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.6.3/1-1: Gene mutation test at the TK-locus of L5178Y cells with 1,2-benzisothiazolin-3-one: Cloning efficiency and mutant frequency data

Treatment	Dose (mMol/L)	S9-mix	Suspension growth	Cloning efficiency	Relative total growth[1] (%)	Mutant cloning efficiency ($x10^6$)	Mutant frequency[2] ($x10^6$)
MMS	0.1	-	8.53	0.43	45	784	1818
BIT	10	-	discarded*				
BIT	5	-	discarded*				
BIT	2.5	-	discarded*				
BIT	1.25	-	discarded*				
BIT	0.63	-	2.33	0.00	0.0	0.0	0.0
BIT	0.31	-	2.98				
BIT	0.16	-	3.72	0.00	0.0	0.0	
BIT	0.078	-	1.57	0.00	0.0	0.0	0.0
BIT	0.039	-	4.94	0.65	39	285	437
BIT	0.020	-	11.98	0.81	117	191	236
BIT	0.010	-	16.98	0.90	184	127	142
BIT	0.0049	-	18.78	0.91	207	158	173
BIT	0.0024	-	16.21	0.85	167	147	173
DMSO	0	-	9.16	0.80	88	180	226
DMSO	0	-	9.81	0.95	112	127	134
MCA	10 µg/mL	+	17.48	0.60	36	823	1376
BIT	10	+	1.01	0.00	0.0	0.0	0.0
BIT	5	+	discarded*				
BIT	2.5	+	0.96	0.00	0.0	0.0	
BIT	1.25	+	discarded*				
BIT	0.63	+	0.85	0.00	0.0	1.3	
BIT	0.31	+	discarded*				

Treatment	Dose (mMol/L)	S9-mix	Suspension growth	Cloning efficiency	Relative total growth[1] (%)	Mutant cloning efficiency ($x10^6$)	Mutant frequency[2] ($x10^6$)
BIT	0.16	+	2.64	0.43	3.9	450	1044
BIT	0.078	+	15.14	0.69	36	407	588
BIT	0.039	+	25.95	0.70	63	199	283
BIT	0.020	+	29.49	0.84	85	127	151
BIT	0.010	+	30.71	0.96	102	140	146
BIT	0.0049	+	33.26	0.78	90	147	188
BIT	0.0024	+	32.63	0.82	92	97	118
DMSO	0	+	32.89	0.81	92	147	182
DMSO	0	+	35.27	0.90	109	140	157

*Culture discarded because of toxicity
[1] Values are given relative to that of the vehicle negative control.
[2] Mutant frequency per 106 clonable cells

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.3/2 *IN VITRO* GENE MUTATION ASSAY IN MAMMALIAN CELLS (OVARY, CHINESE HAMSTER)

Annex Point IIA VI.6.6.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: July 22 – August 15, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Company with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD guideline 476.	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazol-3-(2*H*)-one (BIT)	
3.1.1	Lot/Batch number	BT 17301	
3.1.2	Specification	Please refer to Doc. III-A 2/1	
3.1.2.1	Description	Beige to light brown coloured, moist paste	
3.1.2.2	Purity	97.42% (w/w) on dry basis	
3.1.2.3	Stability	The test substance solutions were prepared just before use.	
3.2	**Study Type**	*In vitro* mammalian cell gene mutation test	
3.2.1	Organism/cell type	Mammalian cell lines: CHO-K1, ovary, Chinese hamster, obtained from American Type Culture Collection, 12301, Parklawn Drive, Rockville, Maryland 20852, USA	

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.3/2 *IN VITRO* GENE MUTATION ASSAY IN MAMMALIAN CELLS (OVARY, CHINESE HAMSTER)

Annex Point IIA VI.6.6.3

3.2.2	Deficiencies / Proficiencies	Hypoxanthine-Guanine Phosphoribosyl-Transferase (HPRT) deficient
3.2.3	Metabolic activation system	S9 mix

Liver microsomal enzymes were prepared from male Wistar rats that had been injected intraperitoneally with Aroclor 1254 in groundnut oil. Five days after injection, the rats were sacrificed by cervical dislocation, swabbed with 70% alcohol and their livers excised under aseptic conditions. The liver from each rat was placed in a pre-weighed blackened beaker containing ice cold 0.15 KCl. After weighing, the liver was rinsed with 0.15 M KCl and chopped into fine pieces. It was then transferred to a sterile homogenization vessel, 0.15 M KCl was added and the liver was homogenized by holding the homogenization vessel within an ice container. The homogenate was transferred to a cold beaker, KCl was added and the container was kept in the refrigerator. The homogenate for different rat livers was centrifuged in a refrigerated centrifuge. The supernatant was decanted and aliquots transferred into cryovials, frozen and stored at -68 to -76 ºC. The S9 mix was checked for sterility and its ability to metabolise was characterized. The protein contents of the S9 fraction were determined as follows:

Batch 14	24.8 mg/mL
Batch 15	22.4 mg/mL

Prior to use, the S9 was mixed 1:9 (S9: co-factor solution). The co-factor solution contained the following:

Components	Preliminary cytotoxicity test	Trial I	Trial II
NADP 4 mM	25 mg	16 mg	22 mg
Glucose-6-phosphate 5 mM	14 mg	9 mg	12 mg
Magnesium chloride 8 mM	13 mg	8 mg	11 mg
Potassium chloride	20 mg	12 mg	17 mg

Section A6 **Toxicological and Metabolic Studies**

Subsection A6.6.3/2 ***IN VITRO* GENE MUTATION ASSAY IN MAMMALIAN CELLS (OVARY, CHINESE HAMSTER)**

Annex Point IIA VI.6.6.3

		33 mM			
		Phosphate buffer saline (pH 7.4)	8 mL	5 mL	7 mL

3.2.4 Positive control

Without metabolic activation: Ethylmethanesulphonate at 0.4 µL/mL

With metabolic acitvation: Benzo (a) pyrene at 3 µg/mL

3.3 Application of test substance

3.3.1 Concentrations

Preliminary cytotoxicity test:

1, 2, 4, 6, 8 and 10 µg/mL

Cell treatment with metabolic activation:

Trial I and II: 0.95, 1.90, 3.80 and 7.60 µg/mL

Negative control: DMSO

Positive control: Benzo (a) pyrene at 3 µg/mL

Cell treatment without metabolic activation:

Trial I: 0.95, 1.90, 3.80 and 7.60 µg/mL

Trial II: 0.75, 1.65, 3.63 and 8.00 µg/mL

Negative control: DMSO

Positive control: Ethylmethane sulphonate at 0.4 µL/mL

3.3.2 Way of application

Assessment of cytotoxicity

Exponentially growing CHO-KI cells were plated at a density of 5 x 10^5 cells/25 cm^2 flask in F12 FBS 10. The medium volume was made up to 5 mL and incubated for 23 hours. The target cells in duplicate cultures were exposed to different concentrations of the test item and solvent control for 5 hours at 37 ± 1 ºC.

The treatment medium consisted of 5 mL of F12 FBS 10 without metabolic activation and 4.5 mL F12 FBS 10 with metabolic activation and 100 µL of test item diluted to the appropriate concentration with DMSO. In test incorporating metabolic activation, 0.5 mL of S9-mix was added to each flask to give a final concentration of 10% (v/v) in the test suspensions.

After the treatment period, medium from each flask was aspirated, cells washed twice with phosphate buffer saline and cultured in F12 FBS 10 for a further period of 17 hours and 30 minutes.

The cells were trypsinized, detached with 5 mL F12 FBS 10, the replicates were pooled and the cell counts were determined separately using a haemocytometer. The effect of the test substance on cell multiplication was estimated by expressing the number of

		cells in each treated culture as a percentage of the number relative to the DMSO control. The replicate cultures from the controls and each treatment condition were trypsinized, detached with 5 mL of F12 FBS 5, pooled and counted using a haemocytometer. For expression of the mutant phenotype, the cells from the pooled replicates were subcultured in 10 mL of F12 FBS 5 in duplicate, at density of approximately 10^6 cells/90 mm dish. Subculturing as above at 2-3 days intervals was done for 7-9 day expression period. After this time, the mutant phenotype was selected. <u>Assessment of genotoxicity</u> Exponentially growing CHO-KI cells were plated in F12 FBS 10 at a density of 5 x 10^5 cells/25 cm^2 flask. The medium volume was made up to 5 mL and incubated for 23 hours, 30 minutes and 23 hours, 45 minutes for trials I and II respectively. The target cells were exposed to solvent, positive control and four concentrations of the test item for 5 hours at 37 ± 1 ºC. The treatment medium consisted of 5 ml of F12 FBS 10 and 100 µL of test item diluted to the appropriate concentration with DMSO. In test incorporating metabolic activation, 0.5 mL of S9-mix was added to each flask to give a final concentration of 7.5 and 10% (v/v) in the test suspension used in trials I and II, respectively. After the treatment period, medium from each flask was aspirated, cell monolayer was washed twice with phosphate buffer saline and re-fed with 5ml F12 FBS 10 for a further period of 17 hours, 15 minutes and 17 hours, 45 minutes for Trials I and II, respectively. For selection of the 6-Thioguanine resistant phenotype, the replicates from controls and each treatment condition were pooled and replated, in quintuplicate, at a density of approximately 2 X 10^6 cells/90 mm dish in F12 FBS 10 containing 20 µM 6-Thioguanine and incubate for 10 days. For cloning efficiency determination at the time of selection 100 cells/25 cm^2 flask were plated in triplicate in F12 FBS 10 and incubated for 7 days. After the specified incubation time the colonies were stained with methylene blue and counted for both cloning efficiency and mutant selection.
3.3.3	Pre-incubation time	23 hours and 30 minutes for Trial I and 23 hours and 45 minutes for Trial II
3.3.4	Other modifications	None
3.4	**Examinations**	
3.4.1	Number of cells evaluated	2 x 10^6 cells

Section A6 **Toxicological and Metabolic Studies**

Subsection A6.6.3/2 ***IN VITRO* GENE MUTATION ASSAY IN MAMMALIAN CELLS (OVARY, CHINESE HAMSTER)**

Annex Point IIA VI.6.6.3

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No Please refer to Table A6.6.3/2-1.
4.1.2	with metabolic activation	No Please refer to Table A6.6.3/2-1.
4.2	**Cytotoxicity**	Yes In the preliminary cytotoxicity test > 50% inhibition was reached between the test item concentrations of 4 and 6 µg/mL both in the presence and absence of metabolic activation. At the highest tested concentration of 10 µg/mL, growth inhibition by 92.5 and 95.55% was observed when compared to the DMSO control in the presence and absence of metabolic activation, respectively. In Trial I, there was a 43.43 and 39.39% reduction in cell growth when compared to the DMSO control at the highest concentration of 7.60 µg/mL in the presence and absence of metabolic activation, respectively. In Trial II, there was a 5.71 and 29.66 reduction in cell growth when compared to the DMSO control at the highest test concentrations of 7.60 and 8.00 µg/mL in the presence and absence of metabolic activation respectively. Please refer to Table A6.6.3/2-2.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The genotoxicity of BIT was tested *in vitro* using Chinese Hamster ovary cells in both the absence and the presence of a metabolic activation system to examine the ability to induce gene mutations. The study was conducted according to OECD guideline 476 and is described under point 3 with no deviations.
5.2	**Results and discussion**	In the preliminary cytotoxicity test > 50% inhibition was reached between the test item concentrations of 4 and 6 µg/mL both in the presence and absence of metabolic activation. At the highest tested concentration of 10 µg/mL, growth inhibition by 92.5 and 95.55% was observed when compared to the DMSO control in the presence and absence of metabolic activation, respectively. In Trial I, there was a 43.43 and 39.39% reduction in cell growth when compared to the DMSO control at the highest concentration of

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.6.3/2 **Annex Point IIA VI.6.6.3**	***IN VITRO* GENE MUTATION ASSAY IN MAMMALIAN CELLS (OVARY, CHINESE HAMSTER)**

		7.60 µg/mL in the presence and absence of metabolic activation, respectively. In Trial II, there was a 5.71 and 29.66 reduction in cell growth when compared to the DMSO control at the highest test concentrations of 7.60 and 8.00 µg/mL in the presence and absence of metabolic activation respectively. The test substance did not cause a significant increase in the frequency of the mutants with or without metabolic activation when compared to solvent control. Please refer to Tables A6.6.3/2-1 and A6.6.3/2-2.
5.3	**Conclusion**	It was concluded that under the test conditions used in this study, BIT is not mutagenic to Chinese Hamster ovary cells.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted.*
Conclusion	*Applicant's version is adopted.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.6.3/2-1: Gene mutation test with BIT: Cloning efficiency and mutant frequency data

Trial	Treatment (µg/mL)	S9-mix	Total number of mutants counted	Absolute cloning efficiency	Mutants/10^6 survivors
I	DMSO (0.1 mL)	+	4	95	4
I	0.95	+	3	83	4
I	1.90	+	1	70	1
I	3.80	+	5	59	8
I	7.60	+	6	58	10
I	Benzo (a) pyrene	+	96	50	192*
I	DMSO (0.1 mL)	-	7	95	7
I	0.95	-	4	77	5
I	1.90	-	3	68	4
I	3.80	-	6	60	10
I	7.60	-	2	55	4
I	Ethylmethanesulphonate	-	279	46	607*
II	DMSO (0.1 mL)	+	19	93	20
II	0.95	+	9	86	10
II	1.90	+	18	73	25
II	3.80	+	15	64	23
II	7.60	+	13	60	22
II	Benzo (a) pyrene	+	107	53	202*
II	DMSO (0.1 mL)	-	7	89	8
II	0.75	-	6	84	7
II	1.65	-	5	72	7
II	3.63	-	7	58	12
II	8.00	-	6	58	10
II	Ethylmethanesulphonate	-	382	52	735*

*Significantly higher than the control by Dunnett's test

Table A6.6.3/2-2: Gene mutation test with 1,2-benzisothiazolin-3-one: Cytotoxicity data

Trial	Treatment(µg/mL)	S9-mix	Cell count ($x10^6$/flask)	Control %
Preliminary	DMSO (0.1 mL)	+	2.40	100.00
Preliminary	1	+	2.37	98.75
Preliminary	2	+	2.08	86.67
Preliminary	4	+	1.22	50.83
Preliminary	6	+	1.13	47.08
Preliminary	8	+	1.03	42.92
Preliminary	10	+	0.18	07.50
Preliminary	DMSO (0.1 mL)	-	2.92	100.00
Preliminary	1	-	2.55	87.33
Preliminary	2	-	1.92	65.75
Preliminary	4	-	1.97	67.47
Preliminary	6	-	1.38	47.26
Preliminary	8	-	1.12	38.36
Preliminary	10	-	0.13	04.45
I	DMSO (0.1 mL)	+	2.74	100.00
I	0.95	+	2.35	85.77
I	1.90	+	1.83	66.79
I	3.80	+	1.65	60.22
I	7.60	+	1.55	56.57
I	Benzo (a) pyrene	+	1.65	60.22
I	DMSO	-	2.31	100.00
I	0.95	-	1.96	84.85
I	1.90	-	2.05	88.74
I	3.80	-	1.90	82.25
I	7.60	-	1.40	60.61

Trial	Treatment(µg/mL)	S9-mix	Cell count ($x10^6$/flask)	Control %
I	Ethylmethanesulphonate	-	1.70	73.59
II	DMSO	+	2.10	100.00
II	0.95	+	2.25	107.14
II	1.90	+	1.90	90.48
II	3.80	+	2.09	99.52
II	7.60	+	1.98	94.29
II	Benzo (a) pyrene	+	2.30	109.52
II	DMSO	-	2.90	100.00
II	0.75	-	2.40	82.76
II	1.65	-	2.18	75.17
II	3.63	-	2.23	76.90
II	8.00	-	2.04	70.34
II	Ethylmethanesulphonate	-	2.31	79.66

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.4 Genotoxicity *In Vivo* micronucleus assay

Annex Point IIA VI.6.6.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: December 3, 2001 – January 18, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Company with letter of access	DOW	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD guideline 474 and EU guideline B.12.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, the following deviation was noted: The mean body weight and standard deviation were given rather than the individual weights of the animals, including body weight range. This deviation is minor and is not considered to compromise the scientific validity of this study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-one	
3.1.1	Lot/Batch number	BT 12000	
3.1.2	Specification	Please refer to Doc. III-A 2/2	
3.1.2.1	Description	Brown solid	
3.1.2.2	Purity	98%	
3.1.2.3	Stability	Not relevant, single dose only	
3.1.2.4	Maximum tolerable dose	500 mg/kg bw	
3.2	**Test Animals**		

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.4 Genotoxicity *In Vivo* micronucleus assay

Annex Point IIA VI.6.6.4

3.2.1	Species	Mouse
3.2.2	Strain	Swiss Mice, Charles River, CD-1 strain
3.2.3	Source	Charles River Deutschland, Sulzfeld, Germany
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Young adult Males: 30.5 – 32.2 g (range of means) Females 25.0 – 25.8 g (range of means)
3.2.6	Number of animals per group	Range finding: 3 groups of 2 animals/sex/group Main study: Vehicle control and 500 mg/kg bw: 10 animals/sex/group 125, 250 mg/kg bw and positive control: 5 animals/sex/group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Number of applications	Range Finding study: single application Main micronucleus test: single application
3.3.2	Interval between applications	Not applicable
3.3.3	Postexposure period	24 and 48 h after treatment
3.3.4	Type	Gavage
3.3.5	Concentration	Range finding study: 300, 700 and 2000 mg/kg bw Main micronucleus test: 0, 125, 250 and 500 mg/kg bw
3.3.6	Vehicle	Corn oil
3.3.7	Concentration in vehicle	0, 6.25, 12.5 and 25 mg/mL
3.3.8	Total volume applied	20 mL/kg bw
3.3.9	Controls	Vehicle
3.4	**Examinations**	

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.4 Genotoxicity *In Vivo* micronucleus assay

Annex Point IIA VI.6.6.4

3.4.1	Clinical signs	Yes, 1-4 hours after treatment and daily thereafter
3.4.2	Tissue	Bone marrow
	Number of animals:	All animals
	Number of cells:	The number of polychromatic and normochromatic erythrocytes (PE and NE, respectively) were recorded in 1000 erythrocytes per animal. If micronuclei were observed, the incidence of micronucleated polychromatic erythrocytes (MPE) was recorded in a total of 2000 PE per animal and the number of micronucleated normochromatic erythrocytes (MNE) was recorded in the number of NE.
	Time points:	At 24 hours 10 vehicle controls (5/sex), 30 treated animals (5/sex/dose) and 10 positive controls (5/sex). At 48 hours, 10 vehicle controls (5/sex) and 10 treated animals at the 500 mg/kg bw dose level.
	Type of cells	Erythrocytes in bone marrow
	Parameters:	Number of polychromatic and normochromatic erythrocytes Micronucleated normochromatic erythrocytes /normochromatic erythrocytes ratio Micronucleated polychromatic erythrocytes/2000 polychromatic erythrocytes ratio
3.5	**Further remarks**	Animals were weighed just before dosing.
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	Vehicle control, 125 and 250 mg/kg bw: no effects No clinical signs were observed 500 mg/kg bw: The following clinical signs were observed: dyspnoea, hunched posture, sluggishness, blepharospasm, piloerection and 3 animals were found dead
4.2	**Haematology / Tissue examination**	No statistically significant differences in the incidence of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes and in the number of polychromatic erythrocytes per 1000 erythrocytes were observed in the treated groups when compared to the vehicle controls. Please refer to Tables A6.6.4-1 and A6.6.4-2.
4.3	**Genotoxicity**	No
4.4	**Other**	None
		5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.4 Genotoxicity *In Vivo* micronucleus assay

Annex Point IIA VI.6.6.4

5.1	**Materials and methods**	The genotoxicity of 1,2-benzisothiazolin-3-(2*H*)-one was investigated by orally dosing mice with the test substance at the concentrations of 0, 125, 250 and 500 mg/kg bw and then examining the bone marrow erythrocytes for the induction of micronuclei. The study was conducted according to OECD guideline 474 and EU guideline B.12 and is described under point 3. The following deviation was noted: The mean body weight and standard deviation were given rather than the individual weights of the animals, including body weight range. However, this deviation is minor and is not considered to compromise the scientific validity of this study.
5.2	**Results and discussion**	No clinical effects were observed in the vehicle control, 125 and 250 mg/kg bw groups. At the 500 mg/kg bw dose level, clinical signs such as dyspnoea, hunched posture, sluggishness, blepharospasm, piloerection were observed and 3 animals were found dead. No statistically significant differences in the incidence of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes and in the number of polychromatic erythrocytes per 1000 erythrocytes were observed in the treated groups when compared to the vehicle controls. Results are summarised in Tables A6.6.4-1 and A6.6.4-2.
5.3	**Conclusion**	It is concluded that 1,2-benzisothiazolin-3-one did not produce micronuclei in polychromatic erythrocytes in the bone marrow of both male and female mice at dose levels up to 500 mg/kg bw.
5.3.1	Reliability	1
5.3.2	Deficiencies	One deviation was noted and is outlined under points 2.3 and 5.1. However, it does not compromise the scientific validity of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted*
Conclusion	*Applicant's version is adopted.*
Reliability	*1*

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.4 Genotoxicity *In Vivo* micronucleus assay

Annex Point IIA VI.6.6.4

Acceptability	*Acceptable*
Remarks	

Table A6.6.4-1: Micronucleus test in vivo for 1,2-benzisothiazolin-3-one: numbers of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes

Group		Negative control mean ± SD	125 mg/kg bw mean ± SD	250 mg/kg bw mean ± SD	500 mg/kg bw mean ± SD	Positive control mean ± SD
Sex	Sacrifice (h)					
Male	24	3.8 + 1.3	2.8 + 2.4	3.8 + 1.1	1.8 + 0.8	38.0* + 19.8
Male	48	1.8 + 0.8			2.4 + 0.5	
Female	24	3.0 + 2.3	2.6 + 1.8	2.8 + 0.8	2.6 + 0.9	34.6*** + 8.0
Female	48	3.0 + 1.7			3.0 + 1.0	

* $p < 0.05$
*** $p < 0.001$

Table A6.6.4-2: Micronucleus test in vivo for 1,2-benzisothiazolin-3-one: numbers of polychromatic erythrocytes per 1000 erythrocytes

Group		Negative control mean ± SD	125 mg/kg bw mean ± SD	250 mg/kg bw mean ± SD	500 mg/kg bw mean ± SD	Positive control mean ± SD
Sex	Sacrifice (h)					
Male	24	522.4 + 85.0	576.6 + 52.1	495.4 + 69.5	555.0 + 101.5	505.0 + 31.4
Male	48	464.4 + 63.9			444.0 + 25.9	
Female	24	577.4 + 60.3	560.6 + 69.9	562.2 + 41.1	547.2 + 59.2	529.2 + 60.3
Female	48	450.8 + 57.0			513.4 + 80.8	

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.5 Genotoxicity *in vivo*

Annex Point IIA VI.6.6.5 ***In-vivo* Unscheduled DNA Synthesis Test with Mammalian Liver Cells**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: January 3 – March 12, 2002	
1.2	**Data protection**	Yes	
1.2.4	Data owner	Troy Chemical Company BV	
1.2.5	Company with letter of access	DOW	
1.2.6	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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD guideline 486.	
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	BT 12000	
3.1.2	Specification	Please refer to Doc. III-A 2/2	
3.1.2.1	Description	Brown solid powder	
3.1.2.2	Purity	98%	
3.1.2.3	Stability	Not relevant, single dose only	
3.1.2.4	Maximum tolerable dose	400 mg/kg bw	
3.2	**Test Animals**		
3.2.1	Species	Rat	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.6.5	**Genotoxicity *in vivo***
Annex Point IIA VI.6.6.5	***In-vivo* Unscheduled DNA Synthesis Test with Mammalian Liver Cells**

3.2.2	Strain	Wistar outbred: strain Crl:[WI] WU BR
3.2.3	Source	Charles River Deutschland, Sulzfeld, Germany
3.2.4	Sex	Range Finding study: male and female Main test: male
3.2.5	Age/weight at study initiation	Range finding study: 7 weeks old Main test: 8 weeks old Range finding study: males 203.5 – 229.0 g females 150.3 – 181.3 g Main test: males 225.9 – 291.6 g
3.2.6	Number of animals per group	Range finding study: 3 groups of 2 animals/sex/group Main test: negative control and test substance: 6 animals/group positive control: 2 animals/group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Number of applications	Range Finding study: single application Main test: single application
3.3.2	Interval between applications	Not applicable
3.3.3	Postexposure period	Range Finding study: 48 h after treatment Main test: 2-4 and 12-16 h after treatment
3.3.4	Type	Gavage
3.3.5	Concentration	Range finding study: 200, 400 and 800 mg/kg bw Main test: 0, 200 and 400 mg/kg bw
3.3.6	Vehicle	Corn oil
3.3.7	Concentration in vehicle	Range Finding study: 10, 20 and 40 mg/mL Main test: 0, 10 and 20 mg/mL
3.3.8	Total volume applied	20 mL/kg bw

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.6.5	**Genotoxicity *in vivo***
Annex Point IIA VI.6.6.5	***In-vivo* Unscheduled DNA Synthesis Test with Mammalian Liver Cells**

3.3.9	Controls	Vehicle
3.4	**Examinations**	
3.4.3	Clinical signs	Yes Range finding: twice during the first 4 hours, at 24 and 48 hours after treatment Main test: at 1-4 and 12-16 hours after treatment
3.4.4	Tissue	Liver
	Number of animals:	All animals
	Number of cells:	5×10^5
	Time points:	2-4 and 12-16 h after treatment
	Type of cells	Hepatocytes
	Parameters:	Net nuclear grains
3.5	**Further remarks**	Range finding: Body weights were recorded prior to treatment (Day 0) and on Day 3. Main test: Body weights were recorded prior to treatment (Day 0).
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	Control groups: no effects No clinical signs were observed. Treated groups: 1 h after administration sluggishness was observed but it later subsided and nothing else was observed.
4.2	**Haematology / Tissue examination**	At both time points, the treated groups and the vehicle control group always yielded ≤ 0 net nuclear grains. The test substance did not increase the mean net nuclear grains compared to the vehicle control. Please refer to Table A6.6.5-1.
4.3	**Genotoxicity**	No
4.4	**Other**	None
		5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6 Toxicological and Metabolic Studies

Subsection A6.6.5 Genotoxicity *in vivo*

Annex Point IIA VI.6.6.5 ***In-vivo* Unscheduled DNA Synthesis Test with Mammalian Liver Cells**

5.1	**Materials and methods**	The genotixicity of 1,2-Benzisothiazolin-3-(2*H*)-one was investigated by dosing rats orally with the test substance at the concentrations of 0, 200 and 400 mg/kg bw and recording any unschedueled DNA synthesis in rat hepatocytes. The study was conducted according to OECD guideline 486 and is described under point 3 with no deviations.
5.2	**Results and discussion**	No clinical signs were observed in the control groups. In the treated groups, sluggishness was observed one hour after administration, but it later subsided and nothing else was observed. At both time points, the treated groups and the vehicle control group always yielded < 0 net nuclear grains. The test substance did not increase the mean net nuclear grains compared to the vehicle control. Therefore, 1,2-benzisothiazolin-3-(2*H*)-one does not induce unscheduled DNA synthesis. Please refer to Table A6.6.5-1.
5.3	**Conclusion**	It is concluded that the test substance 1,2-benzisothiazolin-3-(2*H*)-one did not induce unscheduled DNA synthesis (UDS) in rat hepatocytes after short-term *in vivo* exposure of rats, under the conditions used in the present study.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted*
Conclusion	*Applicant's version is adopted.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.6.5-1: Summary of unscheduled DNA synthesis data with 1,2-benzisothiazolin-3-one in rat liver cells *in vivo*

Animal	Treatment	Mean nuclear grain count	Mean cytoplasm grain count	Mean net grains per nucleus	% cells in repair	Mean nuclear grain count of cells in repair
1	Vehicle control	22.45 ± 2.45	30.68 ± 2.60	-8.23 ± 0.16	0.00	0.00 ± 0.00
2		18.50 ± 1.90	25.90 ± 2.35	-7.40 ± 0.45	0.00	0.00 ± 0.00
3		25.51 ± 1.91	36.06 ± 1.36	-10.55 ± 0.55	0.00	0.00 ± 0.00
4		20.97 ± 1.34	28.78 ± 0.25	-7.81 ± 1.60	0.00	0.00 ± 0.00
5		17.39 ± 2.67	24.67 ± 3.52	-7.28 ± 0.85	0.00	0.00 ± 0.00
6*	Test substance 200 mg/kg bw	15.52	22.61	-7.09	0.00	0.00
7*		21.32	28.78	-7.46	0.00	0.00
8		16.83 ± 0.13	24.79 ± 1.57	-7.96 ± 1.70	0.00	0.00 ±0.00
9		17.09 ±1.68	24.39 ± 3.58	-7.30 ± 1.90	0.00	0.00 ± 0.00
10		19.38 ± 1.90	27.13 ± 2.45	-7.75 ± 0.55	0.00	0.00 ± 0.00
11		17.83 ± 1.06	26.12 ± 4.47	-8.29 ± 3.41	0.00	0.00 ± 0.00
12	Test substance 400 mg/kg bw	16.28 ± 2.69	22.88 ± 3.85	-6.60 ± 1.16	0.00	0.00 ± 0.00
13		16.94 ± 0.93	25.93 ±6.07	-8.99 ± 0.27	1.00	3.00 ± 4.24
14		18.69 ± 5.50	26.31 ± 4.94	-7.62 ±0.57	0.00	0.00 ± 0.00
15		17.50 ± 0.11	25.36 ± 1.39	-7.86 ± 1.27	0.00	0.00 ± 0.00

Animal	Treatment	Mean nuclear grain count	Mean cytoplasm grain count	Mean net grains per nucleus	% cells in repair	Mean nuclear grain count of cells in repair
16		21.89 ± 1.65	30.42 ±1.30	-8.53 ± 0.35	2.00	2.5 ± 3.54
17		16.65 ± 3.32	24.62 ± 4.33	-7.97 ± 1.00	0.00	0.00 ± 0.00
18*	2-AFF 50 mg/kg bw	20.50	17.60	2.90	34.00	7.41
19		17.78 ± 0.85	18.30 ±2.46	-0.52 ± 1.61	18.00	6.47 ± 0.66
20**	DMN 10 mg/kg bw				Average > 90***	
21						

* = only one slide could be scored
** = cells could not be counted electronically, because cells were too heavily labelled
*** = visually estimated > 90% in repair

Section A6 **Subsection A6.6.6** **Annex Point IIA6.6.6**	**Toxicological and Metabolic Studies** **Germ cell effect**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	BIT was negative in two *in-vitro* gene mutation studies in bacteria summarised under point IIIA, 6.6.1/1 and 6.6.1/2. Positive results were obtained in two *in vitro* studies: the *in vitro* cytogenicity study in mammalian cells and the gene mutation test at the TK-locus summarised under IIIA, 6.6.2/1 and 6.6.3/1, respectively**.** Negative results were obtained in the *in vitro* cytogenicity study in mammalian cells and the gene mutation test in Chinese Hamster ovary cells summarised under IIIA, 6.6.2/2 and IIIA, 6.6.3/2. Furthermore, results of the two *in vivo* genotoxicity studies summarised under IIIA, 6.6.4 and IIIA, 6.6.5 concluded that BIT is not genotoxic. Most importantly, the toxicokinetics/metabolism study performed in rats demonstrated that BIT is quantitatively absorbed which assures its bioavailability (point IIIA, 6.2/1). Therefore it is concluded that no further work is required on genotoxicity to assess possible germ cell effects.	
Undertaking of intended data submission [ ]	Not relevant	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*October 2008*
Evaluation of applicant's justification	*Applicant's justification is accepted.*
Conclusion	*Applicant's is exempted of the second in vivo genotoxicity study.*
Remarks	

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.6.7 **Annex Point IIA6.6.7**	**If the results are negative for the three tests 6.6.1, 6.6.2 and 6.6.3, then further testing is normally only required if metabolites of concern are formed in mammals**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	BIT was negative in two *in-vitro* gene mutation studies in bacteria summarised under point IIIA, 6.6.1/1 and 6.6.1/2. Positive results were obtained in two *in vitro* studies: the *in vitro* cytogenicity study in mammalian cells and the gene mutation test at the TK-locus summarised under IIIA, 6.6.2/1 and 6.6.3/1, respectively**.** Negative results were obtained in the *in vitro* cytogenicity study in mammalian cells and the gene mutation test in Chinese Hamster ovary cells summarised under IIIA, 6.6.2/2 and IIIA, 6.6.3/2. Furthermore, results of the two *in vivo* genotoxicity studies summarised under IIIA, 6.6.4 and IIIA, 6.6.5 concluded that BIT is not genotoxic. Furthermore, results of the two *in vivo* genotoxicity studies summarised under IIIA, 6.6.4 and IIIA, 6.6.5 concluded that BIT is not genotoxic. Therefore, it is concluded that no further work is required on genotoxicity. Furthermore, there are no metabolites of concern for which further testing would be required.	
Undertaking of intended data submission [ ]	Not applicable	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*October 2008*
Evaluation of applicant's justification	*Applicant's justification is accepted.*
Conclusion	*Applicant is exempted to assay genotoxicity in BIT metabolites.*
Remarks	

Section 6	**Toxicological and Metabolic Studies**	
Subsection A6.7	**Carcinogenicity study**	
Annex Point IIA6.7		
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	In order to avoid unnecessary vertebrate testing, Troy Chemical Company BV propose to waive carcinogenicity studies, based on the following arguments: - No genotoxic potential for humans was identified. The genotoxicity studies were performed in agreement with the OECD test guidelines (Please see Table A6.7-1). Results from two *in vitro* bacterial reverse mutation tests showed that BIT did not cause a positive response with any of the tester stains either in the presence or absence of metabolic activation. In one *in vitro* mammalian chromosomal aberration study, BIT was shown to induce chromosomal aberrations when tested in the absence of S-9 metabolic activation. In a second *in vitro* mammalian chromosomal aberration study, BIT did not induce chromosomal aberrations in human lymphocytes either in the absence and presence of metabolic activation. In one *in vitro* gene mutation assay in mammalian cells, BIT was also shown to be mutagenic at the TK-locus of mouse lymphoma L5178Y cells both in the presence and absence of metabolic activation. In a second *in vitro* gene mutation assay in mammalian cells, BIT was not mutagenic to Chinese Hamster ovary cells, both in the absence and presence of metabolic activation. Furthermore, in a micronucleus assay, BIT did not produce micronuclei in polychromactic erythrocytes and in an UDS test, BIT did not induce unscheduled DNA synthesis. Therefore, although positive results were shown in some of the *in vitro* tests presented, there are negative *in vivo* results which confirm that BIT is not genotoxic, based on the results from the two *in vivo* studies, BIT is not deemed to be genotoxic. - Mechanisms of toxicological effects, with indications of non-genotoxic carcinogenicity, were not observed in subchronic toxicity studies. The most clearly understood effects of non-genotoxic agents are on cell proliferation where they may act as mitogens, cytotoxins or as perturbers of the normal process of growth control interfering with endocrine system. The histopathology determinations carried out in the 90 day toxicity study in rats (IIIA, 6.4.1/1), showed that no adverse effects, such as hyperplastic or preneoplastic responses that are assumed to be related to tumour growth, were observed after treatment with BIT. The NOAEL for BIT was considered to be 27.5 mg/kg bw/day based on a slight reduction in mean body weight, increased cholesterol in males at 75 mg/kg bw/day and decreased RBC in females at 75 mg/kg bw/day. No carcinogenic effects were noted. No changes at gross pathology and histopathology were reported. In an oral 90-day study in the dog summarised under point IIIA, 6.4.1/2, 1,2-benzisothiazol-3(2*H*)-one (BIT) was administered	

Section 6	**Toxicological and Metabolic Studies**
Subsection A6.7	**Carcinogenicity study**
Annex Point IIA6.7	

in the diet to 4 beagle dogs/sex/group at 300, 1000 and 3000 ppm (which is equivalent to 11, 37 and 106 mg/kg bw/day in male and 11, 38 and 89 mg/kg bw/day in females) for a minimum of 90 days. All animals survived throughout the study. There were no treatment related clinical observations or changes in haematology or urinalysis parameters or organ weights. There were no ophthalmic, macroscopic or microscopic findings. The only changes noted were lower body weights at 3000 ppm in males and females and lower food consumption at 3000 ppm in females when compared to control animals. Based on the above, the NOAEL of 1000 ppm (which is equivalent to 37 mg/kg bw/day in males and 38 mg/kg bw/day in females). In the 90-day dermal study in rat (IIIA, 6.4.2), changes in some of the clinical chemistry parameters such as albumin and mean total protein were reported at 1000 mg/kg bw/day in females. The NOAEL was established to be 300 mg/kg bw/day. However, there were no carcinogenic effects reported. A review of the available literature did not highlight any carcinogenic potential for BIT.

- There are no structural alerts for carcinogenicity of the active substance BIT. A structure-activity relationship (SAR) analysis was carried out on a series of isothiazolinone compounds (MIT, CMIT and OIT) using the cat-SAR model (Barbee, S., 2005). MIT, CMIT, OIT and BIT are structurally related. This model is based on the identification of structural features of the compounds in the learning sets that are capable of differentiating the active and inactive compounds. Four models were used in these analyses, i.e., the rat model using the CPDB (Carcinogenic Potency Database), the mouse model using the CPDB, the model in the rat using data from MIT and CMIT and the model in the mouse using data from OIT. Individual predictions were based on the analysis of multiple fragments of the molecules. Each fragment is derived from several compounds with similar carcinogenic or non-carcinogenic activity. The validity of cat-SAR was checked based on the analysis of BIT for allergic dermatitis in humans and the model identified that isothiazolinone compounds could induce contact dermatitis. Based on the results from the four models, it was predicted that BIT is not carcinogenicity.

Table A6.7-1: Summary of genotoxicity studies with BIT

Test	Test system	Concentration tested	Result	Reference
In vitro bacterial mutagenicity	*Salmonella typhimurium :* TA 1535, TA 1537, TA 98 and TA 100 *E. coli*: WP2 uvrA	200, 66.7, 22.2, 7.41, and 2.47 µg/plate with and without metabolic activation	Negative	[redacted] (2002)

Section 6	**Toxicological and Metabolic Studies**
Subsection A6.7	**Carcinogenicity study**
Annex Point IIA6.7	

In vitro bacterial mutagenicity	*Salmonella typhimurium :* TA 1537, TA 1535, TA 98, TA 100 and TA 102	0, 3.125, 6.25, 12.5, 25 and 50 µg/plate with and without metabolic activation	Negative	[redacted] (2003)
In vitro mammalian chromosomal aberration	Chinese hamster ovary cells	2.9, 5.9 and 11.8 µg/ml with metabolic activation and 1.5, 2.9 and 5.9 µg/mg without metabolic activation	Positive	[redacted] (2002)

Table A6.7-1 continued

In vitro mammalian chromosomal aberration	Human blood lymphocytes	0, 0.0015, 0.003, 0.006 mg/ml with and without metabolic activation	Negative	[redacted] (2003)
In vitro mammalian cell gene mutation	Mouse lymphoma L5178Y cells (TK locus)	0.37, 0.74, 1.5, 2.9, 5.9, 12, 24, 47, 94, 189, 378, 755, and 1510 µg/mL with and without metabolic activation	Positive	[redacted], (2002)
In vitro mammalian cell gene mutation	*Chinese Hamster ovary cells*	Trial I and II: 0.95, 1.90, 3.80 and 7.60 µg/mL with metabolic activation	Negative	[redacted] (2003)

Section 6	**Toxicological and Metabolic Studies**
Subsection A6.7	**Carcinogenicity study**
Annex Point IIA6.7	

		Trial I: 0.95, 1.90, 3.80 and 7.60 µg/mL without metabolic activation Trial II:0.75, 1.65, 3.63 and 8.00 µg/mL without metabolic activation		
In vivo micronucleus test	*Swiss Mice, Charles River, CD-1 strain (erythrocytes in bone marrow)*	125, 250 and 500 mg/kg bw	Negative	[redacted]., (2002)
In vivo unscheduled DNA synthesis	*Rat, Wistar outbred: strain Crl:[WI] WU BR (hepatocytes)*	200 and 400 mg/kg bw	Negative	[redacted] (2002)

BIT is manufactured outside the EU and is used as a preservative. The product [redacted] and the product type PT 13 "Metalworking fluids" are intended to be supported by the dossier. Preservatives are added to metalworking fluids to preserve them in their action of cooling, lubricating and carrying cuttings from mechanical cutting operations. Metal is shaped by moving past a cutting tool, or the cutting tool moves around or past metal. Metalworking fluid is supplied at the cutting tool for cooling, lubrication and swarf removal. The end-use products containing BIT are intended for professionals. There are three different population groups that may be exposed to BIT: industrial and professional users and the general public *via* indirect exposure as a result of use.

The preservative [redacted] containing 20% w/w BIT is incorporated by industrial users into the different products by simple dilution into them. Industrial users manufacture metalworking fluid concentrates adding the preservative [redacted] to the preparation to obtain a concentration up to 0.4% w/w BIT. Professionals dilute

Section 6	**Toxicological and Metabolic Studies**
Subsection A6.7	**Carcinogenicity study**
Annex Point IIA6.7	

the metalworking fluid concentrate to obtain the metalworking fluid emulsion containing up to 0.02% w/w of the preservative BIT and use the metalworking emulsion in different metalworking activities.

A study on dermal absorption has been summarised under point IIIA, 6.2/2. The dermal absorption value was established to be approximately 28.86% for the high dose and can therefore be rounded to 30%.

The NOAEL to be compared with the estimated exposures must be the lowest NOAEL value obtained in the most sensitive species. Therefore, the most appropriate NOAEL is obtained from a teratogenicity study in the rabbit which is summarised under point IIIA, 6.8.1/1. The NOAEL for maternal toxicity was determined to be 6 mg/kg bw/day.

The exposure to BIT was calculated based in the selected models and default values from the User Guidance (2002). Metalworking fluids is the only use which is taken into account for PT 13. The primary exposures have been calculated for industrial users when they dilute the product [redacted] to manufacture the end-use products and for professionals when diluting and using the metalworking fluids containing BIT. The secondary exposures for adults, children and infants have been calculated for the post-application period of the end-use products.

For metalworking fluids, the estimated primary exposures when taking into account industrial and professional users range from 0.0001 to 0.0443 with margins of safety (MOS) ranging from 60000 to 135 indicating an acceptable risk. The estimated secondary exposures for adults, children and infants range from 0.0005 to 0.0014 (MOS ranging from 12000 to 4286 indicating an acceptable risk). These values have been obtained taking into account the worst-case scenario. However, they are well above the MOS of 1000. It is important to outline that the total exposure when taking into account the equilibrium vapour concentration of BIT is much lower than the estimated total exposure when taking into account the default values stated in the guidelines and therefore, the MOS are higher.

It is important to outline that the total exposure, when taking into account the equilibrium vapour concentration of BIT, is much lower than the estimated total exposure when taking into account the default values stated in the guidelines and therefore, the MOS are higher.

It is concluded that the risk is acceptable for both primary and secondary exposures when taking into account the end-use products containing BIT.

In conclusion, there are no ethical grounds (that would not contravene the requirements of Directive 86/609/EC which advises against unnecessary testing using animals) for performing further studies on animals. It is therefore proposed that no additional investigations are required to address this point.

Section 6	**Toxicological and Metabolic Studies**
Subsection A6.7	**Carcinogenicity study**
Annex Point IIA6.7	
Undertaking of intended data submission [ ]	Not applicable
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Evaluation of applicant's justification	*Applicant version is adopted.*
Conclusion	*Applicant's is exempted of carcinogenity studies.*
Remarks	

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.1/1 Teratogenicity Study

Annex Point IIA VI.6.8.1 **6.8.1 Teratogenicity test in rabbits**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: September 15, 2006 – December 27, 2006	
1.2	**Data protection**	Yes	
3.2.1	Data owner	ROHM & HAAS	
3.2.2	Companies with letter of access	Troy Chemical Company BV	
3.2.3	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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to OECD guideline 414.	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	Yes, the following deviation was noted: Not all the females underwent an acclimatization period of 5 days recommended by the guideline since they were received on gestation days 1, 2 or 3. This deviation is minor and is not considered to compromise the scientific validity of the study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one	
3.1.1	Lot/Batch number	Lot no. 2005-051	
3.1.2	Specification	As given under point 3.1.2.2	
3.1.2.1	Description	Off-white powder containing lumps	
3.1.2.2	Purity	89.8%	

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.1/1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Teratogenicity test in rabbits

3.1.2.3	Stability	Duplicate samples for resuspension, homogeneity and stability determinations at 0.4 mg/mL were collected from the top and bottom strata of an aliquot of the approximate volume needed for one day of dose administration from a formulation following 11 days of refrigerated storage. Samples for concentration analysis were collected from the middle stratum of each dosing formulation prepared during the in-life phase of the study. The analysed dosing formulations were within 85 – 115% of the target concentration and were homogeneous and stable for at least 11 days.
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand white
3.2.3	Source	Denver, Pennsylvania facility of Covance Research Products, USA
3.2.4	Sex	Females
3.2.5	Age/weight at study initiation	5 months 2913 – 4041 g on gestation day 0
3.2.6	Number of animals per group	4 groups of 25 females/group
3.2.7	Control animals	Yes
3.2.8	Mating period	Time-mating
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of exposure	Once daily on day 6-28 post mating
3.3.2	Postexposure period	1 days (animals were necropsied on Day 29)
3.3.3	Type	Gavage
3.3.4	Concentration	Range finding: 10, 30, 60 and 90 mg/kg bw/day Main study: 0, 2, 6 and 25 mg/kg bw/day

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Annex Point IIA VI.6.8.1 6.8.1 Teratogenicity test in rabbits

3.3.5	Vehicle	0.5% carboxymethylcellulose and 0.1% polysorbate 80
3.3.6	Concentration in vehicle	0, 0.4, 1.2, 5 mg/mL
3.3.7	Total volume applied	5 mL/kg
3.3.8	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	Individual maternal body weights were recorded on days 0, 4, and daily from day 6 to 29 of gestation.
3.4.2	Food consumption	Food consumption data were recorded during days 0, and daily on days 4 to 29 of gestation.
3.4.3	Clinical signs	Twice daily for mortality and moribundity. Individual detailed clinical observations were recorded on days 4-29 of gestation. Animals were also observed for signs of toxicity, at the time of dose administration and/or approximately 1 hour following dose administration.
3.4.4	Examination of uterine content	All surviving rabbits were euthanised on gestation day 29. The contents of the thoracic, abdominal and pelvic cavities were examined. Post mortem findings were correlated with ante mortem comments and abnormalities were recorded. The uterus and ovaries were exposed and excised and the uterus was weighed. The number of corpora lutea on each ovary was recorded. The number and location of all foetuses, early and late resorptions and the total number of implantation sites were recorded. The placentae were also examined. The individual uterine distribution of implantation sites was documented. All implantation sites including resorptions were numbered in consecutive order beginning with the left distal to the left proximal to the right distal uterine horn. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulphide solution for detection of early implantation loss. Maternal tissues were preserved in 10% neutral-buffered formalin for possible future histopathological examination.
3.4.5	Maternal organ weights	
3.4.5.1		Each viable foetus was examined externally and weighed. The detailed external examination included the eyes, palate and external orifices. Degrees of autolysis and gross examinations were recorded for late resorptions.
3.4.5.2	Skeleton	Yes, the heads of half the foetuses in each litter were examined by a mid-coronal slice. All the foetuses were examined externally.

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.1/1 Teratogenicity Study

Annex Point IIA VI.6.8.1 **6.8.1 Teratogenicity test in rabbits**

3.4.5.3	Soft tissue	Yes, the heads from half the foetuses in each litter were removed from the carcasses and placed in Bouin's solution for soft tissue examination. Microscopic examination of folded retinas was performed. Each viable foetus was subjected to a visceral examination and the sex was determined. All carcasses were eviscerated, skinned and fixed in 100% ethyl alcohol then macerated in potassium hydroxide and stained with Alizarin Red S.
3.5	**Further remarks**	A gross necropsy was performed on females that died during the course of the study. The abdominal, pelvic and thoracic cavities were opened and the organs were examined. Gross lesions were recorded. The number and location of implantation sites, corpora lutea and viable foetuses were recorded. Recognisable foetuses were euthanised, examined externally and preserved.
		4 RESULTS AND DISCUSSION
4.1	**Maternal toxic effects**	2 mg/kg bw/day: No test substance related clinical observations were observed. No test substance related effects on mean maternal body weights, bodyweight gains, net body weights, net body weight gains or gravid uterine weights were noted. No test substance related effects on mean food consumption were observed. No test substance related internal findings were noted at necropsy. 6 mg/kg bw/day: Two females were found dead on gestation days 22 and 28, respectively. These deaths were not considered to be related to the test substance. Decreased defecation was observed in 5 females from gestation days 8-29 but this was not considered treatment related. Nine females had hair loss in the urogenital area during gestation days 22-24. No test substance related effects on mean maternal body weights, bodyweight gains, net body weights, net body weight gains or gravid uterine weights were noted. No test substance related effects on mean food consumption were observed. No test substance related internal findings were noted at necropsy. 25 mg/kg bw/day: Dark red areas were found on the lung of one female but were not considered treatment related. Decreased defection was observed in 13 females from gestation days 8-29 which corresponds to periods of reduced food consumption in many of the same females. Nine females had hair loss in the urogenital area during gestation days 22-24. A test substance related mean body weight loss was observed during gestation days 6-9. Statistically significant decreases were

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.1/1 Teratogenicity Study

Annex Point IIA VI.6.8.1 **6.8.1 Teratogenicity test in rabbits**

		observed in mean body weights from day 13 to 25. Mean gravid uterine weight was similar to the control group. Mean food consumption was statistically significantly lower during gestation days 6-9, 9-12 and 12-20 when compared to the control group. At necropsy on gestation day 29, four females had dark red discolouration in areas of the stomach. These findings were considered treatment related. Please refer to Tables A6.8.1/1-1 and A6.8.1/1-2.
4.2	**Teratogenic / embryo toxic effects**	2 mg/kg bw/day: No test substance related effects on intrauterine growth and survival was noted. No test substance related external malformations or external developmental variations were observed. No test substance related skeletal malformation or developmental variations were observed. 6 mg/kg bw/day: No test substance related effects on intrauterine growth and survival was noted. No test substance related external malformations or external developmental variations were observed. No test substance related soft tissue malformation or developmental variations were observed. No test substance related skeletal malformation or developmental variations were observed. 25 mg/kg bw/day: The mean male foetal weight was statistically significantly lower than the control group. The mean female and the mean combined-sex foetal weights were lower than the control group but the differences were not statistically significant. No effects on sex ratio or foetal survival were noted. No test substance related external malformations or external developmental variations were observed. No test substance related soft tissue malformation or developmental variations were observed. No test substance related skeletal malformation or developmental variations were observed.
4.3	**Other effects**	No

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.1/1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Teratogenicity test in rabbits

5.1	**Materials and methods**	The teratogenicity of 1,2-benzisothiazolin-3-one was investigated by oral administration to 4 groups of rabbits (25 females/group) at the following concentrations: 0, 2, 6 and 25 mg/kg bw/day followed by full examination of the females and the foetuses. This study was conducted according to OECD guideline 414 and is described under point 3 with the following deviation: Not all the females underwent an acclimatisation period of 5 days recommended by the guideline since they were received on gestation days 1, 2 or 3. However, this deviation is minor and is not considered to compromise the scientific validity of the study.
5.2	**Results and discussion**	No treatment related effects were observed in body weights, food consumption, clinical observations, gravid uterine weight or necropsy in the 2 or 6 mg/kg bw/day groups. Please refer to Tables A6.8.1/1-1 and A6.8.1/1-2. In the 6 mg/kg bw/day group, two females were found dead on gestation days 22 and 28, respectively. However, these deaths were not related to the test substance. Decreased defecation was observed in 5 females from gestation days 8-29 but this finding was not considered treatment related. Nine females had hair loss in the urogenital area during gestation days 22-24. No test substance related effects on intrauterine growth, survival, external malformations or external developmental variations, skeletal malformation or developmental variations were observed in the 2 or 6 mg/kg bw/day groups. In the 25 mg/kg bw/day group, dark red areas were found on the lung of one female but this finding was not considered treatment related. Decreased defecation was observed in 13 females from gestation days 8-29 which corresponds to periods of reduced food consumption in many of the same females. Nine females had hair loss in the urogenital area during gestation days 22-24. A test substance related mean body weight loss was observed during gestation days 6-9. Lower mean body weight gains were observed during gestation days 9-12 and 12-20 and the mean net body weight gain was statistically significantly lower than the control. Mean food consumption was statistically significantly lower during gestation days 6-9, 9-12 and 12-20 when compared to the control group. At necropsy on gestation day 29, four females had dark red discolouration in areas of the stomach. These were the only findings considered as test substance related. Mean gravid uterine weight was similar to the control group The mean male foetal weight in the 25 mg/kg bw/day group was statistically significantly lower than the control group. The mean female and the mean combined-sex foetal weight were lower than the control group but the differences were not statistically significant. No effects on sex ratio or foetal survival were noted. No treatment related external malformations or external developmental variations, soft tissue malformation or developmental variations or skeletal malformation or

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.8.1/1	**Teratogenicity Study**
Annex Point IIA VI.6.8.1	**6.8.1 Teratogenicity test in rabbits**

		developmental variations were observed.
5.3	**Conclusion**	Based on statistically significant reductions in mean body weight gain and food consumption (accompanied by decreased defecation in a majority of the animals) at 25 mg/kg bw/day, a dosage level of 6 mg/kg bw/day was considered to be the no observable adverse effect level (NOAEL) for maternal toxicity. In the absence of test substance-related effects on intrauterine growth and survival as well as foetal morphology, a dosage level of 25 mg/kg bw/day was considered to be the NOAEL for embryo/foetal development toxicity when 1,2-Benzisothiazolin-3-one was administered orally by gavage to pregnant New Zealand White rabbits.
5.3.1	LO(A)EL maternal toxic effects	25 mg/kg bw/day
5.3.2	NO(A)EL maternal toxic effects	6 mg/kg bw/day
5.3.3	LO(A)EL embryo toxic / teratogenic effects	Not determined
5.3.4	NO(A)EL embryo toxic / teratogenic effects	25 mg/kg bw/day
5.3.5	Reliability	1
5.3.6	Deficiencies	One deviation was noted and is outlined under points 2.3 and 5.1. However, it does not compromise the scientific validity of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted.*
Conclusion	*Applicant's conclusion is adopted.*
Reliability	*1*

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.1/1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Teratogenicity test in rabbits

Acceptability	*Acceptable*
Remarks	

Table A6.8.1/1-1: Mean maternal body weights in the teratogenicity study with 1,2-Benzisothiazolin-3-one

Day of gestation	Dosage level (mg/kg bw/day)			
	Group mean maternal body weights (g)			
	0 (Control)	2	6	25
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0	3349 ± 263.2	3341 ± 267.1	3284 ± 239.8	3310 ± 258.5
4	3317 ± 237.3	3273 ± 295.5	3258 ± 214.6	3281 ± 232.1
6	3389 ± 237.1	3332 ± 281.1	3296 ± 204.5	3326 ± 218.9
7	3399 ± 223.0	3336 ± 278.1	3306 ± 210.1	3312 ± 234.4
8	3406 ± 218.9	3362 ± 237.9	3315 ± 210.6	3289 ± 235.8
9	3418 ± 218.5	3376 ± 242.3	3320 ± 218.4	3285 ± 238.7
10	3428 ± 210.1	3386 ± 251.5	3340 ± 213.9	3278 ± 247.5
11	3435 ± 224.7	3405 ± 244.3	3351 ± 218.6	3278 ± 259.1
12	3459 ± 221.0	3438 ± 273.4	3373 ± 220.8	3287 ± 259.5
13	3498 ± 219.3	3452 ± 264.9	3395 ± 224.1	3299* ± 268.2
14	3529 ± 223.0	3484 ± 263.6	3419 ± 234.6	3325* ± 259.7
15	3560 ± 221.4	3512 ± 275.6	3450 ± 238.4	3329* ± 270.3
16	3580 ± 221.4	3518 ± 227.0	3462 ± 240.3	3342* ± 270.5
17	3591 ± 219.6	3526 ± 276.1	3476 ± 233.2	3346* ± 271.9
18	3600 ± 220.7	3534 ± 279.7	3487 ± 236.3	3356* ± 274.7
19	3608 ± 215.8	3546 ± 274.6	3496 ± 241.8	3366* ± 270.8
20	3627 ± 216.0	3567 ± 285.0	3512 ± 238.8	3377* ± 270.7
21	3652 ± 221.6	3577 ± 289.4	3528 ± 245.5	3400* ± 265.3

Day of gestation	Dosage level (mg/kg bw/day)			
	Group mean maternal body weights (g)			
	0 (Control)	2	6	25
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
22	3673 ± 217.6	3604 ± 297.0	3560 ± 255.4	3424* ± 262.2
23	3696 ± 228.1	3630 ± 295.4	3577 ± 255.9	3451* ± 263.8
24	3711 ± 236.7	3639 ± 302.8	3597 ± 268.1	3483* ± 268.7
25	3733 ± 240.7	3649 ± 312.6	3610 ± 268.4	3504* ± 262.9
26	3739 ± 239.0	3656 ± 312.0	3619 ± 267.1	3516 ± 271.7
27	3737 ± 241.6	3668 ± 310.1	3617 ± 276.2	3526 ± 275.8
28	3746 ± 253.9	3675 ± 310.5	3645 ± 264.1	3544 ± 278.0
29	3765 ± 265.8	3703 ± 297.4	3654 ± 258.3	3566 ± 279.0

* Significantly different from the control group at 0.05 using Dunnett's test

Table A6.8.1/1-2: Mean maternal food consumption in the teratogenicity study with 1,2-benzisothiazolin-3-one (g/animal/day)

Day of gestation	Dosage level (mg/kg bw/day)			
	0 (Control)	2	6	25
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
4-6	137 ± 19.3	134 ± 28.9	142 ± 12.0	139 ± 19.1
6-9	145 ± 12.1	139 ± 16.9	144 ± 12.4	104* ± 40.0
9-12	142 ± 15.1	139 ± 19.2	137 ± 19.2	95* ± 38.9
12-20	143 ± 12.8	132 ± 26.1	130 ± 26.0	105* ± 40.7
20-29	118 ± 27.2	108 ± 27.5	110 ± 29.6	115 ± 15.6
6-29	133 ± 13.7	124 ± 19.7	125 ± 19.2	107* ± 23.0

* Significantly different from the control group at 0.05 using Dunnett's test

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.1/2 Teratogenicity Study in rats

Annex Point IIA VI.6.8.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: February 21 – July 12, 1994	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Company with letter of access	DOW	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted according to EPA FIFRA guideline 83-3 which is equivalent to OECD guideline 414.	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	Yes, the following deviation was noted: The body weights and food consumption were recorded every 4 days rather than every 3 days. This deviation is minor and is not considered to compromise the scientific validity of the study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazol-3-(2*H*)-one ([redacted])	
3.1.1	Lot/Batch number	60793	
3.1.2	Specification	Please refer to Doc. III-A 2/2	
3.1.2.1	Description	Tan powder	
3.1.2.2	Purity	100%	
3.1.2.3	Stability	Three sets of samples were collected from the low and high dose levels mixed for the homogeneity analysis. One set was analysed on the day of mixing, the second set was stored refrigerated for 10 days and analysed before the initiation of treatment and the last set was stored in a freezer for 7 weeks and then analysed. The results indicated that the test substance was stable in the carrier under the conditions of the study.	

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.1/2 Teratogenicity Study in rats

Annex Point IIA VI.6.8.1

3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Crl : CD®BR VAF / Plus®
3.2.3	Source	Charles River Laboratories, Inc., Portage, Michigan, USA
3.2.4	Sex	Females
3.2.5	Age/weight at study initiation	12 weeks old at time of mating 198.5 to 264.5 g on gestation day 0
3.2.6	Number of animals per group	4 group of 25 females/group
3.2.7	Control animals	Yes
3.2.8	Mating period	One female and one male rat of the same strain were placed together for mating. The occurrence of copulation was determined by daily inspection for a copulatory plug or sperm using a vaginal smear. The day evidence of mating was detected was designated as day 0 of gestation.
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of exposure	Once daily on day 6-15 post mating
3.3.2	Postexposure period	5 days (animals were necropsied on Day 20)
3.3.3	Type	Gavage
3.3.4	Concentration	0, 10, 30 and 90 mg/kg bw
3.3.5	Vehicle	0.5% carboxymethylcellulose
3.3.6	Concentration in vehicle	0, 2, 6, 18 mg/mL
3.3.7	Total volume applied	5 mL/kg
3.3.8	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	Individual maternal body weights were recorded on days 0, 6, 8, 12, 16 and 20 of gestation.

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.1/2 Teratogenicity Study in rats

Annex Point IIA VI.6.8.1

3.4.2	Food consumption	Food consumption data were recorded during days 0 – 6, 6 – 8, 8 – 12, 12 – 16 and 16 – 20 of gestation.
3.4.3	Clinical signs	Twice daily for mortality and moribundity. Additional signs of poor health and abnormal behaviour were recorded as they were observed. A detailed examination was done on each animal on days 0, 6, 8, 12, 16 and 20 of gestation
3.4.4	Examination of uterine content	Uterus weight Number and location of implantation sites, live and dead foetuses, early and late resorptions and any abnormalities were recorded. Uteri with no visible implantations were excised and stained for detection of implantations and confirmation of pregnancy.
3.4.5	Examination of foetuses	
3.4.5.1	General	Individual foetal weight, sex ratio, external abnormalities
3.4.5.2	Skeleton	Yes, half of the live foetuses from each litter
3.4.5.3	Soft tissue	Yes, half of the live foetuses from each litter
3.5	**Further remarks**	Ovaries were examined for the number of corpora lutea. Maternal tissues were examined macroscopically for abnormal thoracic, abdominal or pelvic viscera. Abnormal tissues were preserved
		4 RESULTS AND DISCUSSION
4.1	**Maternal toxic effects**	90 mg/kg bw/day: Two animals were found dead between days 8 and 13 of gestation and two animals were sacrificed moribund on days 8 and 9. Dry brown material around the nasal area, excessive salivation, audible breathing, gasping, and anogenital hair coat staining were observed. The animals that died were cold to touch prior to death. These findings were considered treatment related. Significant reductions in mean body weights were observed at days 12, 16 and 20 of gestation. Mean body weight changes were significantly reduced. Mean food consumption was significantly lower throughout the dosing period. Following completion of dosing, food consumption was comparable to that of the controls. No significant differences in gravid uterine weights were observed. Corrected body weights and net body weights from day 0 were significantly reduced due to the significantly lower mean body weights on gestation day 0. Congestion or dark reddened lungs were observed at necropsy for all animals. Intestines and stomachs were distended with gas and reddened areas in the intestines were found. These findings were considered to be treatment related.

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Annex Point IIA VI.6.8.1

		30 mg/kg bw/day: One animal was found dead on day 13 of gestation. Dry brown material around the nasal area, audible breathing, and anogenital coat staining were noted. These findings were considered treatment related. No significant differences in mean body weights and food consumption were observed. No significant differences in gravid uterine weights were observed. Congestion or dark reddened lungs were observed at necropsy for all animals. Intestines and stomachs were distended with gas and reddened areas in the intestines were found. These findings were considered to be treatment related. 10 mg/kg bw/day: No treatment related observations. No significant differences in mean body weights and food consumption were observed. No significant differences in gravid uterine weights were observed. Please refer to Tables A6.8.1/2-1 and A6.8.1/2-2.	
4.2	**Teratogenic / embryo toxic effects**	No significant differences in co-variant mean adjusted foetal body weights were observed. No treatment related foetal external abnormalities were observed. No treatment related differences in the foetal litter incidences of soft tissue abnormalities were observed. The foetal incidence of unossified sternebrae was increase in the 90 mg/kg bw/day group but there were no significant differences in the litter incidence of this abnormality. No significant differences in mean percent pre-implantation loss, post-implantation loss, percent live foetuses, sex ratio or resorptions were observed. There was an increase in the mean percent of early resorptions and a decrease in the mean percent of live foetuses at 90 mg/kg bw/day dose level but this finding was attributed to one animal that had no viable foetuses.	
4.3	**Other effects**	No	
		5 APPLICANT'S SUMMARY AND CONCLUSION	

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.1/2 Teratogenicity Study in rats

Annex Point IIA VI.6.8.1

5.1	**Materials and methods**	The teratogenicity of 1,2-Benzisothiazolin-3-one was investigated by oral administration to 4 groups of rats (25 females/group) at the following concentrations: 0, 10, 30 and 90 mg/kg bw/day followed by full examination of the dams and the foetuses. This study was conducted according to EPA FIFRA guideline 83-3 which is comparable to OECD guideline 414 and is described under point 3. The following deviation was noted: The body weights and food consumption were recorded every 4 days rather than every 3 days. However, this deviation is minor and is not considered to compromise the scientific validity of the study.
5.2	**Results and discussion**	Two animals were found dead in the 90 mg/kg bw/day group between days 8 and 13 of gestation and two animals were sacrificed moribund on days 8 and 9. In the 30 mg/kg bw/day group, one animal was found dead on day 13 of gestation. These deaths and moribund conditions were considered treatment related. For the 90 mg/kg bw/day group, dry brown material around the nasal area, excessive salivation, audible breathing, gasping, and anogenital hair coat staining were observed. The two animals that died were cold to touch prior to death. For the 30 mg/kg bw/day group, dry brown material around the nasal area, audible breathing, and anogenital coat staining were noted but to a lesser degree. These findings were considered treatment related. Significant reductions in mean body weight were present for the 90 mg/kg bw/day animals at days 12, 16 and 20 of gestation. Mean body weight changes were significantly reduced for the 90 mg/kg bw/day group. Mean food consumption was significantly lower for the 90 mg/kg bw/day group throughout the dosing period. Following completion of dosing, food consumption was comparable to that of the controls. No significant differences in gravid uterine weights were observed. Corrected body weights and net body weights from day 0 were significantly reduced at 90 mg/kg bw/day due to the significantly lower mean body weights for the 90 mg/kg bw/day females on gestation day 0. Congestion or dark reddened lungs were observed at necropsy for all animals in the 30 and 90 mg/kg bw/day groups. Intestines and stomachs were distended with gas and reddened areas in the intestines were found. All these findings were considered treatment related. No treatment related foetal external abnormalities or differences in the foetal litter incidences of soft tissue abnormalities were observed. The foetal incidence of unossified sternebrae was

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.1/2 Teratogenicity Study in rats

Annex Point IIA VI.6.8.1

		increase in the 90 mg/kg bw/day group but there were no significant differences in the litter incidence of this abnormality. No significant differences in mean percent pre-implantation loss, post-implantation loss, percent live foetuses, sex ratio or resorptions were observed. Please refer to Tables A6.8.1/2-1 and A6.8.1/2-2.
5.3	**Conclusion**	Under the conditions of this study, the no observable adverse effect level (NOAEL) for developmental toxicity for 1,2-Benzisothiazolin-3-one was established at 90 mg/kg bw/day.
5.3.1	LO(A)EL maternal toxic effects	30 mg/kg bw/day
5.3.2	NO(A)EL maternal toxic effects	10 mg/kg bw/day
5.3.3	LO(A)EL embryo toxic / teratogenic effects	Not determined
5.3.4	NO(A)EL embryo toxic / teratogenic effects	90 mg/kg bw/day
5.3.5	Reliability	1
5.3.6	Deficiencies	One deviation was noted and is outlined under points 2.3 and 5.1. However, it does not compromise the scientific validity of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted*
Conclusion	*Applicant's conclusion is adopted.*
Reliability	*1*

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.1/2 Teratogenicity Study in rats

Annex Point IIA VI.6.8.1

Acceptability	*Acceptable*
Remarks	

Table A6.8.1/2-1: Summary of maternal antemortem and necropsy observations in the teratogenicity study with 1,2-Benzisothiazolin-3-one

Observation	Maternal antemortem observations			
	Dosage Level (mg/kg bw/day)			
	0 (Control)	10	30	90
Death and moribund	0	0	1	4
Cold to touch	0	0	0	2
Dry brown material around nasal area	0	0	1	1-3
Excessive salivation (clear)	0	0	0	1
Audible breathing	0	0	1	1-3
Gasping	0	0	0	1-2
Anogenital hair coat staining	0	0	1	1
Observation	**Maternal necropsy observations**			
	Dosage Level (mg/kg bw/day)			
	0 (Control)	10	30	90
Congested lungs	0	0	0	4
Dark reddened lungs	0	0	1	1
Distended intestines	0	0	1	3
Distended stomach	0	0	1	2
Reddened intestines	0	0	0	2

Table A6.8.1/2-2: Mean maternal body weights and body weight changes in the teratogenicity study with 1,2-Benzisothiazolin-3-one

Day of gestation	Dosage level (mg/kg bw/day)			
	Group mean maternal body weights (g)			
	0 (Control)	10	30	90
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0	232.43 ± 13.97	234.65 ± 9.44	232.09 ± 11.15	227.55 ± 15.36
6	259.69 ± 15.40	266.66 ± 10.27	261.77 ± 13.66	254.90 ± 15.62
8	265.54 ± 15.88	271.38 ± 11.17	266.40 ± 13.38	253.78 ± 22.41
12	285.45 ± 14.69	289.03 ± 11.28	281.79 ± 17.75	264.83** ± 24.43
16	309.06 ± 14.41	316.98 ± 12.71	310.00 ± 19.17	279.67** ± 34.32
20	369.20 ± 21.25	379.48 ± 18.60	372.75 ± 23.22	340.76* ± 39.65
Days of gestation	**Group mean maternal body weights changes (g)**			
0 to 6	27.26 ± 7.61	32.01 ± 7.40	29.68 ± 9.35	27.36 ± 6.11
6 to 8	5.85 ± 3.97	4.72 ± 3.97	4.64 ± 4.43	-1.12** ± 10.18
8 to 12	19.91 ± 5.93	17.66 ± 4.59	15.39 ± 11.07	8.10** ± 12.67
12 to 16	23.61 ± 8.04	27.94 ± 4.56	26.48 ± 8.30	13.81* ± 15.94
16 to 20	60.14 ± 11.35	62.50 ± 8.42	62.75 ± 7.35	61.09 ± 9.90
6 to 16	49.37 ± 11.85	50.32 ± 6.60	48.03 ± 9.05	23.67** ± 23.72
0 to 20	136.77 ± 21.65	144.83 ± 16.35	140.26 ± 18.72	111.21** ± 30.79

Significantly different from control:
* $p < 0.05$
** $p < 0.01$

Section A6 Toxicological and Metabolic Studies

Subsection A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 Two generation study in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
		Dates of experimental work: July 14, 2006 – June 25, 2007	
1.2	**Data protection**	Yes	
1.2.1	Data owner	ROHM & HAAS	
1.2.2	Company with letter of access	Troy Chemical Company BV	
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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was carried out according to OECD Guideline 416.	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one	
3.1.1	Lot/Batch number	Lot No. 2005-051	
3.1.2	Specification	As given under point 3.1.2.2	
3.1.2.1	Description	Off-white powder containing lumps	
3.1.2.2	Purity	89.8%	
3.1.2.3	Stability	The test article was stored at room temperature and was considered stable under this condition. Duplicate samples from the top, middle and bottom strata of the formulations prepared with aqueous 0.5% w/v carboxymethylcellulose (CMC) and 0.1% (v/v) Tween 80 at nominal test article concentrations of 1, 2.5 and 6.5 mg/ml were stored refrigerated for 10 days and analysed to assess test article stability. The mean concentration after 10 days of refrigerated storage ranged from 104 to 105% of the time-zero values which met the requirement for stability.	

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3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Crl:CD(SD)
3.2.3	Source	Charles River Laboratories, Inc., Raleigh, North Carolina, USA
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	8 weeks Males: 261 – 356 g Females: 176 – 229 g
3.2.6	Number of animals per group	4 groups of 30 animals/sex/group
3.2.7	Mating	After a minimum of 70 days of treatment, the animals were paired on a 1:1 basis. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in vaginal lavage. The day when evidence of mating was identified was termed gestation day 0. The animals were separated, and the female was housed in an individual cage with nesting material. Each generation was mated once to produce 1 litter per generation. All animals were randomly selected for pairing, avoiding sibling mating.
3.2.8	Duration of mating	When evidence of mating was not apparent after 14 days or 3 oestrous cycles, the female was placed in an individual cage with nesting material with no further opportunity for mating.
3.2.9	Deviations from standard protocol	None that negatively impact the quality or integrity of the data or the outcome of the study.
3.2.10	Control animals	Yes
3.2.11	Litter size	Each litter was adjusted by use of a computer generated random selection program to 8 pups, to yield when possible 4 males and 4 females. Standardization was not performed on litters with fewer than 8 pups.
3.3	**Administration/ Exposure**	Oral
3.3.1	Animal assignment to dosage groups	Animals in good health and meeting acceptable body weight requirements were selected for the study and randomly allocated to experimental groups by a computer randomization procedure.

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3.3.2	Duration of exposure before mating	F_0 females and males were dosed for at least 70 days prior to mating. Dosing administration continued throughout mating and though the day prior to euthanasia, for a total of 138 to 140 doses. F_1 pups selected for mating were administered the test article following weaning beginning on postnatal day (PND) 22. Dosing administration continued throughout mating, gestation and lactation through the day prior to euthanasia, for a total of 131 to 147 and 134 to 146, for males and females respectively.
3.3.3	Duration of exposure in general P, F1, F2 males, females	Over a two generation period Oral
3.3.4	Type	Gavage
3.3.5	Doses	0, 10, 25 and 65 or 50 mg/kg bw/day. In the high dose group, a dose of 65 mg/kg bw/day was administered to animals in the F_0 generation through study day 21. Due to excessive toxicity, a dose of 50 mg/kg bw/day was administered thereafter to animals in the F_0 and F_1 generations.
3.3.6	Vehicle	0.5% carboxymethylcellulose (CMC) and 0.1% Tween 80 in water.
3.3.7	Concentration in vehicle	0, 1, 2.5 and 6.5 or 5.0 mg/ml. In the high dose group, a concentration of 6.5 mg/L was administered to animals in the F_0 generation through study day 21. Due to excessive toxicity, a concentration of 5.0 mg/L was administered thereafter to animals in the F_0 and F_1 generations.
3.3.8	Total volume applied	0.01 mL/g
3.3.9	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Clinical signs and mortality	Yes, all animals were observed for appearance, behaviour and pharmacotoxic signs at the time of and/or approximately 1 hour following dose administration. Detailed physical examinations were recorded weekly for all parental animals. All animals were observed twice daily for mortality and moribundity.
3.4.2	Body weight	Males: F_0 and F_1 weights were recorded weekly throughout the study beginning 1 week prior to dosing. Females: F_0 and F_1 weights were recorded weekly beginning 1 week prior to dosing until evidence of copulation was observed.

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		Once evidence of mating was observed, body weights were recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and lactation days 1, 4, 7, 14 and 21.
3.4.3	Food/water consumption	Males: Individual F_0 and F_1 food consumption was measured weekly beginning 1 week prior to dosing until pairing. Food intake was not recorded during mating period. Following mating food consumption was measured weekly until scheduled necropsy. Females: Individual F_0 and F_1 food consumption was measured weekly beginning 1 week prior to dosing until pairing. Food intake was not recorded during mating period. Following mating food consumption was recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and lactation days 1, 4, 7, 14 and 21.
3.4.4	Oestrus cycle and mating performance	Vaginal lavages were performed daily and the slides were evaluated to assess the regularity and duration of the oestrous cycles of each F_0 and F_1 female for 21 days prior to mating and continuing until evidence of mating was observed or until the end of the mating period. Vaginal lavages were also performed on the day of necropsy to determine the stage of oestrous.
3.4.5	Duration of gestation	All females were allowed to deliver naturally and rear their young to weaning. During the period of expected parturition, the females were observed twice daily for initiation and completion of parturition and for signs of dystocia.
3.4.6	Sperm parameters	Each male pup was observed for balanopreputial separation beginning on PND 35. Examination of the pups continued daily until balanopreputial separation was present. The reproductive tract of each F_0 and F_1 male was exposed at necropsy. The right testis and epididymis were excised and weighted separately. Sperm motility was determined and analysis of a minimum of 200 motile and non-motile spermatozoa per animal was performed. Sperm morphology was evaluated and abnormal forms of sperm from a differential count of 200 spermatozoa per animal were recorded. The left testis and epididymis from all F_0 and F_1 males were weighed, stored frozen, homogenized and analyzed for determination of homogenization-resistant spermatid count and calculation of sperm production rate.
3.4.7	Offspring	Each litter was examined twice daily for survival. Offspring dying or euthanized in extremis between PND 0 and PND 4 were necropsied. Findings were recorded as either developmental variations or malformations. Detailed necropsy was performed on any pup dying or euthanized after PND 4 and prior to weaning. Changes in appearance or behaviour of the litters were recorded daily and detail physical examinations were performed on PND

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		1, 4, 7, 14 and 21. Any abnormalities in nursing behaviour were recorded. Body weights were recorded on PND 1, 4, 7, 14 and 21. Pups were sexed on PND 0, 4 and 21.
3.4.8	Schedule of sacrifice	All surviving F_0 adults were euthanized after selection of the F_1 generation and completion of a detailed clinical observation. All surviving F_1 adults were euthanized following weaning of the F_2 pups. All remaining non-selected F_1 and F_2 weanlings were euthanized on PND 21. A complete necropsy and selective histopathologic examination were conducted.
3.4.9	Necropsy	A complete necropsy was conducted on all F_0 and F_1 parental animals found dead, euthanized in extremis or at termination. Necropsy included examination of the external surfaces, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, and the thoracic, abdominal and pelvic cavities, including viscera. The number of former implantation sites was recorded for females that delivered or had macroscopic evidence of implantation and the number of unaccounted-for sites was calculated. The numbers of corpora lutea were also recorded for females necropsied through lactation day 4. For females that failed to deliver, a pregnancy status was determined, and any anatomic or pathologic findings that may have interfered with pregnancy were noted. Gross necropsy with emphasis on organs of the reproductive system was performed on all non-selected F_1 and F_2 weanlings on PND 21 and on additional F_1 male and female weanlings that were euthanized from PND 23-27.
3.4.10	Tissues preserved and organ weights	Tissues preserved from F_0: Adrenal glands (2), brain (forebrain, midbrain, hindbrain), cervix, coagulating gland, kidneys (2), liver (sections of 2 lobes), lungs, mammary gland, ovaries and oviducts (2), pituitary gland, prostate gland, seminal vesicles (2), spleen, stomach, testes with epididymides (1) and vas deferens, thyroids with parathyroids (2), uterus with vagina and all gross lesions. Tissues preserved from 1 pup/sex/litter from F_1 and F_2 pups euthanized on PND 21: Adrenal glands (2), cervix, coagulating gland, kidney, liver, mammary gland, ovaries and oviducts (2), pituitary gland, prostate gland, seminal vesicles (2), stomach, testes with epididymides (2) and vas deferens, uterus with vagina and all gross lesions. Organ weights were taken for F_0 and F_1 adults: Adrenal glands, brain, epididymides (total and cauda), kidneys, liver, ovaries, pituitary gland, prostate gland, seminal vesicles and coagulating glands (with accessory fluids), spleen, testes, thyroids and uterus with oviducts and cervix.

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		Organ weights were taken for 1 pup/sex/litter from F_1 and F_2 euthanized on PND 21: brain, spleen and thymus.
3.4.11	Histopathology P and F_1	F_0 and F_1 for the control and the high-dose groups and for all adults found dead or euthanized in extremis: Adrenal glands, cervix, coagulating gland, epididymides (right: caput, corpus and cauda), kidneys, liver, lungs, mammary gland, ovaries, oviducts, pituitary gland, prostate gland, seminal vesicles, stomach, testis (right), uterus, vagina and all gross lesions. Microscopic evaluations were performed on the reproductive organs for F_0 and F_1 parental animals from the low- and mid-dose groups that did not mate or produce offspring or for which oestrous cyclicity, sperm number, motility or morphology were affected.
3.4.12	Histopathology F_1 not selected for mating, F_2	The brain, spleen and thymus were weighted from 1 pup/sex/litter that survived to the scheduled termination on PND 21.
3.4.13	Mating and conception	The following were calculated: Male mating index Female mating index Male fertility index Female fertility index Male copulation index Female conception index
3.4.14	Litter data	The following were calculated: Mean live litter size Postnatal survival between birth and PND 0 or PND 4 Postnatal survival for all other intervals
3.5	**Statistics**	Analyses were conducted using two-tailed tests for a minimum significance level of 5% comparing each test article treated group to the control group by sex. Parental mating, fertility, conception and copulation indices were analyzed using the Chi-square test with Yates' correction factor. Mean parental and offspring body weights and body weight changes, parental food consumption and food efficiency data, oestrous cycle lengths, pre-coital intervals, gestation lengths, former implantation sites, live litter sizes, unaccounted-for sites, numbers of pups born, balanopreputial separation data, vaginal patency data, absolute and relative organ weights, sperm production rates and epididymal and testicular sperm numbers were subjected to a parametric one-way analysis of variance (ANOVA) to determine inter-group differences. If ANOVA revealed statistically significant inter-group variance, Dunnett's test was used to compare the test article-treated groups to the control group. Mean litter proportions of postnatal pup survival and pup sexes

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at birth, percentages of motile sperm and percentages of sperm with normal morphology were subjected to the Kruskal-Wallis nonparametric ANOVA test to determine inter-group differences. If the ANOVA revealed statistically significant inter-group variance, Dunn's test was used to compare the test article-treated groups to the control group. The mean F_1 ovarian primordial follicle count in the high-dose group was subjected to the Kruskal-Wallis nonparametric ANOVA test to determine inter-group differences.

3.6 Further remarks None

4 RESULTS AND DISCUSSION

4.1 Effects

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4.1.1	Parent males	10 mg/kg bw/day: No test article-related mortalities, clinical observations, changes in body and organ weights, effects on reproductive performance and on spermatogenesis endpoints were observed. Hyperplasia and hyperkeratosis of the squamous mucosa in the non-glandular area of the stomach was noted in a few animals. An ulcer was observed in one animal. These microscopic changes were considered to be test article related. 25 mg/kg bw/day: No test article-related changes in body and organ weights, effects on reproductive performance and on spermatogenesis endpoints were observed. Two animals were euthanized in extremis. Hyperplasia and hyperkeratosis of the squamous mucosa in the non-glandular area of the stomach was noted in most animals. Mucosal erosions were observed in the glandular mucosa. Areas of hyperplasia of the surface epithelium occurred in two animals. These microscopic changes were considered to be test article related. 65/50 mg/kg bw/day: No test article-related changes in organ weights, effects on reproductive performance and on spermatogenesis endpoints were observed. Two animals were euthanized in extremis. Statistically significant decreases in mean body weights were noted in the first 3 weeks. Statistically significant decreases in food consumption were observed during days 0-7, 7-14 and 14-21. Hyperplasia and hyperkeratosis of the squamous mucosa in the non-glandular area of the stomach was noted in most animals. There was also a submucosal inflammation observed in most animals as well as other changes including ulcers, erosions and keratinous cysts. Mucosal erosions were observed in the glandular mucosa. Areas of hyperplasia of the surface epithelium occurred in a few animals. An increase incidence of animals had areas of submucosal inflammation with mixed inflammatory cell infiltration. These microscopic changes were considered to be test article related. Results are summarised in Tables A6.8.2-1, A6.8.2-2 and A6.8.2-3.
4.1.2	Parent females	10 mg/kg bw/day: No test article-related mortalities, clinical observations, changes in body and organ weights, effects on reproductive performance and on the number of implantation sites were observed. Hyperplasia and hyperkeratosis of the squamous mucosa in the non-glandular area of the stomach was noted in a few animals.

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		These microscopic changes were considered to be test article related. 25 mg/kg bw/day: No test article-related changes in body and organ weights, effects on reproductive performance and on the number of implantation sites were observed. One animal was euthanized in extremis. Hyperplasia and hyperkeratosis of the squamous mucosa in the non-glandular area of the stomach was noted in most animals. Mucosal erosions were observed in the glandular mucosa. These microscopic changes were considered to be test article related. 65/50 mg/kg bw/day: No test article-related changes in organ weights, effects on reproductive performance and on the number of implantation sites were observed. Four animals were euthanized in extremis and 5 animals were found dead. Statistically significant decreases in body weight were noted from day 14 to 49. Statistically significant decreases in food consumption were observed during days 7-14 and 14-21. Hyperplasia and hyperkeratosis of the squamous mucosa in the non-glandular area of the stomach was noted in most animals. There was also a submucosal inflammation observed in most animals as well as other changes including ulcers, erosions and keratinous cysts. Mucosal erosions were observed in the glandular mucosa and one animal had as ulcer in the glandular mucosa. Areas of hyperplasia of the surface epithelium occurred in a few animals. An increase incidence of animals had areas of submucosal inflammation with mixed inflammatory cell infiltration. These changes were considered to be test article related. Results are summarised in Tables A6.8.2-1, A6.8.2-2 and A6.8.2-3.
4.1.3	F_1 males	Litter data: No test article related effects were noted at any of the dose levels. F_1 generation:

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		10 mg/kg bw/day: No test article-related mortalities, clinical observations, changes in body and organ weights, effects on reproductive performance and on spermatogenesis endpoints were observed. Hyperplasia and hyperkeratosis of the non-glandular mucosa was found at a minimal or mild degree. These microscopic changes were considered to be test article related. 25 mg/kg bw/day: No test article-related mortalities, clinical observations, changes in body and organ weights, effects on reproductive performance and on spermatogenesis endpoints were observed. Hyperplasia and hyperkeratosis of the non-glandular mucosa was found. These microscopic changes were considered to be test article related. 50 mg/kg bw/day: No test article-related changes in body and organ weights, effects on reproductive performance and on spermatogenesis endpoints were observed. Two animals were found dead. Evidence of test article related irritation was noted occasionally during the dose administration period. Five animals had thickened stomachs at scheduled necropsy. Hyperplasia and hyperkeratosis of the non-glandular mucosa was found. Submucosal inflammation, keratinous cysts, erosions and ulcers were found and were considered to be test article related. Results are summarised in Table A6.8.2-1.
4.1.4	F_1 females	Litter data:

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		No test article related effects were noted at any of the dose levels. F_1 generation: 10 mg/kg bw/day: No test article-related mortalities, clinical observations, changes in body and organ weights, effects on reproductive performance and on the number of implantation sites were observed. Hyperplasia and hyperkeratosis of the non-glandular mucosa was found at a minimal or mild degree. These microscopic changes were considered to be test article related. 25 mg/kg bw/day: No test article-related mortalities, clinical observations, changes in body and organ weights, effects on reproductive performance and on the number of implantation sites were observed. Hyperplasia and hyperkeratosis of the non-glandular mucosa was found. These microscopic changes were considered to be test article related. 50 mg/kg bw/day: No test article-related changes in organ weights, effects on reproductive performance and on the number of implantation sites were observed. Two animals were found dead and one animal was euthanized in extremis during the dose administration period. Evidence of test article related irritation was noted occasionally during the dose administration period. A lower mean body weight gain was noted during the first week of the dose administration period. One animal had thickened stomachs at scheduled necropsy. Hyperplasia and hyperkeratosis of the non-glandular mucosa was found. Submucosal inflammation, keratinous cysts, erosions and ulcers were found and were considered to be test article related. Results are summarised in Table A6.8.2-1.
4.1.5	F_2 males	No test article related effects were noted at any of the dose levels.
4.1.6	F_2 females	No test article related effects were noted at any of the dose levels.
4.2	**Other**	None
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	1,2-Benzisothiazolin-3-one was administered to 4 groups of 30 Crl:CD(SD) rats/sex/group at concentrations of 0, 10, 25 and 65/50 mg/kg bw/day over a two generation period. The study was carried out according to OECD Guideline 416 and is described under section 3 with no deviations.
5.2	**Results and discussion**	During the first three weeks of the study, the 65/50 mg/kg bw/day group was administered 65 mg/kg bw/day. This dose

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level proved to be excessive based on moribundity in some animals and lower food consumption and body weight gain during this period. On study day 22, the dose level was lowered to 50 mg/kg bw/day.

No treatment related effects were observed on oestrous cyclicity, reproductive performance, parturition, the mean number of unaccounted-for sites, spermatogenic endpoints, mean number of implantation sites, number of pups born and organ weights in the F_0 and F_1 generations.

There were no F_1 or F_2 pup clinical observations, effects on mean pup body weights, changes in organ weights or internal findings that could be related to test article administration.

Some deaths that occurred and some cases of euthanasia in extremis in the F_0 males in the 25 mg/kg bw/day group and in both sexes in both generations in the 65/50 mg/kg bw/day group were treatment related. Clinical findings in these cases were related to the known irritant properties of the test article. Microscopic findings of stomach lesions were also observed in these animals at necropsy.

Thickened stomachs were found at scheduled necropsy in some animals from both sexes of 50 mg/kg bw/day groups in the F_1 adults. Test article related microscopic changes in the stomach including hyperplasia and hyperkeratosis of the non-glandular mucosa, erosions, ulcers, submucosal inflammation and areas of hyperplasia of the surface epithelium of the glandular mucosa were observed in some animals at all dosage levels in both sexes of the F_0 and F_1 adults. The incidence and severity of the lesions observed in the gastric mucosa generally occurred in a dose-related manner with only a few rats in the 10 mg/kg bw/day group being affected with minimal of mild changes. These changes are consistent with an irritating effect caused by the test article on the gastric mucosa.

A statistically significant decrease in mean body weights, body weight gains, food consumption and food efficiency were noted in the 65/50 mg/kg bw/day group during the first 3 weeks. This was considered to be test article related.

F_1 postnatal survival in the 65/50 mg/kg bw/day group was reduced due to total litter loss in 2 females. F_2 postnatal survival was unaffected by administration of the test article to the F_1 parental animals.

5.3 Conclusion

Under the conditions of this study, the NOAEL of 1,2-Benzisothiazolin-3-one when administered to male and female rats was 50 mg/kg bw/day with respect to reproductive parameters (the highest dose tested). Based on the moribund condition of two F_0 males in the 25 mg/kg bw/day group and mortality in both sexes in both generations in the 65/50 mg/kg bw/day group and adverse clinical signs of significant reductions in mean body weight and food consumption and changes and irritation in the stomach in these animals prior to death, the NOAEL for the F_0 and F_1 generations was 10 mg/kg

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		bw/day. The NOAEL for neonatal toxicity was 25 mg/kg bw/day. The test article produced dose-related microscopic changes in the stomach indicative of an irritation effect at all dose levels in both generations. Given that only minimal to mild gastric mucosa changes were noted at 10 mg/kg bw/day, this dose was considered a minimal-effect level (MEL) with respect to local irritation at the site of dosing.
5.3.1	LO(A)EL	
5.3.1.1	Parent males	25 mg/kg bw/day (for toxicity) > 50 mg/kg bw/day (for reproductive parameters)
5.3.1.2	Parent females	25 mg/kg bw/day (for toxicity) > 50 mg/kg bw/day (for reproductive parameters)
5.3.1.3	F_1 males	25 mg/kg bw/day (for parental toxicity) > 50 mg/kg bw/day (for reproductive parameters)
5.3.1.4	F_1 females	25 mg/kg bw/day (for parental toxicity) > 50 mg/kg bw/day (for reproductive parameters)
5.3.1.5	F_2 males	50 mg/kg bw/day (for neonatal toxicity)
5.3.1.6	F_2 females	50 mg/kg bw/day (for neonatal toxicity)
5.3.2	NO(A)EL	
5.3.2.1	Parent males	10 mg/kg bw/day (for toxicity) 50 mg/kg bw/day (for reproductive parameters)
5.3.2.2	Parent females	10 mg/kg bw/day (for toxicity) 50 mg/kg bw/day (for reproductive parameters)
5.3.2.3	F_1 males	10 mg/kg bw/day (for parental toxicity) 25 mg/kg bw/day (for neonatal toxicity) 50 mg/kg bw/day (for reproductive parameters)
5.3.2.4	F_1 females	10 mg/kg bw/day (for parental toxicity) 25 mg/kg bw/day (for neonatal toxicity) 50 mg/kg bw/day (for reproductive parameters)
5.3.2.5	F_2 males	25 mg/kg bw/day (for neonatal toxicity)
5.3.2.6	F_2 females	25 mg/kg bw/day (for neonatal toxicity)
5.3.3	Reliability	1

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5.3.4	Deficiencies	None

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Materials and Methods	*Applicant's version is accepted.*
Results and discussion	*Applicant's version is accepted.*
Conclusion	*Applicant's conclusion is adopted.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A6.8.2-1: Summary of mortality data with 1,2-benzisothiazolin-3-one

Animals	Disposition	0 mg/kg bw/day	10 mg/kg bw/day	25 mg/kg bw/day	65/50 mg/kg bw/day
F_0 males	Found dead	0/30	0/30	0/30	0/30
	Euthanized in extremis	0/30	1/30	2/30	2/30
	Scheduled euthanasia	30/30	29/30	28/30	28/30
F_0 females	Found dead	0/30	0/30	0/30	5/30
	Euthanized in extremis	0/30	0/30	1/30	4/30
	Scheduled euthanasia	30/30	30/30	29/30	21/30
F_1 males	Found dead	0/30	0/30	0/30	2/30
	Euthanized in extremis	0/30	0/30	0/30	0/30

	Scheduled euthanasia	30/30	30/30	30/30	28/30
F_1 females	Found dead	0/30	1/30	1/30	2/30
	Euthanized in extremis	0/30	0/30	0/30	1/30
	Scheduled euthanasia	30/30	29/30	29/30	27/30

Table A6.8.2-2: Summary of male and female body weight values (g) with 1,2-benzisothiazolin-3-one – F_0 weekly (Mean and standard deviation)[a]

Days of study	0 mg/kg bw/day		10 mg/kg bw/day		25 mg/kg bw/day		65/50 mg/kg bw/day	
	Male	Female	Male	Female	Male	Female	Male	Female
0	315 ± 19.4	203 ± 12.2	315 ± 18.8	204 ± 12.8	314 ± 18.7	203 ± 12.3	312 ± 20.2	202 ± 12.4
7	360 ± 24.7	220 ± 15.6	353 ± 21.0	221 ± 14.5	355 ± 24.7	220 ± 14.6	341* ± 26.3	214 ± 11.8
14	398 ± 29.8	236 ± 19.9	391 ± 24.5	238 ± 16.0	389 ± 27.9	238 ± 16.3	372* ± 29.4	225* ± 18.7
21	431 ± 35.9	251 ± 21.3	442 ± 29.0	250 ± 15.8	418 ± 33.9	251 ± 15.0	400* ± 33.8	232* ± 19.7
28	455 ± 38.9	261 ± 23.4	448 ± 31.0	264 ± 18.8	443 ± 34.5	260 ± 17.9	427* ± 35.2	245* ± 19.3
35	475 ± 46.1	269 ± 25.1	472 ± 34.4	272 ± 18.8	468 ± 36.7	271 ± 18.9	454 ± 39.5	254* ± 23.9
42	496 ± 42.9	276 ± 24.0	489 ± 36.7	279 ± 16.4	490 ± 38.7	280 ± 19.8	470 ± 44.2	263* ± 16.7
49	515 ± 48.6	284 ± 25.7	508 ± 36.6	285 ± 19.6	512 ± 42.6	286 ± 20.1	489 ± 47.7	270* ± 18.3

a: Only the period that had test material related effects is reported in this table
*: Significantly different from the control group at 0.05 using Dunnett's test

Table A6.8.2-3: Summary of male and female food consumption (g/animal/day) with 1,2-benzisothiazolin-3-one – F_0 weekly (Mean and standard deviation)[a]

Days of study	0 mg/kg bw/day		10 mg/kg bw/day		25 mg/kg bw/day		65/50 mg/kg bw/day	
	Male	Female	Male	Female	Male	Female	Male	Female
0	28 ± 2.4	18 ± 1.6	27 ± 2.0	19 ± 1.5	27 ± 2.3	19 ± 1.5	25* ± 2.6	18 ± 2.0
7	28 ± 2.5	19 ± 2.2	28 ± 1.8	20 ± 1.7	28 ± 2.6	19 ± 1.4	26* ± 2.2	17* ± 2.3
14	29 ± 2.8	20 ± 2.0	28 ± 1.9	20 ± 1.4	28 ± 3.1	19 ± 2.0	27 ± 2.9	17* ± 2.0
21	28 ± 3.1	20 ± 2.5	29 ± 2.5	21 ± 1.8	28 ± 3.4	20 ± 2.2	27 ± 2.9	19 ± 1.9

a: Only the period that had test material related effects is reported in this table
*: Significantly different from the control group at 0.05 using Dunnett's test

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.9	**Neurotoxicity**	
Annex Point IIIA 6.9		
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	This point is not relevant as it is only required for substances of similar or related structures to those capable of inducing (delayed) neurotoxicity such as organophosphates and carbamates. In the acute oral study summarised under point IIIA, 6.1.1, clinical signs such as hypoactivity, hunched posture, pain reflex absent, righting reflex absent were noted at dose levels much higher than those expected in an occupational scenario. Therefore, such effects which are considered to be a result of overt acute systemic toxicity rather than a consequence of neurotoxicity, are not sufficient to conclude on potential neurotoxic effects. Furthermore, results from the ADME study did not show that BIT can cross the cerebral barrier. Effects of BIT on the neurons would be unlikely. No neurological effects have been reported in either the acute dermal study in rabbits or the 90-day oral study in rats. It is worth noting that the 90-day oral study investigated possible neurological effects such as motor activity, sensory re-activity, grip strength and hind limb foot splay. These observations were all normal throughout the study. Furthermore, the developmental toxicity study did not show any neurological effects in rats or rabbits. Therefore, it is concluded that BIT is not neurotoxic and no study to investigate the potential acute or subchronic neurotoxicity of BIT is required.	
Undertaking of intended data submission [ ]	Not applicable	

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Evaluation of applicant's justification	*Applicant's justification is accepted.*
Conclusion	*Applicant isexemted of the neurotoxicity study.*
Remarks	

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.10 **Annex Point IIIA 6.10**	**Mechanistic study**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	It is proposed that sufficient information is provided to explain the effects and to address the metabolism of 1,2-benzisothiazol-3-(2*H*)-one in mammals. Please refer to Doc. III-A, 6.2/1. It is proposed that additional studies are not required in the case of BIT.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*October 2008*	
Evaluation of applicant's justification	*Applicant's justification is accepted.*	
Conclusion	*Applicant isexemted of the mechanistic studies.*	
Remarks		

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.11 **Annex Point IIIA 6.11**	**Studies on other routes of administration (parenteral routes)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	For existing active substances, these studies on alternative routes only need to be submitted if the data already exists. New studies are normally only required in exceptional circumstances. The oral, dermal and inhalation routes of exposure in the case of 1,2-benzisothiazol-3-(2*H*)-one (BIT) have been investigated in the available studies and are expected to be the major and most relevant routes of exposure for the intended use(s) of BIT within PT 2. It is proposed that additional studies are not required in the case of BIT.	
Undertaking of intended data submission [ ]	Not relevant	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*October 2008*	
Evaluation of applicant's justification	*Applicant's justification is accepted.*	
Conclusion	*Applicant isexemted of studies on other routes of administration.*	
Remarks		

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.12.1 **Annex Point IIIA 6.12.1**	**Medical surveillance data on manufacturing plant personnel**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Troy Chemical Company BV have been working with BIT for many years. Over the years, no serious health problems have been recorded in workers of either company.	
Undertaking of intended data submission [ ]	Not relevant	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*October 2008*	
Evaluation of applicant's justification	*Applicant's justification is accepted.*	
Conclusion	*Applicant is exempted to display medical surveillance data on manufacturing plant personnel.*	
Remarks		

Section A6 **Subsection A6.12.2** **Annex Point IIIA 6.12.2**	**Toxicological and Metabolic Studies** **Direct observation (clinical cases, poisoning incidents)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	No clinical cases or poisoning incidents have been reported for any of the Troy Chemical Company BV employees.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*October 2008*	
Evaluation of applicant's justification	*Applicant's justification is accepted.*	
Conclusion	*Applicant is exempted to display clinical cases or poisonings incidents.*	
Remarks		

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.12.3	**Medical data – Worker Health Incidences**	
Annex Point IIIA 6.12.3		
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	[redacted]. *et al*, (1981) Occupational dermatitis due to 1,2-Benzisothiazolin-3-one. Contact Dermatitis:7:145-147 reports that three men working as mould makers in the pottery industry acquired contact allergic dermatitis due to releasing oil which contained BIT at up to 1.6%. Observations included rashes and eczema of the hands, fingers and forearms. All three individuals had positive responses to the patch test. A patch test conducted on 45 human volunteers tested BIT at 500 ppm in propylene glycol. Several volunteers were sensitized. Please note that the sensitisation/allergenicity observations which have been recorded are summarised in documents IIIA 6.12.6-1, IIIA 6.12.6-2, IIIA 6.12.6-3 and IIIA 6.12.6-4.	
Undertaking of intended data submission [ ]	Not applicable	

Evaluation by Competent Authorities

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Evaluation of applicant's justification	*The justification is supported on previously published data. This information allows justifying the absence of health records.*
Conclusion	*The available information allows concluding, without health records form industry or any other source, that a commercial product containing diluted BIT caused three cases of sensitization in humans.*
Remarks	*The information about patch test conducted on 45 human volunteers is not displayed in the document IV and could not be evaluated. Therefore, this information will not be taken into consideration.*

Section A6 **Subsection A6.12.4** **Annex Point IIIA 6.12.4**	**Toxicological and Metabolic Studies** **EPIDEMIOLOGICAL STUDIES ON THE GENERAL POPULATION**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	To our knowledge, no epidemiological studies are available for 1,2-benzisothiazol-3-(2*H*)-one (BIT).	
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*October 2008*
Evaluation of applicant's justification	*Applicant's justification is accepted.*
Conclusion	*Applicant is exempted to display epidemiological studies on the general population.*
Remarks	

Section A6 **Subsection A6.12.5** **Annex Point IIAV1.6.9.5**	**Toxicological and Metabolic Studies** **DIAGNOSIS OF POISONING INCLUDING SPECIFIC SIGNS OF POISONING AND CLINICAL TESTS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Eye exposure: Risk of serious damage to eyes. Skin exposure: Brief contact may cause severe skin irritation with pain and local redness. Prolonged or widespread skin contact may result in absorption of harmful amounts. May cause sensitisation by skin contact. Inhalation exposure: At room temperature, exposure to vapour is minimal due to low volatility; single exposure is not likely to be hazardous. Ingestion: Harmful if swallowed. Swallowing may result in irritation or burns of the mouth, throat, and gastrointestinal tract.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Ocotber 2008.*	
Evaluation of applicant's justification	*This is information never might be displayed in a justification for nonsubmission of data form because is information about hazards of exposure through several routes and really does not help to the diagnostic of poisonings.*	
Conclusion	*The displayed information is not suitable for diagnosis of poisonings caused by BIT.*	
Remarks		

Section A6 **Subsection A6.12.6/1** **Annex Point IIAVI.6.9.6**	**Toxicological and Metabolic Studies** **SENSITISATION/ALLERGENICITY OBSERVATIONS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	[redacted] After defining the optimal patch test concentration, the prevalence of 1,2-benzisothiazolin-3-one contact allergy both in persons at occupational risk and in random dermatological patients, without clear occupational risk, was analysed. [redacted]	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.12.6/1	**SENSITISATION/ALLERGENICITY OBSERVATIONS**
Annex Point IIAVI.6.9.6	
	[redacted]
Undertaking of intended data submission [ ]	Not applicable
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008.*
Evaluation of applicant's justification	*The justification is supported on previously published data. This information allows justifying the absence of sensitization/allergenicity observations.*
Conclusion	*All information supplied in the detailed justification and its conclusions are accepted.*
Remarks	

Section A6 **Subsection A6.12.6/2** **Annex Point IIAVI.6.9.6**	**Toxicological and Metabolic Studies** **SENSITISATION/ALLERGENICITY OBSERVATIONS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	[redacted] (1992), Allergic contact dermatitis due to 1,2-benzisothiazolin-3-one in paint manufacture **Case Report** [redacted]	
Undertaking of intended data submission [ ]	Not applicable	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date *October 2008.*

Evaluation of applicant's justification *The justification is supported on previously published data. This information allows justifying the absence of sensitization/allergenicity observations.*

Conclusion *All information supplied in the detailed justification and its conclusions are accepted.*

Remarks

Section A6 **Subsection A6.12.6/3** **Annex Point IIAVI.6.9.6**	**Toxicological and Metabolic Studies** **SENSITISATION/ALLERGENICITY OBSERVATIONS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*October 2008.*	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.12.6/3 **Annex Point IIAVI.6.9.6**	**SENSITISATION/ALLERGENICITY OBSERVATIONS**
Evaluation of applicant's justification	*The justification is supported on a non-published report which summarizes the main results previously published in the scientific literature. This information, together with information displayed in documents A6.12.6.1 and A6.12.6.2 allows justifying the absence of sensitization/allergenicity observations.*
Conclusion	*All information supplied in the detailed justification and its conclusions are accepted.*
Remarks	

Section A6 **Subsection A6.12.6/4** **Annex Point IIAV1.6.9.6**	**Toxicological and Metabolic Studies** **SENSITISATION/ALLERGENICITY OBSERVATIONS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*October 2008.*	
Evaluation of applicant's justification	*The justification is supported on previously published data. This information might allow justifying the absence of sensitization/allergenicity observations.*	
Conclusion	*The corresponding document IV was not supplied by the applicant. Thus this information could not be evaluated and was not taken into consiederation.*	
Remarks		

Section A6 **Subsection A6.12.7** **Annex Point IIAV1.6.9.7**	**Toxicological and Metabolic Studies** **SPECIFIC TREATMENT IN CASE OF AN ACCIDENT OR POISONING: FIRST AID MEASURE, ANTIDOTES AND MEDICAL TREATMENT, IF KNOWN**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Ensure it is safe to approach the casualty. Before giving first aid always put on personal protective equipment if you are likely to be contaminated. Always seek medical advice in cases of serious personal contamination. Remove any contaminated clothing. Keep the patient at rest and if possible under shelter. If drowsy or unconscious, place the casualty in the recovery position, maintain an open airway and loosen any constrictive clothing at neck and waist. If breathing ceases or weakens, immediately apply artificial resuscitation. If the person is conscious and breathing, apply first aid as follows: Inhalation: Move to fresh air and seek medical advice. Skin Contact: Immediately wash skin with soap and plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Obtain medical attention without delay. Wash clothing before reuse. Items which cannot be decontaminated, including leather articles such as shoes, belts, and watchbands should be disposed of properly. Eye Contact: Immediately flush eyes with water; remove contact lenses, if present, after the first 5 minutes, then continue to flush eyes for at least 15 minutes. Obtain medical attention without delay, preferably from an ophthalmologist. Ingestion: Do not induce vomiting. Give one cup (240 mL) of water or milk if available and transport to a medical facility. Do not give anything by mouth to an unconscious person. Due to irritant properties, swallowing may result in burns/ulceration of mouth, stomach and lower gastrointestinal tract with subsequent stricture. Aspiration of vomitus may cause lung injury. Suggest endotracheal /esophageal control if lavage is done.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*October 2008.*	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.12.7 **Annex Point IIAV1.6.9.7**	**SPECIFIC TREATMENT IN CASE OF AN ACCIDENT OR POISONING: FIRST AID MEASURE, ANTIDOTES AND MEDICAL TREATMENT, IF KNOWN**
Evaluation of applicant's justification	*This is information never might be displayed in a justification for nonsubmission of data form because is information about specific treatment in case of an accident or poisoning.*
Conclusion	*All information supplied in the detailed justification is accepted.*
Remarks	

Section A6 **Subsection A6.12.8** **Annex Point IIA6.9.8**	**Toxicological and Metabolic Studies** **PROGNOSIS FOLLOWING POISONING**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	No significant acute or chronic health effects are anticipated from exposures incidental to normal industrial handling.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*27 October 2008*	
Evaluation of applicant's justification	*Applicant justification is accepted.*	
Conclusion	*Applicant is exempted to supply prognosis following poisoning.*	
Remarks		

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.13 **Annex Point IIIA VI. 2**	**TOXIC EFFECTS ON LIVESTOCK AND PETS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X] **Limited exposure [X]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	1,2-benzisothiazol-3(2*H*)-one (BIT) is intended for use as a preservative in metalworking fluids at very low levels. It is not intended for use in spaces in which animals are housed, kept or transported and exposure of drinking water or feeding-stuffs is not anticipated. It is not used in the manufacture of feeding troughs, animal bedding or beehives. Furthermore, the highest concentration of BIT in the end-use products is only 0.4% in the metalworking fluid concentrate. Therefore, it is considered that the exposure is negligible. The acute and sub acute effects of this preservative on the target species have been discussed in relation to tests on laboratory rodents. In addition the toxic response of BIT has been summarised for various species that are known to be representative and used to extrapolate effects to humans. Therefore, there is sufficient information available to address toxicity effects on livestock and pets. Based on results of acute toxicity tests in the rat, BIT is classified as harmful if swallowed and remains unclassified by the dermal route. It is not a skin irritant. However, it is considered an eye irritant. BIT is also a dermal sensitiser. A 90-day oral study in the rat is available and is summarised under Doc IIIA, 6.4.1. Only minimal signs of toxicity such a slight reduction in mean body weight, an increase in cholesterol in males at 75 mg/kg bw/day and a decrease in RBC in females at 75 mg/kg bw/day (the highest dose tested) were noted in this study. A 90 day dermal study in the rat is available and is summarised under Doc. III-A, 6.4.2. A small number of clinical signs such as swollen eyes, tremors, piloerection and soiling of the anogenital area were noted in a few animals in all groups. However most animals appeared normal throughout the study. An oral 90-day study in the dog is available and is summarised under point IIIA, 6.4.1/2. There were no treatment related clinical observations or changes in haematology or urinalysis parameters or organ weights. There were no ophthalmic, macroscopic or microscopic findings. The only changes noted were lower body weights at 3000 ppm in males and females and lower food consumption at 3000 ppm in females when compared to control animals. A SAR analysis has predicted that BIT is not carcinogenic. Furthermore, BIT is neither genotoxic, nor teratogenic. It is considered that sufficient data is already available to conclude that it would not be toxic to livestock and pets in the unlikely event of these being exposed.	

Section A6	**Toxicological and Metabolic Studies**
Subsection A6.13	**TOXIC EFFECTS ON LIVESTOCK AND PETS**
Annex Point IIIA VI. 2	
	In conclusion, there are no ethical grounds (that would not contravene the requirements of Directive 86/609/EC which advises against unnecessary testing using animals) for performing further studies on animals. It is therefore proposed that no additional investigations are required to address this point.
Undertaking of intended data submission [ ]	Not applicable
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2008*
Evaluation of applicant's justification	*Applicant's justification is accepted.*
Conclusion	*Applicant is exempted to study toxic effects on livestock and pets.*
Remarks	

Section A6	Toxicological and Metabolic Studies	
Subsection A6.14 **Annex Point IIIA 6.14**	**OTHER TESTS RELATED TO THE EXPOSURE OF HUMANS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	It is proposed that other tests related to the exposure of humans are not required for 1,2-benzisothiazol-3(2H)-one (BIT) based on the low human exposure expected during the use of BIT as a preservative in biocides. No cases of poisoning or clinical cases due to worker exposure have been reported during the manufacturing process at Troy Chemical Company BV over many years. From this we can also conclude that they are no reaction products or by-products of toxicological concern contained in BIT. The synthesis pathway for BIT is presented as confidential information in Doc. III – Business confidential information together with identified impurities.	
Undertaking of intended data submission [ ]	Not applicable	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*October 2008*
Evaluation of applicant's justification	*Applicant's justification is accepted.*
Conclusion	*Applicant is exempted of other test related to the exposure of humans.*
Remarks	

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.15	**Food and feedingstuffs**	
Annex Point IIIA6.15		
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Proposed acceptable residue levels are not required for product type 13 (metalworking fluids) according to Directive 98/8/EC. Exposure of food and feedingstuffs to the product is unlikely to occur considering its use pattern. Furthermore, the claimed label states that the biocidal product must be kept away from food, drink and animal feedingstuff and as such BIT will not be used for direct application to food or feeding stuffs. The biocidal product [redacted] contains only 20% w/w BIT (industrial uses). Industrial users manufacture metalworking fluid concentrates adding the preservative [redacted] to the preparation to obtain a concentration up to 0.4% w/w BIT. Professionals dilute the metalworking fluid concentrate to obtain the metalworking fluid emulsion containing up to 0.04% w/w of the preservative BIT and use the metalworking emulsion in different metalworking activities. It is not classified as harmful to humans but it is considered as an irritant to skin and may cause serious damage to the eyes and is therefore, assigned Xn, R22, R41 and R43. Therefore, it is reasonable to conclude that exposure of food and feedingstuffs to the active substance will not occur considering the use pattern of the biocidal product and tests to determine residues in food and feedingstuffs are unnecessary. This justification also applies to point IIIA, 6.15.1 to 6.15.6.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*October 2008*	
Evaluation of applicant's justification	*Applicant justification is accepted.*	
Conclusion	*Applicant is exempted of studies on food and feedingstuffs.*	
Remarks		

Section A6	**Toxicological and Metabolic Studies**	
Subsection A6.16 Annex Point IIIA6.3.5-IIIA11.2	**ANY OTHER TESTS RELATED TO THE EXPOSURE OF THE ACTIVE SUBSTANCE TO HUMANS, IN ITS PROPOSED BIOCIDAL PRODUCTS, THAT ARE CONSIDERED NECESSARY**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	No other additional tests relating to exposure of 1,2-benzisothiazol-3(2*H*)-one (BIT), other than those outlined in previous data points, are considered necessary at this time.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*27 October 2008*	
Evaluation of applicant's justification	*Applicant's justification is accepted.*	
Conclusion	*Applicant is exempted to display any other test related to the exposure of the active substance to humans.*	
Remarks		

Section A6 **Subsection A6.17** **Annex Point IIIA6.17**	**Toxicological and Metabolic Studies** **If the active substance is to be used in products for action against plants then tests to assess toxic effects of metabolites from treated plants where different from those identified in animals shall be required**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Since this PT 13 submission does not support the use of 1,2-benzisothiazol-3(2*H*)-one (BIT) for action against plants, tests to assess toxic effects of metabolites on treated plants are not required in this case.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*27 October 2008*	
Evaluation of applicant's justification	*Applicant's justification is accepted.*	
Conclusion	*Applicant is exempted to perform test to assess toxic effects of metabolites from treated plants where different from those identified in animals.*	
Remarks		

Section A6 **Subsection A6.18** **Annex Point IIIAVI.6**	**Toxicological and Metabolic Studies** **SUMMARY OF MAMMALIAN TOXICOLOGY AND CONCLUSIONS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	For details on mammalian toxicology and conclusions, please refer to Doc. II-A.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*October 2008*	
Evaluation of applicant's justification	*Applicant's justification is accepted.*	
Conclusion	*Applicant is exempted to perform the summary of mammalian toxicology and conclusions because enough information is displayed in Doc. II-A.*	
Remarks		

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.1/1 ABIOTIC

Annex Point IIA7.6.2.1 Hydrolysis as a function of ph and identification of breakdown products (01)

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: January 02, 2002 – January 24, 2002.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access	Rohm and Haas	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on OECD guideline 111 Hydrolysis as a Function of pH.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, this study deviates from OECD guideline 111 in the following respects: 1. A buffer solution at pH 5.0 was used instead of at pH 4.0 2. The study was carried out for 7 days instead of 5 days 3. No DT_{50} or r^2 values were reported 4. No graph of test substance concentration versus time was reported However, these deviations are not considered to compromise the scientific validity of this study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisotiazolin-3-one	
3.1.1	Lot/Batch number	BT 12000	
3.1.2	Specification	Please refer to Doc. III-A, 2/2	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**	
Subsection A7.1.1.1.1/1	**ABIOTIC**	
Annex Point IIA7.6.2.1	**Hydrolysis as a function of ph and identification of breakdown products (01)**	
3.1.3 Purity	98 %	
3.1.4 Further relevant properties	The solubility of 1,2-benzisothiazolin-3-one in water is 1.15 g/L.	
3.2 Reference substance	No	
3.2.1 Initial concentration of reference substance	Not applicable	
3.3 Test solution	Please refer to Tables A7.1.1.1.1/1-1 and A7.1.1.1.1/1-2	
3.4 Testing procedure		
3.4.1 Test system	Please refer to Table A7.1.1.1.1/1-3	
3.4.2 Temperature	50 °C ± 1 °C	
3.4.3 pH	5, 7 and 9	**X**
3.4.4 Duration of the test	7 days	**X**
3.4.5 Number of replicates	Two samples were taken for each pH at each sampling period.	
3.4.6 Sampling	Duplicate samples were taken on day 0, day 3 and day 7 for each pH value.	
3.4.7 Analytical methods	The concentration of 1,2-benzisothiazolin-3-one in aqueous solutions was determined using High Performance Liquid Chromatography with UV detection. Quantitation occurred using the calibration graph constructed from the 1,2-benzisothiazolin-3-one calibration solutions. The following chromatographic conditions were used: Column: Hypersil BDS C18 (5 µm) 250 x 4.6 mm Mobile Phase: 54.5 % Milli-Q water 45 % methanol 0.5 % acetic acid Injection volume: 20 µL Flow: 1.0 mL/min Detection: 318 nm (DAD)	

Section A7		**Ecotoxicological Profile Including Environmental Fate and Behaviour**	
Subsection A7.1.1.1.1/1		**ABIOTIC**	
Annex Point IIA7.6.2.1		**Hydrolysis as a function of ph and identification of breakdown products (01)**	
3.5	**Preliminary test**	Yes pH 5.0 0.1 mol/L monopotassium citrate + 0.1 mol/L sodium hydroxide pH 7.0 0.1 mol/L monopotassium phosphate + 0.1 mol/L sodium hydroxide pH 9.0 0.1 mol/L boric acid in 0.1 mol/L potassium chloride + 0.1 mol/L sodium hydroxide	
		4 RESULTS	
4.1	**Concentration and hydrolysis values**	Please refer to Tables A7.1.1.1.1/1-4 and A7.1.1.1.1/1-5	
4.2	**Hydrolysis rate constant (k_H)**	Not documented	
4.3	**Dissipation time**	1,2-benzisothiazolin-3-one was found to be hydrolytically stable at pH 5, 7 and 9 as the percentage hydrolysis after 7 days at 50 °C was less than 10 %.	
4.4	**Concentration – time data**	Please refer to Tables A7.1.1.1.1/1-4 and A7.1.1.1.1/1-5	
4.5	**Specification of the transformation products**	No transformation products were detected	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Two 5 mL samples of 1,2-benzisothaizolin-3-one/DMF stock solution were made up 50 mL with buffer solutions at pH 5, 7 and 9. The test was carried out at 50 °C and the concentartion of 1,2-benzisothiazolin-3-one in the samples was determined at t = 0, t = 3 days and t = 7 days using HPLC. This study was conducted according OECD method 111 and is described under section 2 with the following deviations: 1. A buffer solution at pH 5.0 was used instead of at pH 4.0 2. The study was carried out for 7 days instead of 5 days 3. No DT_{50} or r^2 values were reported 4. No graph of test substance concentration versus time was reported However, these deviations are not considered to compromise the scientific validity of this study.	**X**

Section A7		**Ecotoxicological Profile Including Environmental Fate and Behaviour**	
Subsection A7.1.1.1.1/1		**ABIOTIC**	
Annex Point IIA7.6.2.1		**Hydrolysis as a function of ph and identification of breakdown products (01)**	
5.2	**Results and discussion**	The percentage hydrolysis after 7days at 50 °C was 2.4 %, 2.9 % and 2.3 % for hydrolysis solutions at pH 5.0, pH 7.0 and pH 9.0, respectively. Since the percentage hydrolysis of 1,2-benzisothiazolin-3-one in all buffer solutions is less than 10 % after incubation at 50 °C for 7 days, the test substance 1,2-benzisothiazolin-3-one is considered to be hydrolytically stable at pH 5.0, 7.0 and 9.0.	
5.2.1	k_H	Not documented	
5.2.2	DT_{50}	Not documented	
5.2.3	r^2	Not documented	
5.3	**Conclusion**	1,2-benzisothiazolin-3-one is considered to be hydrolytically stable at pH 5.0, 7.0 and 9.0.	
5.3.1	Reliability	1	**X**
5.3.2	Deficiencies	Yes Deficiencies were noted and outlined under points 2.3 and 5.1. However, they do not compromise the scientific validity of this study.	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2009*
Materials and Methods	*Applicant's version is accepted but with the following comments:* *3.4.3. There is no data for pH 4, which can affect the validity of this study. Nevertheless, this pH value is include in the second study reported (7.1.1.1.1./2).* *3.4.4. OCDE method 111 specifies that the duration of the test should be 5 days, instead of the 7 days reported, but this do not compromise the scientific validity of this study.*

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.1.1.1.1/1	**ABIOTIC**
Annex Point IIA7.6.2.1	**Hydrolysis as a function of ph and identification of breakdown products (01)**

Results and discussion	*Adopt applicant's version*
Conclusion	*1,2-benzisothiazolin-3-one is considered to be hydrolytically stable at pH 5.0, 7.0 and 9.0.*
Reliability	*2*
Acceptability	*Acceptable, despite the lack of data for hydrolysis at pH = 4.*
Remarks	*BIT concentration used in this study is approximately 10 times lower than the half-saturated concentration specified in OCDE method 111. However, this concentration employed in this study is around 400 times higher than LOD. Thus, this do not compromise the validity of the study.*

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

pH	Type of buffer (final molarity)	Composition
5	Not described	1 L of 0.1 mol/L monopotassium citrate + 934 mL of 0.1 mol/L sodium hydroxide made up to 2 L with Milli-Q water
7	Not described	2 L of 0.1 mol/L monopotassium phosphate + 1184 mL of 0.1 mol/L sodium hydroxide made up to 4 L with Milli-Q water
9	Not described	426 mL of 0.1 mol/L sodium hydroxide + 1 L of 0.1 mol/L boric acid (in 0.1 mol/L potassium chloride) made up to 2 L with Milli-Q water
The pH of the bulk buffer solutions were adjusted to 4.0 ± 0.2, 7.0 ± 0.2, and 9.0 ± 0.2.		

Table A7.1.1.1.1/1-2: Description of test solution

Criteria	Details
Purity of water	Milli-Q water
Preparation of test medium	A stock solution containing 9.97g 1,2-benzisothiazolin-3-one/1 DMF was prepared by transferring 249.15 mg 1,2-benzisothiazolin-3-one in a 25 mL volumetric flask. The flask was brought to volume with DMF. For each hydrolysis solution 5 ml of this stock solution was transferred to a 50 mL volumetric flask and each flask was made up to volume with buffer solutions pH 5.0, pH 7.0 and pH 9.0 respectively. For each study sample 4 mL of the diluted stock solution was transferred in a 100 mL volumetric flask and each flask was made up to a volume with buffer solutions with pH 5.0, pH 7.0 and pH 9.0, respectively and each flask was homogenised. The prepared concentration in the three hydrolysis solutions was 39.9 mg 1,2-benzisothiazolin-3-one/ 1 buffer solution. In order to exclude the occurrence of biodegradation of the test substance, glassware was sterilised at 120 °C for at least 30 minutes before use. All hydrolysis solutions were sterilised by filtration over a 0.45 µm filter. Oxygen was excluded by bubbling nitrogen through the solutions for 5 minutes. Subsequently the sample vials (10 mL) were filled with the hydrolysis solutions with a minimum of headspace in the vials and closed with a teflon coated crimpcap closure. The test was carried out in the dark to avoid photolytic ineterference.
Test concentrations (mg a.i./L)	39.9 mg 1,2-benzisothiazolin-3-one/ 1 buffer solution
Temperature (ºC)	50 °C ± 1 °C
Controls	In order to determine the between-run variation, QC samples containing 39.9 mg 1,2-benzisothiazolin-3-one/1 pH 7.0 buffer solution, were prepared on t = 0 and stored in the refrigerator. A QC sample was analysed on each day of analysis of the study samples.
Identity and concentration of co-solvent	Not applicable
Replicates	Yes, two samples were taken for each pH at each sampling period.

Table A7.1.1.1.1/1-3: Description of test system

Criteria	Details
Glassware	25 mL, 50 mL and 100 mL volumetric flasks.
Other equipment	HPLC with UV detection, pH meter
Method of sterilization	Glassware was sterilised at 120 °C for at least 30 minutes before use.

Table A7.1.1.1.1/1-4: Hydrolysis of 1,2-benzisothiazolin-3-one at pH 5, 7 and 9

Study Samples	Measured concentration of 1,2-benzisothiazolin-3-one in study samples (mg/L)			Percentage hydrolysis after 7 days (%)
	t = 0	t = 3 days	t = 7 days	
pH 5.0	39.7	38.6	38.8	2.4
	39.7	38.6	38.8	
	Mean: 39.7	Mean: 38.6	Mean: 38.8	
pH 7.0	39.3	38.2	38.2	2.9
	39.3	38.3	38.1	
	Mean: 39.3	Mean: 38.3	Mean: 38.2	
pH 9.0	38.2	37.6	37.3	2.3
	38.2	37.6	37.2	
	Mean: 38.2	Mean: 37.6	Mean: 37.3	

Table A7.1.1.1.1/1-5: Results of pH determinations of the incubated study samples

Day	pH of incubated solutions at pH 5.0	pH of incubated solutions at pH 7.0	pH of incubated solutions at pH 9.0
0	5.02	7.09	9.00
3	4.99	7.03	8.97
	4.96	7.03	8.97
7	4.95	7.03	8.99
	4.96	7.04	8.99

Note: On day 0 the pH was determined in the hydrolysis solution imediately prior to filling the sample vials. On days 3 and 7 the pH was determined in both sample vials.

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.1/2 ABIOTIC

Annex Point IIA7.6.2.1 Hydrolysis as a function of ph and identification of breakdown products (02)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: August 14, 2002 – August 28, 2002.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on OECD guideline 111 Hydrolysis as a Function of pH.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, this study deviates from OECD guideline 111 in the following respects: 1. The buffer solutions were scaled up by a factor of 10 However, this deviation is not considered to compromise the scientific validity of this study.	**X**
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-(2*H*)-one	
3.1.1	Lot/Batch number	BT17301	
3.1.2	Specification	Please refer to Doc. III-A, 2/1	
3.1.3	Purity	97.42 %	
3.1.4	Further relevant properties	Not applicable	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.1.1.1.1/2	**ABIOTIC**
Annex Point IIA7.6.2.1	**Hydrolysis as a function of ph and identification of breakdown products (02)**

3.2	**Reference substance**	No	
3.2.1	Initial concentration of reference substance	Not applicable	
3.3	**Test solution**	Please refer to Tables A7.1.1.1.1/2-1 and A7.1.1.1.1/2-2	
3.4	**Testing procedure**		
3.4.1	Test system	Please refer to Table A7.1.1.1.1/2-3	
3.4.2	Temperature	50 °C ± 0.5 °C	
3.4.3	pH	4, 7 and 9	
3.4.4	Duration of the test	5 days	
3.4.5	Number of replicates	One	**X**
3.4.6	Sampling	Duplicate sampling took place at day 0 hours, 4 hours and 5 days.	
3.4.7	Analytical methods	All the samples for analytical method validation and the test samples were analysed by HPLC. The following chromatographic conditions were used: Column: C18 (24 cm x 4.6 mm (id) x 5 µm particle size) Mobile Phase: 15 % acetonitrile 85 % water 1.5 % acetic acid Injection volume: 20 µL Flow: 1.7 mL/min Detection: SPD 10A UV-VIS detector with CLASS LC-10 software	
3.5	**Preliminary test**	Yes pH 4: 4 mL 0.1 N sodium hydroxide solution + 500 mL 0.1 M potassium biphthalate (potassium hydrogen phthalate) and made up to 1000 mL using sterile distilled water	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**	
Subsection A7.1.1.1.1/2	**ABIOTIC**	
Annex Point IIA7.6.2.1	**Hydrolysis as a function of ph and identification of breakdown products (02)**	
	pH 7: 296.3 mL 0.1 N sodium hydroxide solution + 500 mL 0.1 M monopotassium phosphate solution and made up to 1000 mL using sterile distilled water pH 9: 213.0 mL 0.1 N sodium hydroxide solution + 500 mL 0.1 M boric acid in 0.1 M potassium chloride solution and made up to 1000 mL using sterile distilled water	
	4 RESULTS	
4.1 **Concentration and hydrolysis values**	Please refer to Table A7.1.1.1.1/2-4	
4.2 **Hydrolysis rate constant (k_H)**	Not documented	
4.3 **Dissipation time**	1,2-benzisothiazolin-3-(2*H*)-one was found to be hydrolytically stable at pH 4, 7 and 9 as the percentage hydrolysis after 5 days at 50 °C was less than 10 %.	
4.4 **Concentration – time data**	Please refer to Table A7.1.1.1.1/2-4	
4.5 **Specification of the transformation products**	No transformation products were detected	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 **Materials and methods**	The hydrolysis of 1,2-benzisothiazol-3-(2*H*)-one was examined by adding BIT stock solution to sterilized buffer solutions at pH 4, 7 and 9. The test concentration was 4 mg BIT/L. The test solutions were analysed using HPLC at 0 hours, 4 hours and 5 days. This study was conducted according OECD method 111 and is described under section 2 with the following deviations: 1. The buffer solutions were scaled up by a factor of 10 However, this deviation is not considered to compromise the scientific validity of this study.	
5.2 **Results and discussion**	The hydrolysis of 1,2-benzisothiazol-3-(2*H*)-one (BIT) was 0.74, 0.25 and 0.76 % after four hours of incubation at 50 ± 0.5 °C and 4.22, 2.99 and 1.27 % after five days at pH 4, 7 and 9, respectively. The percent hydrolysis data revealed that degradation of BIT was less than 10 % in the preliminary test (5 days at 50 °C).	
5.2.1 k_H	Not documented	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**	
Subsection A7.1.1.1.1/2	**ABIOTIC**	
Annex Point IIA7.6.2.1	**Hydrolysis as a function of ph and identification of breakdown products (02)**	
5.2.2	DT_{50}	> 1 year
5.2.3	r^2	Not documented
5.3	**Conclusion**	As the hydrolytic degradation of BIT was less than 10 % after 5 days at 50 °C, it can be concluded that the theoretical half-life of BIT will be > 1 year at 25 °C. Therefore, BIT is considered to be hydrolytically stable.
5.3.1	Reliability	1
5.3.2	Deficiencies	Yes Deficiencies were noted and outlined under points 2.3 and 5.1. However, they do not compromise the scientific validity of this study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*October 2012*
Materials and Methods	*Applicant's version is accepted but with the following remarks:* *-The buffer solutions were scaled up by a factor of 10* *-3.4.5. Number of replicates: Only one replicate was used. However, in the full study report there are two replicates for each pH value.*
Results and discussion	*Adopt applicant's version.*
Conclusion	*As the hydrolytic degradation of BIT was less than 10 % after 5 days at 50 °C, it can be concluded that the theoretical half-life of BIT will be > 1 year at 25 °C. Therefore, BIT is considered to be hydrolytically stable.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	*BIT concentration used in this study is approximately 100 times lower than the half-saturated concentration specified in OCDE method 111. However, the*

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.1.1.1.1/2	**ABIOTIC**
Annex Point IIA7.6.2.1	**Hydrolysis as a function of ph and identification of breakdown products (02)**

	concentration employed in this study is around 40 times higher than LOD. Thus, this do not compromise the validity of the study. *Key Study.*

Table A7.1.1.1.1/2-1: Type and composition of buffer solutions

pH	Type of buffer (final molarity)	Composition
4	Not described	4.0 mL 0.1 N sodium hydroxide solution + 500 mL 0.1 M potassium biphthalate (potassium hydrogen phthalate) solution made up to 1000 mL with sterile distilled water.
7	Not described	296.3 mL 0.1 N sodium hydroxide solution + 500 mL 0.1 M monopotassium phosphate solution made up to 1000 mL with sterile distilled water.
9	Not described	213.0 mL 0.1 N sodium hydroxide solution + 500 mL 0.1 M boric acid in 0.1 M potassium chloride solution made up to 1000 mL with sterile distilled water.

Table A7.1.1.1.1/2-2: Description of test solution

Criteria	Details
Purity of water	Sterile distilled water
Preparation of test medium	Stock solution: The stock solution was prepared by adding 41.06 mg BIT to 5 mL acetonitrile. The volume was then made up to the mark with acetonitrile. Test solution: The test solutions were prepared by adding 0.5 mL BIT stock solution in seperate 50 mL volumetric flasks containing 10 mL sterilized buffer solutions, pH 4, 7 and 9, respectively. The volume was made up to 50 mL with sterilized buffer (pH 4, 7 and 9) in duplicate. The headspace of the flask was flushed with nitrogen to remove any residual oxygen. The test vessels were sealed with sterilised tube and polyurethane foam. All the operations were performed under aspectic condition in a laminar flow chamber.
Test concentrations (mg a.i./L)	4 mg BIT/L
Temperature (ºC)	50 °C ± 0.5 °C
Controls	Five different concentrations of BIT reference standard, 8, 6, 4, 2 and 1ppm were prepared by serial dilution of the BIT reference standard stock solution (99.4 mg/L) with mobile phase. The standard solutions were injected on to the HPLC in duplicate and the mean areas were plotted against concentration (ppm) to obtain the regression equation.
Identity and concentration of co-solvent	Not applicable
Replicates	Yes, two

Table A7.1.1.1.1/2-3: Description of test system

Criteria	Details
Glassware	25 mL, 50 mL and 100 mL volumetric flasks.
Other equipment	HPLC, pH meter
Method of sterilization	Autoclaving at 121 °C and 15 lbs/in^2 pressure for 15 minutes

Table A7.1.1.1.1/2-4: Calculation of Concentration of BIT in Buffer Solutions (Preliminary Test)

Sampling Interval (Hour/Days)	Buffer Solution (pH)	Replication	Concentration of BIT (mg/L)	Average Concentration of BIT (mg/L)	Dissipation of BIT (%)
0 hours	4	I	4.02	4.03	-
		II	4.04		
	7	I	4.02	4.02	-
		II	4.01		
	9	I	3.94	3.94	-
		II	3.93		
4 hours	4	I	4.01	4.00	0.74
		II	3.99		
	7	I	4.01	4.01	0.25
		II	4.01		
	9	I	3.92	3.91	0.76
		II	3.90		
5 days	4	I	3.88	3.86	4.22
		II	3.84		
	7	I	3.90	3.90	2.99
		II	3.90		
	9	I	3.90	3.89	1.27
		II	3.87		

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/1 ABIOTIC

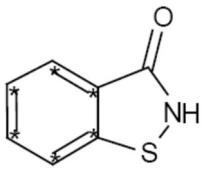
Annex Point IIA7.6.2.2 **Phototransformation in water**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: October 25, 2006 – March 12, 2007	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Rohm and Haas	
1.2.2	Companies with letter of access	Troy Chemical Company B.V.	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on OECD Draft Guideline: Phototransformation of Chemicals in Water - Direct and Indirect Photolysis (August 2000)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazol-3-(2*H*)-one (BIT)	
3.1.1	Lot/Batch number	Radiolabelled BIT: 1069.0005 Non-radiolabelled: 060309/1	
3.1.2	Specification	Please refer to Doc. III-A, 2/2	
3.1.3	Purity	Radiolabelled BIT: 98.3 % (Radiochemical purity) Non-radiolabelled: 99.8 %	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/1 ABIOTIC

Annex Point IIA7.6.2.2 Phototransformation in water

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: October 25, 2006 – March 12, 2007	
3.1.4	Radiolabelling	 * denotes position of ^{14}C-label.	
3.1.5	UV/VIS absorption spectra and absorbance value	300 - 400 nm	
3.1.6	Further relevant properties	Not relevant	
3.2	**Reference substances**	2,3-Dihydroxybenzoic acid, Benzene sulphonamide, Catechol, 2-Sulfobenzoic acid hydrate	
3.3	**Test solution**	Please refer to Table A 7.1.1.1.2/1-1	
3.4	**Testing procedure**		
3.4.1	Test system	Please refer to Table A7.1.1.1.2/1-2	
3.4.2	Properties of light source	Please refer to Table A7.1.1.1.2/1-2	
3.4.3	Determination of irradiance	Please refer to Table A7.1.1.1.2/1-2	
3.4.4	Temperature	20 ± 3°C	
3.4.5	pH	pH 5, 7 and 9	
3.4.6	Duration of the test	30 days	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/1 ABIOTIC

Annex Point IIA7.6.2.2 Phototransformation in water

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: October 25, 2006 – March 12, 2007	
3.4.7	Number of replicates	Duplicate samples	**X**
3.4.8	Sampling	Sampling occurred at 0, 1, 2, 4, 8 hours, 1, 15 and 30 days for pH 5, 0, 0.5, 1, 2 hours, 1, 15 and 30 days for pH 7 and pH 9.	**X**
3.4.9	Analytical methods	Representative samples for each pH were analysed by Liquid Chromatography-Mass Spectroscopy (LC-MS) to confirm the presence of BIT and to elucidate the structures of degradates. BIT confirmation was accomplished using a LC-ion trap-MS and a Restek Ultra Aqueous C-18 column. A linear gradient consisting of water with 0.5% formic acid and methanol with 0.5% formic acid was employed. The flow rate was 0.5 mL/min and this was split approximately 10:1 between a radioactivity detector:mass spectrometer. For metabolite identification, accurate masses were obtained using an LC-Fourier Transform MS. A Phenomenex® Synergi™-polar RP column was employed and a linear gradient consisting of water with 0.5% formic acid and acetonitrile with 0.5% formic acid. The flow rate was 1.0 mL/min which was split approximately 7:1 between a radioactivity detector:mass spectrometer.	
3.5	**Transformation products**	Transformation products tested: Yes	**X**
3.5.1	Method of analysis for transformation products	To identify the 7 degradates, representative samples were analysed by LC-MS.	
		4 RESULTS	
4.1	**Screening test**	Performed. Please refer to Table A7.1.1.1.2-3 and Figures A7.1.1.1.2/1-1 to A7.1.1.1.2/1-3	
4.2	**Actinometer data**	Not applicable	
4.3	**Controls**	Dark control vessels were glass vials sealed with crimped PTFE lined rubber caps. Dark control vessels were used for zero-time, sterility and pH control units.	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/1 ABIOTIC

Annex Point IIA7.6.2.2 **Phototransformation in water**

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: October 25, 2006 – March 12, 2007	
4.4	**Photolysis data**		
4.4.1	Concentration values	Please refer to Table A7.1.1.1.2/1-12	
4.4.2	Mass balance	Please refer to Tables A7.1.1.1.2/1-5 - A7.1.1.1.2/1-7	
4.4.3	k^c_p	Not determined	
4.4.4	Kinetic order	Single first order	
4.4.5	k^c_p / k^a_p	Not applicable	
4.4.6	Reaction quantum yield (ϕ^c_E)	Not determined	**X**
4.4.7	k_{pE}	Not determined	
4.4.8	Half-life ($t_{1/2E}$)	The DT_{50} values of BIT at pH 5, 7 and 9 were 9 hours, 0.7 hours and 0.7 hours respectively.	
4.5	**Specification of the transformation products**	A number of photodegradates were identified by LC-MS, the results of which are presented in Tables A7.1.1.1.2/1-9 - A7.1.1.1.2/1-11 and the photodegradation pathway is presented in Figure A7.1.1.1.2/1-4.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Stock solutions of radiolabelled material (1.19 mg/mL) and non-radiolabelled material (1.2 mg/mL) were prepared. The radiolabelled stock solution was used as the application solution for the 10 µg/mL test vessels. Diluted radiolabelled stock solution was used as the application solution for the 0.1 µg/mL test vessels. Buffer solutions at pH 5, 7 and 9 were prepared in HPLC grade water. 25 mL portions of the buffer solutions were dispensed into irradiation or dark control vessels.	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/1 ABIOTIC

Annex Point IIA7.6.2.2 **Phototransformation in water**

	1 REFERENCE	Official use only
1.1 Reference	[redacted] Dates of experimental work: October 25, 2006 – March 12, 2007	
	For the preliminary test the radiolabelled application solutions (215 µL and 240 µL) were aseptically injected through the vial septum onto the surface of the buffer solutions to give final concentrations in the vessels of 0.1 µg/mL and 10 µg/mL. For the definitive test the radiolabelled stock solution was used as the application solution. A non-radiolabelled application solution was prepared by dissolving BIT (5.794 mg) in acetonitrile (4.8 mL) to produce a 1.207 mg/mL solution. Solutions of radiolabelled or non-radiolabelled BIT were aseptically injected through the vial septum onto the surface of the buffer solutions. Samples were continuously irradiated using Hanau Suntest accelerated exposure machines. Measurements were made using a LI-1800 spectroradiometer. The irradiation vessels were placed under the artificial light and were maintained at 20 ± 3°C. Dark control vessels were maintained in the absence of light at 20 ± 3°C.	
5.2 Results and discussion	The theoretical screen showed that photolysis could account for 100 % loss of test substance, therefore further testing was required. Please refer to Figures A7.1.1.1.2/1-1 to A7.1.1.1.2/1-3. The preliminary test demonstrated that BIT photodegradred rapidly at pH 5, 7 and 9. Please refer to Table A7.1.1.1.2/1-4. **Distribution and Recovery of Applied Radioactivity** Quantitative mass balances were obtained for each of the irradiated and dark control samples at each pH value. At pH 5, a maximum of 4% of the applied radioactivity was present as carbon dioxide for the 30-day irradiated samples. Less than 1% applied radioactivity was trapped as organic volatiles. Mass balance ranged from 91.6% to 100.5% of the applied radioactivity with a mean of 98.8 ± 2.2%. At pH 7, carbon dioxide accounted for 9% applied radioactivity for the 30-day irradiated samples. Less than 1% applied radioactivity was trapped as organic volatiles. Mass balance ranged from 94.7% to 100.5% of the applied radioactivity with a mean of 98.5 ± 1.7%. At pH 9, a maximum of 7% of the applied radioactivity was present as carbon dioxide for the 30-day irradiated samples. Less than 1% applied radioactivity was trapped as organic volatiles. Mass balance ranged from 91.7% to 100.0% of the applied radioactivity with a mean of 97.8 ± 2.1%.	

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Subsection A7.1.1.1.2/1 **ABIOTIC**

Annex Point IIA7.6.2.2 **Phototransformation in water**

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: October 25, 2006 – March 12, 2007	
		Photodegradation of BIT At pH 5, several degradation products were detected, three of which accounted for greater than 10% applied radioactivity. All other degradates accounted for less than 5% applied radioactivity. Two of the degradates accounting for greater than 10% applied radioactivity at pH 5 were also detected at greater than 10% applied radioactivity at pH 7. In addition, a further major degradate was detected. All other degradates accounted for less than 7% applied radioactivity. At pH 9 five degradates accounted for greater than 10% applied radioactivity. No apparent degradation was observed for the dark control samples. Please refer to Tables A7.1.1.1.2/1-5 - A7.1.1.1.2/1-11. **Rate of Degradation of BIT and Degradation Products** The DT_{50} values of BIT at pH 5, 7 and 9 were 9 hours, 0.7 hours and 0.7 hours respectively. Please refer to Table A7.1.1.1.2/1-12.	
5.2.1	k^c_p	Not determined	
5.2.2	K_{pE}	Not determined	
5.2.3	ϕ^c_E	Not determined	
5.2.4	$t_{1/2E}$	The DT_{50} of BIT at pH 5, 7 and 9 were 9 hours, 0.7 hours and 0.7 hours respectively.	
5.3	**Conclusion**	BIT photodegraded very rapidly in buffer solution at pH 5, 7 and 9 at 20 ± 3°C, with no BIT remaining following the equivalent of 30 days natural sunlight. The photodegradation rate was fastest at pH 7 and pH 9. The DT_{50} values of BIT at pH 5, 7 and 9 were 9 hours, 0.7 hours and 0.7 hours respectively. Negligible organic volatiles were detected for each pH; however up to 9% applied radioactivity was present as carbon dioxide. A number of photodegradates were identified by LC-MS. BIT photodegrades very rapidly at pH values that may occur in the environment; therefore it is unlikely that BIT would persist in the aquatic environment.	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/1 ABIOTIC

Annex Point IIA7.6.2.2 Phototransformation in water

	1 REFERENCE	Official use only
1.1 Reference	[redacted] Dates of experimental work: October 25, 2006 – March 12, 2007	
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2012.*
Materials and Methods	*The applicant's version is accepted with the following comments:* ▪ *3.4.7. Samples are duplicated only in Tier 4. In Tier 2, two substance concentrations were tested, but only one replicate for each concentration was analysed.* ▪ *3.4.8. 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 sampling interval. In addition, dark control was only analysed in day 7, instead of being analysed at each sampling interval.* ▪ *3.5. Transformation products are identified and quantified, but there is no information about the degradation rate of these products.*
Results and discussion	▪ *The applicant's version is accepted but with the following comments:* ▪ *The obtained result for K_d in Tier 1 (theoretical screen) is not sufficiently explained. The applicant does not supply the values of the molar absorption coefficient (ελ).* ▪ *4.4.6. The decision of not calculating the reaction quantum yield is not reasoned.*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/1 ABIOTIC

Annex Point IIA7.6.2.2 **Phototransformation in water**

	1 REFERENCE	Official use only
1.1 Reference	[redacted] Dates of experimental work: October 25, 2006 – March 12, 2007	

Conclusion	*The DT_{50} values of BIT at pH 5, 7 and 9 were 9 hours, 0.7 hours and 0.7 hours respectively. Negligible organic volatiles were detected for each pH; however up to 9% applied radioactivity was present as carbon dioxide. A number of photodegradates were identified by LC-MS. BIT photodegrades very rapidly at pH values that may occur in the environment; therefore it is unlikely that BIT would persist in the aquatic environment.*
Reliability	*2*
Acceptability	*The study is considered acceptable despite its methodological deficiencies.*
Remarks	*Key Study.*

Table A7.1.1.1.2/1-1: Description of test solution and controls

Criteria	Details
Purity of water	Rathburn HPLC-grade water.
Preparation of test chemical solution	A stock solution of [^{14}C]-BIT was prepared by dissolving (20.018 mg) in acetonitrile (16.85 mL). Buffer solutions were prepared as follows: pH 5 (0.02M acetate buffer): 1.544 g ammonium acetate was dissolved 1000 mL water. The pH was adjusted by addition of sodium hydroxide solution (0.05M). pH 7 (0.02M phosphate buffer): 2.727 g potassium dihydrogen phosphate was dissolved in 1000 mL water. The pH was adjusted by addition of sodium hydroxide solution (0.05M). pH 9 (0.02M borate buffer): 3.819 g sodium tetraborate decahydrate was dissolved in 1000 mL water. The pH was adjusted by addition of sodium hydroxide solution (0.05M) or hydrochloric acid (0.05M).
Test concentrations (mg a.s./L)	Radiolabelled: 1.19 mg/L Non-radiolabelled: 1.2 mg/mL
Preparation of a.s. solution	Not applicable.
Temperature	20 ± 3°C
Identity and concentration of co-solvent	No applicable
Controls	Dark control vessels were prepared using glass vials sealed with crimped PTFE lined rubber caps.

Table A7.1.1.1.2/1-2: Description of test system

Criteria	Details
Laboratory equipment	Glass vials equipped with quartz glass lids, air inlet and outlet ports and a septum sealed injection port. Vials were sealed with crimped PTFE lined rubber caps. Hanau Suntest accelerated exposure machines were the light source and a LI-1800 spectroradiometer measured the light intensity.
Test apparatus	Samples were continuously irradiated using Hanau Suntest accelerated exposure machines (Heraeus Equipment Ltd, Brentwood, Essex) as the light source. The instruments filter radiation from a xenon burner removing light below 290 nm and resulting in ultra-violet and visible light with a spectral distribution close to that of natural sunlight.
Properties of artificial light source:	
Nature of light source	Xenon burner
Emission wavelength spectrum	300 – 800 nm
Light intensity	42 Wm^{-2}
Filters	Light below 290 nm removed

Table A7.1.1.1.2/1-3: Screening test results

Absorption curve	Please refer to Figures A7.1.1.1.2-1 to A7.1.1.1.2-3
A_λ	give the absorbance at wavelength λ for each replicate and the mean value.
ε_λ^c	give determined molar absorptivity (ε_λ^c) of the test substance (determined from absorption spectra
k_{pEmax}	give the calculated maximum direct aqueous photolysis sunlight rate constant $(K_{pE})_{max}$ for summer and winter solstices using appropriate L_λ values
$t_{1/2Emin}$	give the calculated minimum sunlight half-life in water bodies $(t_{1/2E})_{min}$
L_λ	Give the solar irradiance in water [10^{-3} einsteins cm^{-2} d^{-1}]

Table A7.1.1.1.2/1-4: Recoveries of applied radioactivity for preliminary test (tier 2)

pH	Incubation	Timepoint (days)	% Applied Radioactivity as BIT	
			0.1 µg/mL solution	10 µg/mL solution
5	Light	0	103.4	100.6
	Light	1	6.9	5.8
	Light	2	2.1	ND
	Light	7	ND	ND
	Dark	7	102.6	99.6
7	Light	0	102.9	99.5
	Light	1	3.5	0.4
	Light	2	2.4	0.7
	Light	7	ND	ND
	Dark	7	100.6	97.7
9	Light	0	96.5	100.0
	Light	1	8.4	ND
	Light	2	1.8	0.3
	Light	7	2.6	ND
	Dark	7	99.5	93.1

ND = Not Detected

Table A7.1.1.1.2/1-5: Percent recovery of applied radioactivity from buffer solution (pH 5) treated with [^{14}C]-BIT (Tier 4 definitive test)

	Sampling Interval	Buffer	Unit Rinse	Ethanediol Trap	2% Paraffin in Xylene	Sodium Hydroxide Trap 1	Sodium Hydroxide Trap 2	Foam Bung	Total volatiles	Mass Balance
Light	0 (hours)	99.0	0.4	NA	NA	NA	NA	NA	NA	**99.4**
Light	0 (hours)	99.9	0.5	NA	NA	NA	NA	NA	NA	**100.4**
	Mean	99.5	0.5	NA	NA	NA	NA	NA	NA	**99.9**
Light	2 (hours)	96.5	0.7	ND	ND	ND	ND	0.4	0.4	**97.6**
Light	2 (hours)	98.6	0.7	ND	ND	ND	ND	ND	ND	**99.3**
	Mean	97.6	0.7	ND	ND	ND	ND	0.2	0.2	**98.5**
Light	4 (hours)	98.1	0.4	ND	ND	ND	ND	ND	ND	**98.5**
Light	4 (hours)	100.7	0.4	ND	ND	ND	ND	0.2	0.2	**101.3**
	Mean	99.4	0.4	ND	ND	ND	ND	0.1	0.1	**99.9**
Light	8 (hours)	99.9	0.6	ND	ND	ND	ND	ND	ND	**100.5**
Light	8 (hours)	99.9	0.4	ND	ND	ND	ND	0.2	0.2	**100.5**
	Mean	99.9	0.5	ND	ND	ND	ND	0.1	0.1	**100.5**
Light	1 (day)	97.4	0.4	ND	ND	ND	ND	ND	ND	**97.8**
Light	1 (day)	99.0	0.3	ND	ND	ND	ND	ND	ND	**99.3**
	Mean	98.2	0.4	ND	ND	ND	ND	ND	ND	**98.6**
Light	15 (days)	88.7	0.5	ND	ND	2.2	ND	0.2	2.4	**91.6**
Light	15 (days)	96.6	0.4	ND	ND	1.3	1.1	ND	2.4	**99.4**
	Mean	92.7	0.5	ND	ND	1.8	0.6	0.1	2.4	**95.5**
Light	30 (days)	94.0	0.3	ND	ND	3.3	ND	0.1	3.4	**97.7**
Light	30 (days)	93.7	0.3	ND	ND	3.7	ND	ND	3.7	**97.7**

	Sampling Interval	Buffer	Unit Rinse	Ethanediol Trap	2% Paraffin in Xylene	Sodium Hydroxide Trap 1	Sodium Hydroxide Trap 2	Foam Bung	Total volatiles	Mass Balance
	Mean	93.9	0.3	ND	ND	3.5	ND	0.1	3.6	**97.7**
Dark	30 (days)	98.1	0.5	NA	NA	NA	NA	NA	NA	**98.6**
		100.0	0.5	NA	NA	NA	NA	NA	NA	**100.5**
Dark	30 (days)	99.1	0.5	NA	NA	NA	NA	NA	NA	**99.6**

ND = Not Detected

NA = Not Applicable

Ethanediol trap to collect polar organic volatiles

2 % Paraffin in Xylene trap to collect non polar organic volatiles

2M sodium hydroxide traps to collect carbon dioxide

Table A7.1.1.1.2/1-6: Percent recovery of applied radioactivity from buffer solution (pH 7) treated with [^{14}C]-BIT (Tier 4 definitive test)

	Sampling Interval	Buffer	Unit Rinse	Ethanediol Trap	2% Paraffin in Xylene	Sodium Hydroxide Trap 1	Sodium Hydroxide Trap 2	Foam Bung	Total volatiles	Mass Balance
Light	0 (hours)	99.6	0.3	NA	NA	NA	NA	NA	NA	**99.9**
Light	0 (hours)	99.9	0.5	NA	NA	NA	NA	NA	NA	**100.1**
	Mean	99.8	0.4	NA	NA	NA	NA	NA	NA	**100.2**
Light	0.5 (hours)	98.8	0.4	ND	ND	ND	ND	ND	ND	**99.2**
Light	0.5 (hours)	97.7	0.7	ND	ND	ND	ND	0.3	0.3	**98.4**
	Mean	98.3	0.6	ND	ND	ND	ND	0.2	0.2	**98.8**
Light	1 (hours)	98.8	0.3	ND	ND	ND	ND	ND	0.0	**99.1**
Light	1 (hours)	98.5	0.3	ND	ND	ND	ND	0.8	0.8	**99.6**
	Mean	98.7	0.3	ND	ND	ND	ND	0.4	0.4	**99.4**
Light	2 (hours)	98.1	0.5	ND	ND	ND	ND	ND	ND	**98.6**
Light	2 (hours)	98.1	0.7	ND	ND	ND	ND	ND	ND	**98.8**
	Mean	98.1	0.6	ND	ND	ND	ND	ND	ND	**98.7**
Light	1 (day)	97.3	0.5	ND	ND	0.1	ND	ND	0.1	**97.9**
Light	1 (day)	98.7	0.4	ND	ND	0.1	ND	ND	0.1	**99.2**
	Mean	98.0	0.5	ND	ND	0.1	ND	ND	0.1	**98.6**
Light	15 (days)	87.0	0.4	ND	ND	7.8	ND	0.1	7.9	**95.3**
Light	15 (days)	92.8	0.4	ND	ND	5.6	ND	ND	5.6	**98.8**
	Mean	89.9	0.4	ND	ND	6.7	ND	0.1	6.8	**97.1**
Light	30 (days)	84.7	0.4	ND	ND	11.6	ND	ND	11.6	**96.7**
Light	30 (days)	87.6	0.4	ND	ND	6.6	ND	0.1	6.7	**94.7**

	Sampling Interval	Buffer	Unit Rinse	Ethanediol Trap	2% Paraffin in Xylene	Sodium Hydroxide Trap 1	Sodium Hydroxide Trap 2	Foam Bung	Total volatiles	Mass Balance
	Mean	86.2	0.4	ND	ND	9.1	ND	0.1	9.2	**95.7**
Dark	30 (days)	100.1	0.4	NA	NA	NA	NA	NA	NA	**100.5**
Dark	30 (days)	98.6	0.4	NA	NA	NA	NA	NA	NA	**99.0**
		99.4	0.4	NA	NA	NA	NA	NA	NA	**99.8**

ND = Not Detected

NA = Not Applicable

Ethanediol trap to collect polar organic volatiles

2 % Paraffin in Xylene trap to collect non polar organic volatiles

2M sodium hydroxide traps to collect carbon dioxide

Table A7.1.1.1.2/1-7: Percent recovery of applied radioactivity from buffer solution (pH 9) treated with [^{14}C]-BIT (Tier 4 definitive test)

	Sampling Interval	Buffer	Unit Rinse	Ethanediol Trap	2% Paraffin in Xylene	Sodium Hydroxide Trap 1	Sodium Hydroxide Trap 2	Foam Bung	Total volatiles	Mass Balance
Light	0 (hours)	98.7	0.4	NA	NA	NA	NA	NA	NA	**99.1**
Light	0 (hours)	99.6	0.4	NA	NA	NA	NA	NA	NA	**100.0**
	Mean	99.2	0.4	NA	NA	NA	NA	NA	NA	**99.6**
Light	0.5 (hours)	99.5	0.5	ND	ND	ND	ND	ND	ND	**100.0**
Light	0.5 (hours)	96.7	0.4	ND	ND	ND	ND	0.3	0.3	**97.4**
	Mean	98.1	0.5	ND	ND	ND	ND	0.2	0.2	**98.7**
Light	1 (hours)	96.1	0.3	ND	ND	ND	ND	0.1	0.1	**96.5**
Light	1 (hours)	96.7	0.4	ND	ND	ND	ND	1.1	1.1	**98.2**
	Mean	96.4	0.4	ND	ND	ND	ND	0.6	0.6	**97.4**
Light	2 (hours)	98.7	0.6	ND	ND	ND	ND	ND	ND	**99.3**
Light	2 (hours)	97.2	0.7	ND	ND	ND	ND	ND	ND	**97.9**
	Mean	98.0	0.7	ND	ND	ND	ND	ND	ND	**98.6**
Light	1 (day)	97.5	0.3	ND	ND	0.1	ND	0.2	0.2	**98.0**
Light	1 (day)	99.2	0.5	ND	ND	0.1	ND	ND	ND	**99.7**
	Mean	98.4	0.4	ND	ND	0.1	ND	0.1	0.1	**98.9**
Light	15 (days)	93.2	0.5	ND	ND	2.7	ND	ND	2.7	**96.4**
Light	15 (days)	85.3	2.6	ND	ND	1.9	1.9	ND	3.8	**91.7**
	Mean	89.3	1.6	ND	ND	2.3	1.0	ND	3.3	**94.1**
Light	30 (days)	90.3	0.3	ND	ND	6.4	ND	ND	6.4	**97.0**
Light	30 (days)	88.3	0.3	ND	ND	7.3	ND	ND	7.3	**95.9**

	Sampling Interval	Buffer	Unit Rinse	Ethanediol Trap	2% Paraffin in Xylene	Sodium Hydroxide Trap 1	Sodium Hydroxide Trap 2	Foam Bung	Total volatiles	Mass Balance
	Mean	89.3	0.3	ND	ND	6.9	ND	ND	6.9	**96.5**
Dark	30 (days)	98.1	0.4	NA	NA	NA	NA	NA	NA	**98.5**
Dark	30 (days)	98.7	0.5	NA	NA	NA	NA	NA	NA	**99.2**
		98.4	0.5	NA	NA	NA	NA	NA	NA	**98.9**

ND = Not Detected

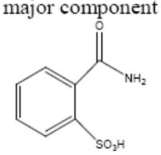
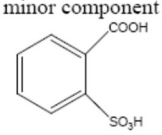
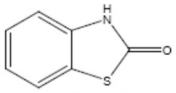
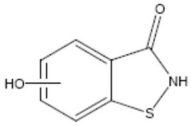
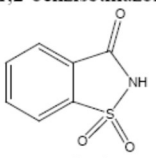
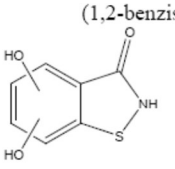
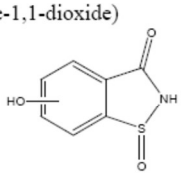
NA = Not Applicable

Ethanediol trap to collect polar organic volatiles

2 % Paraffin in Xylene trap to collect non polar organic volatiles

2M sodium hydroxide traps to collect carbon dioxide

Table A7.1.1.12/1-8: Identified BIT degradates

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)

ND: not detected

Table A7.1.1.1.2/1-9: Percent recovery of applied radioactivity present as [^{14}C]-BIT and degradation products following application of [^{14}C]-BIT to buffer solution (pH 5)

	Sampling Interval	Parent	2-SBAH	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Unknown M	Total other unknowns	Unresolved background	Total
Light	0 (hours)	97.9	ND	ND	ND	ND	ND	ND	ND	ND	1.1	99.0
Light	0 (hours)	99.6	ND	ND	ND	ND	ND	ND	ND	ND	0.3	99.9
	Mean	98.7	ND	ND	ND	ND	ND	ND	ND	ND	0.7	99.5
Light	2 (hours)	83.8	ND	5.6	6.8	ND	ND	ND	ND	ND	0.3	96.5
Light	2 (hours)	87.6	ND	4.2	59.	ND	ND	ND	ND	ND	0.9	98.6
	Mean	85.7	ND	4.9	6.3	ND	ND	ND	ND	ND	0.6	97.6
Light	4 (hours)	74.3	0.6	11.4	12.0	ND	ND	ND	ND	ND	0.2	98.1
Light	4 (hours)	79.3	0.7	9.2	10.7	ND	ND	ND	ND	ND	0.8	100.7
	Mean	76.8	0.6	10.1	11.4	ND	ND	ND	ND	ND	0.5	99.4
Light	8 (hours)	58.1	2.0	18.2	20.4	0.2	ND	0.2	ND	ND	0.8	99.9
	8 (hours)	52.2	3.0	20.9	23.0	0.1	ND	ND	ND	ND	0.6	99.9
	Mean	55.1	2.5	19.6	21.7	0.2	ND	0.1	ND	ND	0.7	99.9
Light	1 (day)	19.2	6.0	35.3	34.4	ND	ND	ND	1.1	ND	1.4	97.4
Light	1 (day)	8.9	9.6	44.6	34.2	ND	ND	ND	1.1	ND	0.6	99.0
	Mean	14.0	7.8	39.9	34.3	ND	ND	ND	1.1	ND	1.0	98.2
Light	15 (days)	ND	17.3	44.9	18.1	2.2	1.1	2.5	2.4	ND	0.3	88.7
Light	15 (days)	ND	16.9	48.6	20.8	2.7	1.0	2.5	2.2	ND	2.0	96.6
	Mean	ND	17.1	46.7	19.4	2.4	1.0	2.5	2.3	ND	1.1	92.7
Light	30 (days)	ND	22.3	49.0	9.1	2.7	1.4	4.9	3.3	ND	1.2	94.0

	Sampling Interval	Parent	2-SBAH	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Unknown M	Total other unknowns	Unresolved background	Total
Light	30 (days)	ND	23.1	50.5	9.1	2.2	0.9	3.3	3.2	1.1	0.4	93.7
	Mean	ND	22.7	49.8	9.1	2.5	1.1	4.1	3.3	0.5	0.8	93.9
Dark	30 (days)	96.9	ND	ND	ND	ND	ND	ND	ND	ND	1.2	98.1
Dark	30 (days)	98.6	ND	ND	ND	ND	ND	ND	ND	0.3	1.0	100.0
		97.8	ND	ND	ND	ND	ND	ND	ND	0.2	1.1	99.1

ND = Not Detected

Table A7.1.1.1.2/1-10: Percent recovery of applied radioactivity present as [^{14}C]-BIT and degradation products following application of [^{14}C]-BIT to buffer solution (pH 7)

	Sampling Interval	Parent	2-SBAH	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Unknown M	Total other unknowns	Unresolved background	Total
Light	0 (hours)	98.9	ND	ND	ND	ND	ND	ND	ND	ND	0.7	99.6
Light	0 (hours)	98.4	ND	ND	ND	ND	ND	ND	ND	ND	1.5	99.9
	Mean	98.7	ND	ND	ND	ND	ND	ND	ND	ND	1.1	99.8
Light	0.5 (hours)	66.1	2.1	0.6	30.0	ND	ND	ND	ND	ND	0.0	98.8
Light	0.5 (hours)	58.2	3.2	1.0	32.7	0.6	ND	ND	0.6	0.4	1.0	97.7
	Mean	62.2	2.6	0.8	31.3	0.3	ND	ND	0.3	0.2	0.5	98.3
Light	1 (hours)	36.0	4.8	1.5	52.3	0.8	ND	1.1	0.7	ND	1.6	98.8
Light	1 (hours)	41.8	4.8	1.6	27.4	0.5	ND	0.6	0.7	ND	1.0	98.5
	Mean	38.9	4.8	1.6	49.9	0.7	ND	0.8	0.7	ND	1.3	98.6
Light	2 (hours)	11.5	11.9	2.5	66.7	2.1	0.7	ND	1.7	ND	1.2	98.1
Light	2 (hours)	14.8	12.2	2.7	64.1	0.9	0.5	ND	1.9	ND	1.0	98.1
	Mean	13.1	12.0	2.6	65.4	1.5	0.6	ND	1.8	ND	1.1	98.1
Light	1 (day)	0.9	26.3	3.5	46.5	4.3	3.0	3.0	6.8	1.4	1.7	97.3
Light	1 (day)	0.6	24.1	3.5	55.5	1.3	2.6	2.5	7.0	0.5	1.2	98.7
	Mean	0.7	25.2	3.5	51.0	2.8	2.8	2.7	6.9	0.9	1.4	98.0
Light	15 (days)	ND	53.0	5.1	ND	3.0	6.3	12.5	5.5	ND	1.7	87.0
Light	15 (days)	ND	59.8	4.2	ND	3.0	5.9	11.3	8.1	ND	0.5	92.8
	Mean	ND	56.4	4.6	ND	3.0	6.1	11.9	6.8	ND	1.1	89.9

	Sampling Interval	Parent	2-SBAH	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Unknown M	Total other unknowns	Unresolved background	Total
Light	30 (days)	ND	48.8	4.0	ND	1.5	6.5	15.8	6.2	0.3	1.6	84.7
Light	30 (days)	ND	57.2	3.5	ND	2.2	5.1	11.7	6.0	0.7	1.2	87.6
	Mean	ND	53.0	3.7	ND	1.8	5.8	13.8	6.1	0.5	1.4	86.2
Dark	30 (days)	99.2	ND	ND	ND	ND	ND	ND	ND	ND	0.9	100.1
Dark	30 (days)	97.1	ND	ND	ND	ND	ND	ND	ND	0.3	1.5	98.6
		98.2	ND	ND	ND	ND	ND	ND	ND	0.2	1.2	99.4

ND = Not Detected

Table A7.1.1.1.2/1-11: Percent recovery of applied radioactivity present as [^{14}C]-BIT and degradation products following application of [^{14}C]-BIT to buffer solution (pH 9)

	Sampling Interval	Parent	2-SBAH	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Unknown M	Total other unknowns	Unresolved background	Total
Light	0 (hours)	97.0	ND	ND	0.4	ND	ND	ND	ND	0.5	0.8	98.7
Light	0 (hours)	98.3	ND	ND	0.3	ND	ND	ND	ND	0.3	0.6	99.6
	Mean	97.7	ND	ND	0.4	ND	ND	ND	ND	0.4	0.7	99.1
Light	0.5 (hours)	49.6	5.5	ND	38.7	1.7	0.3	1.3	1.3	0.2	1.0	99.5
Light	0.5 (hours)	58.5	3.8	ND	30.8	0.4	1.1	0.8	0.9	ND	0.3	96.7
	Mean	54.0	4.6	ND	34.8	1.0	0.7	1.1	1.1	0.1	0.6	98.1
Light	1 (hours)	34.0	7.2	ND	46.8	1.4	1.7	1.7	1.4	0.4	1.6	96.1
Light	1 (hours)	33.0	7.8	ND	50.0	1.4	0.9	1.8	1.3	0.1	0.3	96.7
	Mean	33.5	7.5	ND	48.4	1.4	1.3	1.8	1.3	0.2	1.0	96.4
Light	2 (hours)	20.9	11.4	ND	58.4	2.7	1.5	2.2	0.8	ND	0.9	98.7
Light	2 (hours)	19.3	10.0	ND	59.5	1.8	3.6	1.9	0.9	ND	0.2	97.2
	Mean	20.1	10.7	ND	59.0	2.2	2.5	2.0	0.9	ND	0.5	98.0
Light	1 (day)	ND	27.4	ND	43.2	9.0	4.6	2.8	9.1	0.6	0.8	97.5
Light	1 (day)	0.4	27.1	ND	39.5	10.2	4.9	3.3	10.9	1.1	1.8	99.2
	Mean	0.2	27.2	ND	41.4	9.6	4.8	3.0	10.0	0.8	1.3	98.4
Light	15 (days)	ND	42.7	ND	ND	10.4	9.4	17.2	11.3	1.3	0.8	93.2
Light	15 (days)	ND	36.3	ND	ND	13.9	8.1	14.2	9.4	1.8	1.7	85.3
	Mean	ND	39.5	ND	ND	12.1	8.7	15.7	10.4	1.6	1.3	89.2
Light	30 (days)	ND	39.1	ND	ND	12.6	7.7	27.1	3.2	0.3	0.4	90.3

	Sampling Interval	Parent	2-SBAH	Unknown A	Unknown B	Unknown C	Unknown D	Unknown E	Unknown M	Total other unknowns	Unresolved background	Total
Light	30 (days)	ND	34.7	ND	ND	13.9	7.9	25.2	6.0	ND	0.7	88.3
	Mean	ND	36.9	ND	ND	13.2	7.8	26.1	4.6	0.2	0.5	89.3
Dark	30 (days)	95.4	ND	ND	ND	ND	ND	ND	ND	1.5	1.2	98.1
Dark	30 (days)	95.9	ND	ND	ND	ND	ND	ND	ND	1.5	1.4	98.7
		95.6	ND	ND	ND	ND	ND	ND	ND	1.5	1.3	98.4

ND = Not Detected

Table A7.1.1.1.2/1-12: Rate of degradation of BIT and degradation products

pH	DT_{50}	DT_{75}	DT_{90}	Cmax (%)	R^2	k
5	9 hours	18 hours	30 hours	100.21	0.992445	1.813 day^{-1}
7	0.7 hours	1.4 hours	2.4 hours	99.07	0.996478	22.879 day^{-1}
9	0.7 hours	1.4 hours	2.4 hours	95.44	0.988083	23.833 day^{-1}

Figure A7.1.1.1.2/1-1: UV-VIS absorption spectrum of BIT in pH 5 buffer solution

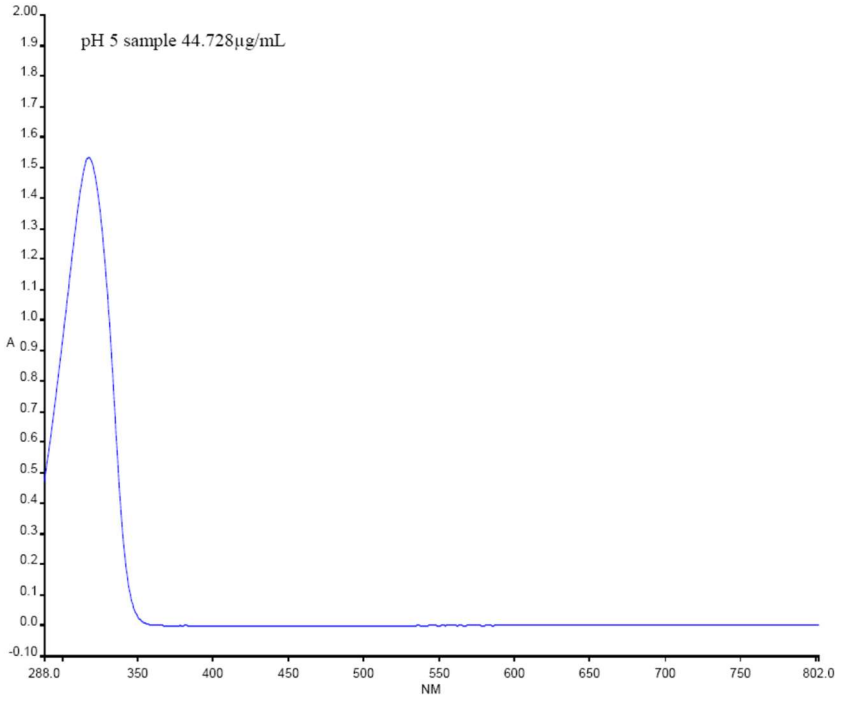


Figure A7.1.1.1.2/1-2: UV-VIS absorption spectrum of BIT in pH 7 buffer solution

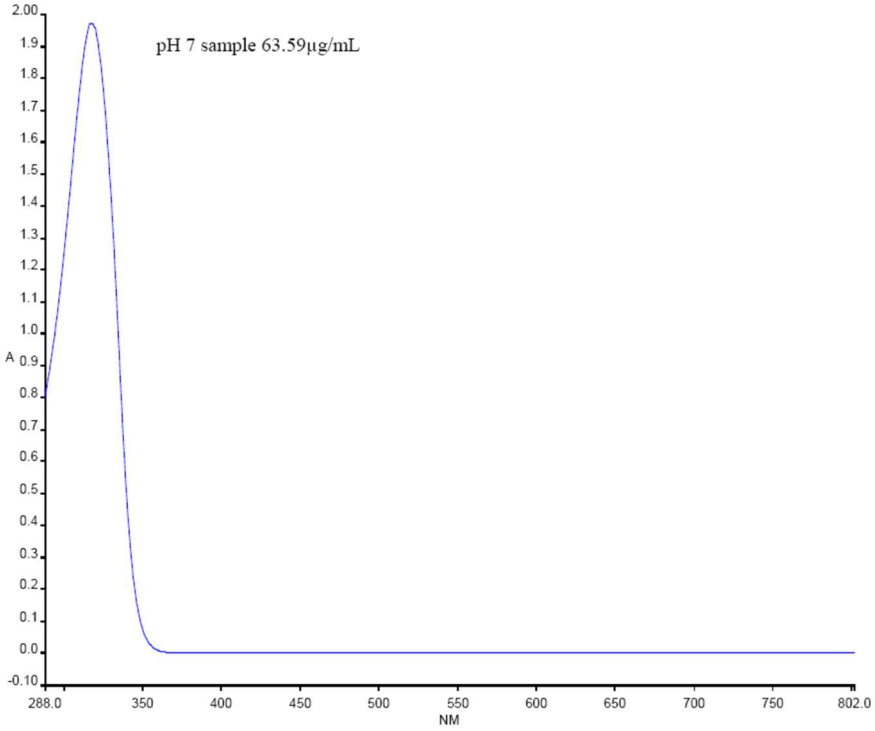


Figure A7.1.1.1.2/1-3: UV-VIS absorption spectrum of BIT in pH 9 buffer solution

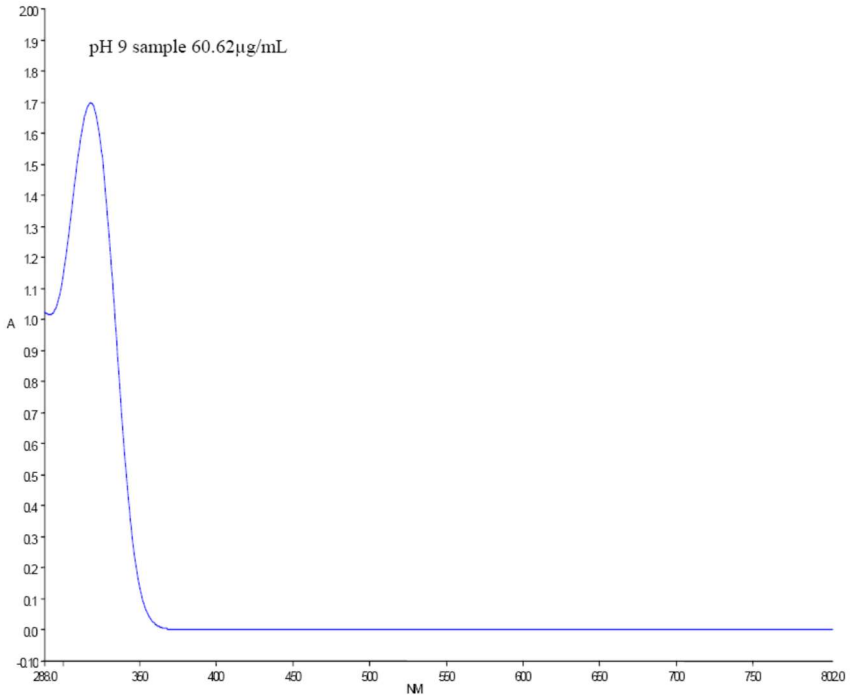
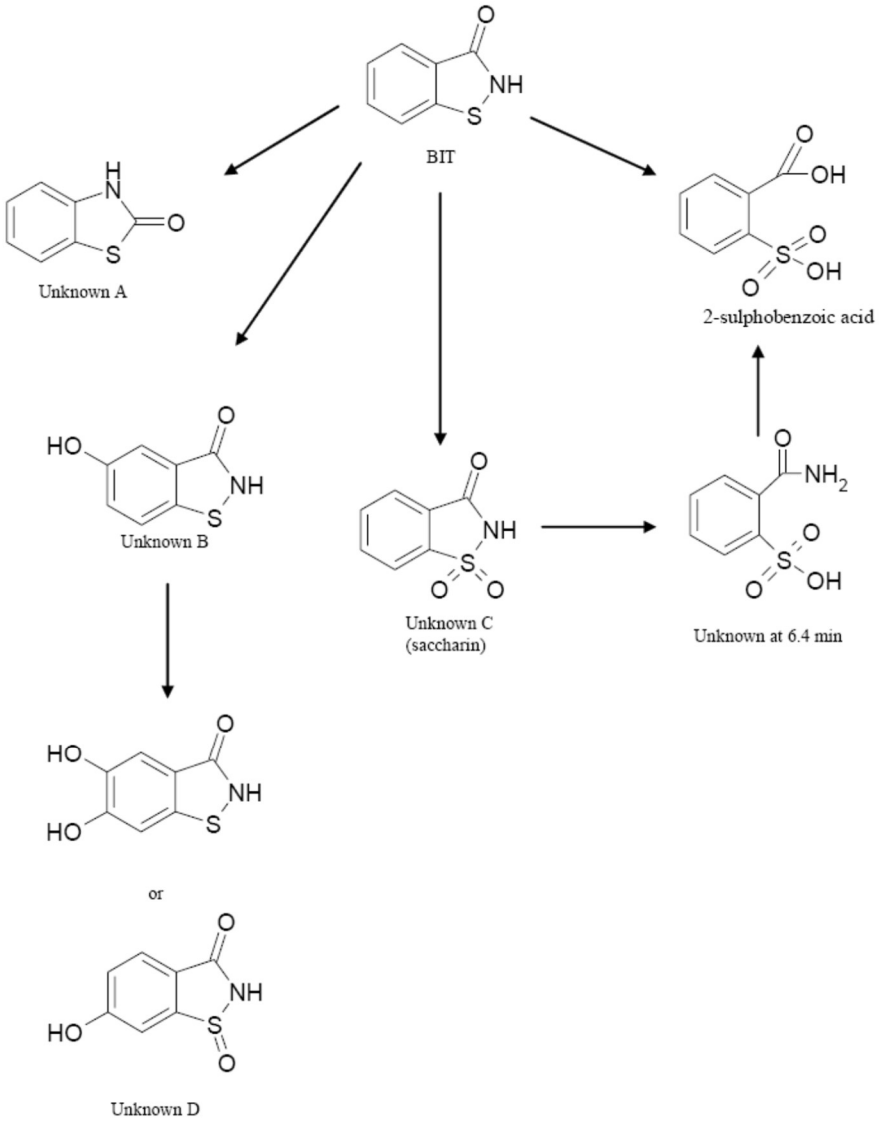


Figure A7.1.1.1.2/1-4: Proposed photodegradation pathway of BIT in buffer solutions



Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/2 ABIOTIC

Annex Point IIA7.6.2.2 Phototransformation in water

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: February 13, 2007 – April 18, 2007	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on OECD Guideline 101 UV/Visible Spectrum US EPA Pesticide Assessment Guidelines, OPPTS 835.2210 and OECD Guidance Document on Direct Phototransformation of Chemicals in Water, OECD/GD(97)21	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, this study deviates from OECD guideline 101 in the following respects: 1. A Tier 3 study was not performed 2. The transformation pathway was not examined However, these deviations are not considered to compromise the scientific validity of this study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-one	
3.1.1	Lot/Batch number	2006-00114-15	
3.1.2	Specification	Please refer to Doc. III-A, 2/1	
3.1.3	Purity	99.5 %	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/2 ABIOTIC

Annex Point IIA7.6.2.2 Phototransformation in water

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: February 13, 2007 – April 18, 2007	
3.1.4	Radiolabelling	200 - 800 nm	
3.1.5	UV/VIS absorption spectra and absorbance value	Solubility in water: 1100 mg/L	
3.2	**Reference substances**	Not applicable	
3.3	**Test solution**	Please refer to Table A 7.1.1.1.2/2-1	
3.4	**Testing procedure**		
3.4.1	Test system	Please refer to Table A7.1.1.1.2/2-2	
3.4.2	Properties of light source	Please refer to Table A7.1.1.1.2/2-2	
3.4.3	Determination of irradiance	Please refer to Table A7.1.1.1.2/2-2	
3.4.4	Temperature	25 ± 1°C	
3.4.5	pH	pH < 2, pH 7 and pH > 10	**X**
3.4.6	Duration of the test	Tier 2, phase 1 experiment: 3 days Tier 2, phase 2 experiment: 2 days	
3.4.7	Number of replicates	Tier 1 and Tier 2, phase 1 experiment: Duplicate samples Tier 2, phase 2 experiment: Triplicate samples	
3.4.8	Sampling	Tier 2, phase 1 experiment: Time zero (T0) and day 3 Tier 2, phase 2 experiment triplicate samples: 3.2 hours, 6 hours, 16 hours, 24 hours and 40 hours Tier 2, phase 2 experiment duplicate samples: T0 and 1 hour	
3.4.9	Analytical methods	HPLC-UV chromatograms of standard curve solutions of the test substance in acetonitrile were obtained. A linear regression equation was calculated relating peak areas to the theoretical concentration of	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/2 ABIOTIC

Annex Point IIA7.6.2.2 Phototransformation in water

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: February 13, 2007 – April 18, 2007	
		1,2-benzisothiazolin-3-one in the curve solutions. This equation and the average peak area for the 1,2-benzisothiazolin-3-one peak in each sample (triplicate or duplicate analyses) were used to calculate the concentration of 1,2-benzisothiazolin-3-one.	
3.5	**Transformation products**	Transformation products tested: No	
3.5.1	Method of analysis for transformation products	Not applicable	
		4 RESULTS	
4.1	**Screening test**	Performed. Please refer to Tables A7.1.1.1.2/2-3 and A7.1.1.1.2/2-4 and Figures A7.1.1.1.2/2-1 to A7.1.1.1.2/2-3.	
4.2	**Actinometer data**	An actinometer was used in tier 2 phase 2	
4.3	**Controls**	Dark control vessels consisted of Pyrex® tubes wrapped with aluminium foil with Teflon®-lined silicon septum screw caps.	
4.4	**Photolysis data**		
4.4.1	Concentration values	Please refer to Tables A7.1.1.1.2/2-5 and A7.1.1.1.2/2-6	
4.4.2	Mass balance	Not applicable	
4.4.3	k^c_p	Not determined	
4.4.4	Kinetic order	Not documented	
4.4.5	k^c_p / k^a_p	Not applicable	
4.4.6	Reaction quantum yield (ϕ^c_E)	Not determined	
4.4.7	k_{pE}	1.09 E+03	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/2 ABIOTIC

Annex Point IIA7.6.2.2 **Phototransformation in water**

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: February 13, 2007 – April 18, 2007	
4.4.8	Half-life ($t_{1/2E}$)	The DT_{50} was << 1 hour of artificial light irradiation and << 2 hours of natural sunlight.	
4.5	**Specification of the transformation products**	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	**Tier 1:** A 7.15 x 10-4 M solution of 1,2-benzisothiazolin-3-one in pH 7 buffer was prepared by diluting 5.43 mg in a 50 mL volumetric flask. Duplicate scans of a 10-fold dilution solution were analyzed from 200-800 nm, recording the absorbance at 1 nm intervals. Aqueous buffered solutions were prepared by combining 2.24 mL of 0.1 M KH_2PO_4 and 2.58 mL of 0.1 M Na_2HPO_4 to a final volume of 100 mL with deionised water. The pH was adjusted to 7.0 as necessary by addition of diluted phosphoric acid. The dose solutions were prepared by transferring aliquots of 1,2-benzisothiazolin-3-one stock solution (11.348 mg/mL in methanol adjusted to 99.5% purity) to volumetric flasks and the solutions brought to volume with sterile pH 7 buffer (test system). **Tier 2:** Samples were prepared by transferring aliquots (5 mL) of the dose solution to sterile quartz or Pyrex sample holders. For the phase 1 experiment, a total of 6 samples were set up for duplicate samplings at T0, and duplicate light exposed and dark control samples following 3 days of continuous exposure. For the phase 2 experiment, triplicate light exposed and triplicate dark control samples were set up for sampling at 5 intervals over a period of 2 days of continuous exposure. Additionally, triplicate samples were prepared for analysis at T0. An additional exposure was carried out by dosing duplicate light exposed samples for a 1 hour irradiation period prior to sampling.	
5.2	**Results and discussion**	**Tier 1:** The UV/Visible spectra of 1,2-benzisothiazolin-3-one in methanol/water (1/4, v/v) at pH < 2 and > 10 and 1,2-benzisothiazolin-3-one in pH 7 buffer are presented in Figures A7.1.1.1.2/2-1 to A7.1.1.1.2/2-3. In acidic pH (pH < 2), the absorption maxima occurred at 200 and 225 nm. Molar absorption coefficients were determined as 14920 and 20332 L mol^{-1} cm^{-1} at 200 and 225 nm,	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/2 ABIOTIC

Annex Point IIA7.6.2.2 Phototransformation in water

	1 REFERENCE	Official use only
1.1 Reference	[redacted] Dates of experimental work: February 13, 2007 – April 18, 2007	
	respectively. In pH 7 buffer, absorption maxima for 1,2-benzisothiazolin-3-one were registered at 200, 224 and 242 nm, with molar absorption coefficients of 20161, 16812 and 9846 L mol^{-1} cm^{-1}, respectively. In basic pH, absorption maxima were at 208-209 nm, 221 nm and 245 nm. Molar absorption coefficients were determined as 12859, 11605 and 11987 L mol^{-1} cm^{-1} at 208, 221 and 245 nm, respectively. The predicted half-life of photolysis of 1,2-benzisothiazolin-3-one in pH 7 buffer was calculated to be 0.0006 days. Please refer to Table A7.1.1.1.2/2-4. **Tier 2, Phase 1 Experiment:** After 3 days of irradiation, the dark control samples contained greater than 94 % of the concentration of 1,2- benzisothiazolin-3-one in the T0 samples. The light exposed samples contained less than 1 % of the concentration of 1,2-benzisothiazolin-3-one in the T0 samples after 3 days of exposure. Please refer to Table A7.1.1.1.2/2-5. **Tier 2, Phase 2 Experiment:** The results showed that less than 4% of the 1,2-benzisothiazolin-3-one initial concentration at T0 remained in the light exposed samples after 3 hours of irradiation. However, analysis of dark control samples at 3 and 16 hours revealed that 1,2-benzisothiazolin-3-one was stable in the dark samples and under storage conditions. Please refer to Table A7.1.1.1.2/2-6. An additional set of duplicate light exposed samples were dosed in pH 7 buffer and exposed to artificial sunlight for 1 hour. After 1 hour of irradiation, only an average of 8.7% of the 1,2-benzisothiazolin-3-one concentration at T0 remained, indicating that 1,2-benzisothiazolin-3-one is unstable in aqueous buffer when exposed to artificial sunlight.	
5.2.1 k^c_p	Not determined	
5.2.2 K_{pE}	1.09 E+03	
5.2.3 ϕ^c_E	Not determined	
5.2.4 $t_{1/2E}$	The DT_{50} was << 1 hour of artificial light irradiation and << 2 hours of natural sunlight.	
5.3 Conclusion	The UV absorption spectra show that 1,2-benzisothiazolin-3-one absorbs a significant amount of light at wavelengths > 290 nm, in the region corresponding to natural sunlight. The estimated maximum half life of 1,2-benzisothiazolin-3-one is 0.0006 days. The Tier 2	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/2 ABIOTIC

Annex Point IIA7.6.2.2 Phototransformation in water

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: February 13, 2007 – April 18, 2007	
		Phase 1 experiment showed little degradation in the dark control samples and > 99% degradation in the light exposed samples after 3 days of irradiation which indicates that hydrolysis is not a competing factor in photolysis of 1,2-benzisothiazolin-3-one. The Tier 2 Phase 2 experiment showed that after 1 hour of irradiation, 1,2-benzisothiazolin-3-one degraded to an average of 8.72% of the concentration at T0. 1,2-benzisothiazolin-3-one is considered unstable in aqueous buffer when exposed to light. The results show that aqueous photolysis of 1,2-benzisothiazolin-3-one is a major degradation pathway for the test substance in the environment.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	Yes, this study deviates from OECD guideline 101 in the following respects: 1. A Tier 3 study was not performed 2. The transformation pathway was not examined However, these deviations are not considered to compromise the scientific validity of this study.	**X**

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*May 2010*
Materials and Methods	*Applicant's version is accepted with the following comment:* *3.4.5. Different pH values are analysed only in Tier 1. Tier 2 experiments are all performed at pH 7.*
Results and discussion	*Applicant's version is accepted.*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.1.2/2 ABIOTIC

Annex Point IIA7.6.2.2 **Phototransformation in water**

	1 REFERENCE	Official use only
1.1 Reference	[redacted] Dates of experimental work: February 13, 2007 – April 18, 2007	

Conclusion	*Applicant's version is accepted with the following remark:* *5.3.2. No information is provided concerning the degradation products of the substance.*
Reliability	*2*
Acceptability	*Acceptable.*
Remarks	*Information about the transformation pathway should be provided, and degradation products should be identified.*

Table A7.1.1.1.2/2-1: Description of test solution and controls

Criteria	Details
Purity of water	Deionised or HPLC-grade water.
Preparation of test chemical solution	A 7.15 x 10^{-4} M solution of 1,2-benzisothiazolin-3-one in pH 7 buffer was prepared by diluting 5.43 mg in a 50 mL volumetric flask. Aqueous buffered solutions were prepared by combining 2.24 mL of 0.1 M KH_2PO_4 and 2.58 mL of 0.1 M Na_2HPO_4 to a final volume of 100 mL with deionised water. The pH was adjusted to 7.0 as necessary by addition of diluted phosphoric acid.
Test concentrations (mg a.s./L)	11.348 mg/mL
Preparation of a.s. solution	Not applicable.
Temperature	25 ± 1°C
Identity and concentration of co-solvent	No applicable
Controls	Dark control vessels consisted of Pyrex® tubes wrapped with aluminium foil with Teflon®-lined silicon septum screw caps.

Table A7.1.1.1.2/2-2: Description of test system

Criteria	Details
Laboratory equipment	Quartz sample tubes, equipped with Teflon®-lined silicon septum screw caps were used for the irradiated samples. For the dark control samples Pyrex® tubes wrapped with aluminium foil with Teflon®-lined silicon septum screw caps were used.
Test apparatus	The artificial light source was a Heraeus Suntest CPS+ unit. The Suntest CPS+ was equipped with a Xenon arc lamp with a quartz glass filter with IRreflective coating and a special UV glass filter blocking the radiation below approximately 290 nm, corresponding to natural sunlight. Light intensity and spectral distribution of the Xenon light source were determined with a LI-COR® LI-1800 spectroradiometer.
Properties of artificial light source:	
Nature of light source	Xenon burner
Emission wavelength spectrum	300 – 800 nm
Light intensity	600 Wm^{-2}
Filters	Light below 290 nm blocked

Table A7.1.1.1.2/2-3: Screening test results

Absorption curve	Please refer to Figures A7.1.1.1.2/2-1 to A7.1.1.1.2/2-3
A_λ	Please refer to Table A7.1.1.1.2/2-4
$\varepsilon_\lambda{}^c$	Please refer to Table A7.1.1.1.2/2-4
k_{pEmax}	1.09 E+04
$t_{1/2max}$	0.0006 days
L_λ	Please refer to Table A7.1.1.1.2/2-4

Table A7.1.1.1.2/2-4: Estimated half-life of 1,2-benzisothiazolin-3-one in pH 7 buffer based on Tier 1 experiment

Wavelength (nm)	Average Absorbance	Average ε_λ[1]	L_λ[2]	$\varepsilon_\lambda L_\lambda$
297.5	0.2137	2990	6.17E-05	1.84E-01
300.0	0.2351	3289	2.69E-04	8.85E-01
302.5	0.2569	3594	8.30E-04	2.98E+00
305.0	0.2805	3924	1.95E-03	7.65E+00
307.5	0.3045	4260	3.74E-03	1.59E+01
310.0	0.3270	4575	6.17E-03	2.82E+01
312.5	0.3442	4815	9.07E-03	4.37E+01
315.0	0.3550	4966	1.22E-02	6.06E+01
317.5	0.3575	5001	1.55E-02	7.75E+01
320.0	0.3520	4924	1.87E-02	9.21E+01
323.0	0.3300	4617	3.35E-02	1.55E+02
330.0	0.2464	3447	1.16E-01	4.00E+02
340.0	0.0819	1146	1.46E-01	1.67E+02
350.0	0.0157	220	1.62E-01	3.56E+01

[1] absorbance at λ/molar concentration
[2] Solar irradiance at 40 °N latitude in summer

$(k_{pE})_{max} = \sum \varepsilon_\lambda L_\lambda = 1.09E+03$
$t_{1/2} = \ln 2/(k_{pE})_{max}$
$t_{1/2} = 0.0006$ days

Table A7.1.1.1.2/2-5: Measured 1,2-benzisothiazolin-3-one concentration in Tier 2 phase 1

Solution	Measured concentration (mg/ml)	% Remaining[1]
Tier 2 phase 1 T0 rep A	0.05096	NA
Tier 2 phase 1 T0 rep B	0.05094	NA
Average	0.05095	100.0
Tier 2 phase 1 Light T3d rep A	0.0001569	0.3
Tier 2 phase 1 Light T3d rep B	0.0001390	0.3
Average	0.000148	0.3
Tier 2 phase 1 Dark T3d rep A	0.04830	94.8
Tier 2 phase 1 Dark T3d rep B	0.04832	94.8
Average	0.048310	94.8

[1] Measured concentration x 100/measured concentration of T0

Table A7.1.1.1.2/2-6: Measured 1,2-benzisothiazolin-3-one concentration in Tier 2 phase 2

Solution	Measured Concentration (mg/mL)	% Remaining[1]
Dose #1		
Tier 2 phase 2 T0 rep A	0.04690	NA
Tier 2 phase 2 T0 rep B	0.04690	NA
Tier 2 phase 2 T0 rep C	0.04686	NA
Average	0.04689	100
Tier 2 phase 2 LT3.2 h rep A	0.001572	3.35
Tier 2 phase 2 LT3.2 h rep B	0.001441	3.07
Tier 2 phase 2 LT3.2 h rep C	0.001790	3.82
Average	0.001601	3.41
Tier 2 phase 2 DT3.2 h rep A	0.04829	103.0
Tier 2 phase 2 DT3.2 h rep B	0.04837	103.2
Tier 2 DT3.2 h rep C	0.04834	103.1
Average	0.04833	103.1
Tier 2 phase 2 DT16 h rep A	0.04840	103.2
Tier 2 phase 2 DT16 h rep B	0.04834	103.1
Tier 2 phase 2 DT16 h rep C	0.04823	102.9
Average	0.04832	103.1
Dose #2		
Tier 2 phase 2 T0	0.04706	100.0
Tier 2 phase 2 LT1 h rep A	0.004702	9.99
Tier 2 phase 2 LT1 h rep B	0.003506	7.45
Average	0.004104	8.72

Figure A7.1.1.1.2/2-1: UV/Visible spectra of 1,2-benzisothiazolin-3-one at pH < 2

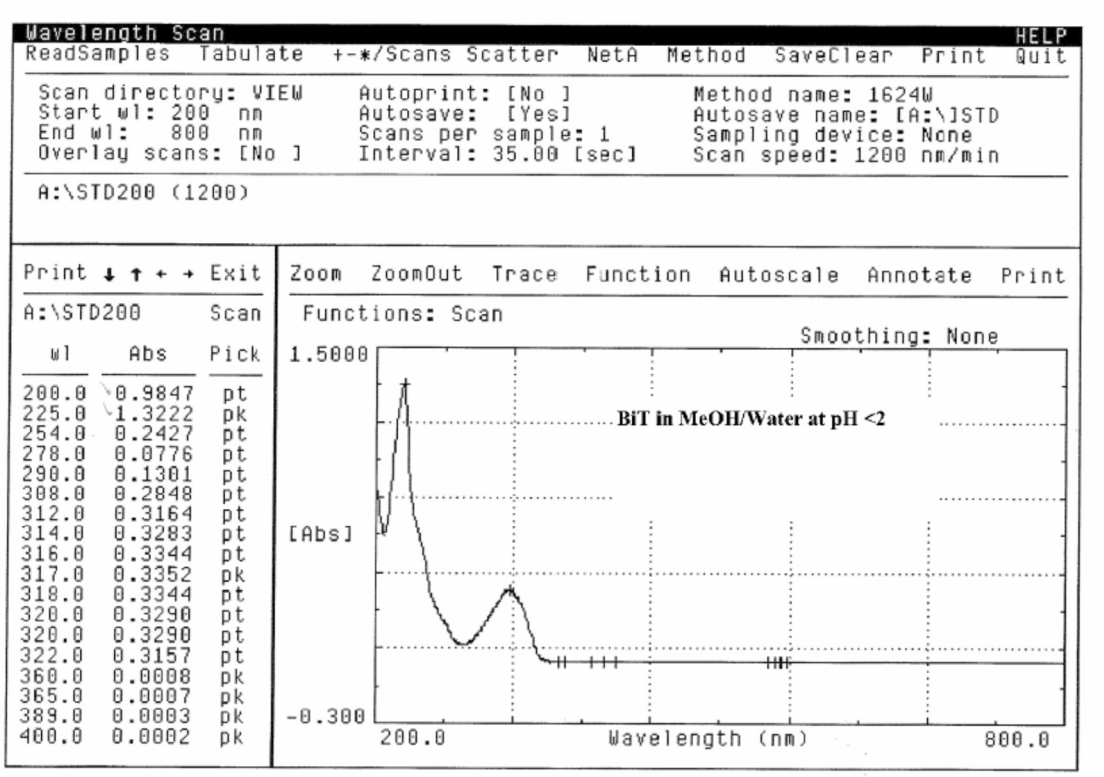


Figure A7.1.1.1.2/2-2: UV/Visible spectra of 1,2-benzisothiazolin-3-one at pH 7

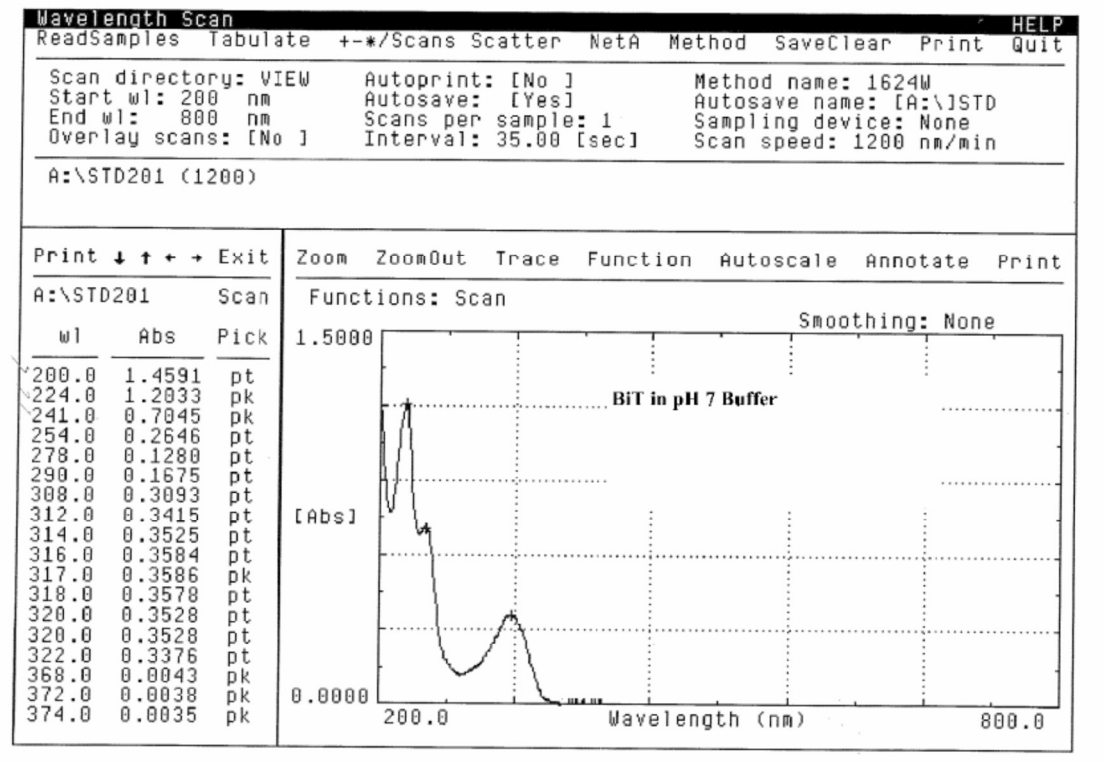
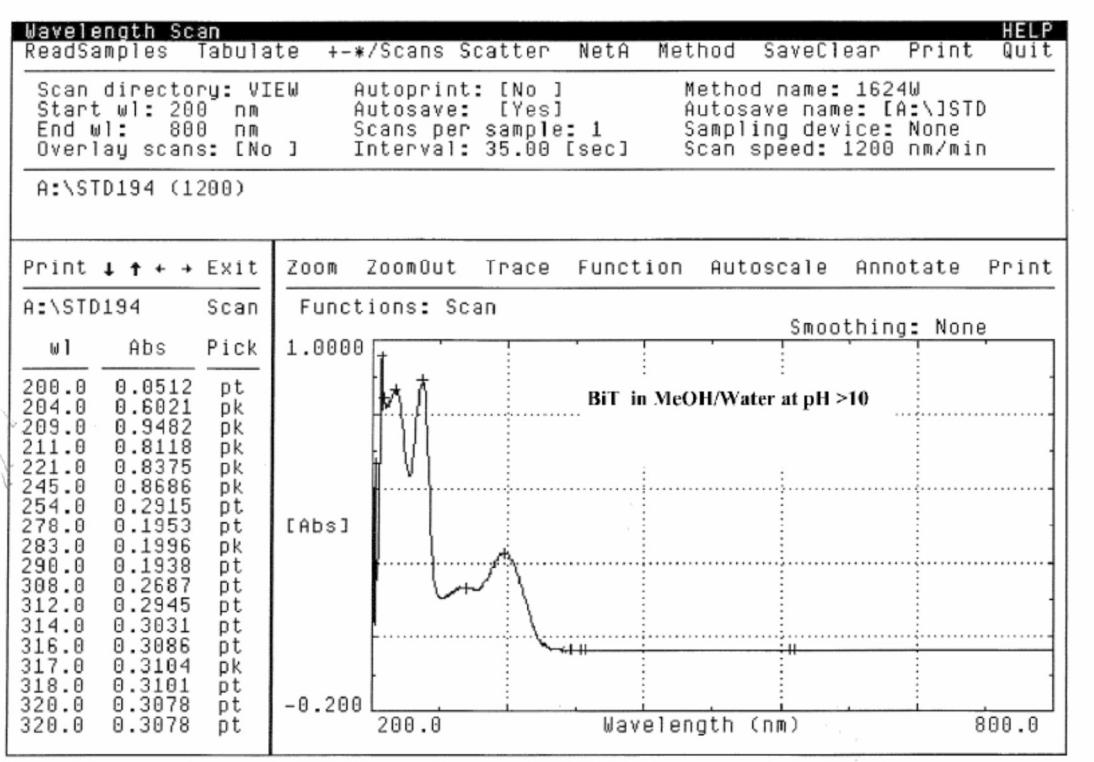


Figure A7.1.1.1.2/2-3: UV/Visible spectra of 1,2-benzisothiazolin-3-one at pH > 10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ReadSamples Tabulate +-*/Scans Scatter NetA Method SaveClear Print Quit
Scan directory: VIEW
Start wl: 200 nm
End wl: 800 nm
Overlay scans: [No]
Autoprint: [No]
Autosave: [Yes]
Scans per sample: 1
Interval: 35.00 [sec]
Method name: 1624W
Autosave name: [A:\]STD
Sampling device: None
Scan speed: 1200 nm/min
A:\STD194 (1200)
Print ↓ ↑ ← → Exit
A:\STD194 Scan
wl Abs Pick
200.0 0.0512 pt
204.0 0.6021 pk
209.0 0.9482 pk
211.0 0.8118 pk
221.0 0.8375 pk
245.0 0.8686 pk
254.0 0.2915 pt
278.0 0.1953 pt
283.0 0.1996 pk
290.0 0.1938 pt
308.0 0.2687 pt
312.0 0.2945 pt
314.0 0.3031 pt
316.0 0.3086 pt
317.0 0.3104 pk
318.0 0.3101 pt
320.0 0.3078 pt
320.0 0.3078 pt
Zoom ZoomOut Trace Function Autoscale Annotate Print
Functions: Scan
Smoothing: None
1.0000
[Abs]
-0.200
BiT in MeOH/Water at pH >10
200.0
Wavelength (nm)
800.0

Section A7
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Annex Point IIA7.6.1.1

Ecotoxicological Profile Including Environmental Fate and Behaviour

BIOTIC

Biodegradability (ready) (01)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]. (2002), Determination of the ready biodegradability of 1,2-benzisothiazolin-3-one in a Closed Bottle Test, TNO Nutrition and Food Research, Department of Environmental Toxicology, Schoemakerstraat 97, P.O. Box 6011, 2600 JA Delft, The Netherlands, unpublished report No.: 01-4004/04 Dates of experimental work: October 12, 2001 – November 9, 2001.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company B.V.	
1.2.2	Companies with letter of access	Rohm and Haas	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on OECD guideline 301D.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, this study deviates from OECD guideline 301D in the following respects: 1. The amount of NH_4Cl in nutrient stock solution A2 was 1.5 g instead of 0.5 g. However, this deviation is not considered to compromise the scientific validity of this study.	**X**
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-one	
3.1.1	Lot/Batch number	BT 12000	
3.1.2	Specification	Please refer to Doc. III-A, 2/2	
3.1.3	Purity	98 %	
3.1.4	Further relevant properties	Not applicable	

Section A7 Subsection A7.1.1.2.1/1 Annex Point IIA7.6.1.1		Ecotoxicological Profile Including Environmental Fate and Behaviour BIOTIC Biodegradability (ready) (01)	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	Yes	
3.1.7	Specific chemical analysis	Not applicable	
3.2	**Reference substance**	Yes, anhydrous sodium acetate	
3.2.1	Initial concentration of reference substance	4.04 mg/L	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	Please refer to Table A7.1.1.2.1/1-1	
3.3.2	Test system	Please refer to Table A7.1.1.2.1/1-2	
3.3.3	Test conditions	Please refer to Table A7.1.1.2.1/1-3	
3.3.4	Method of preparation of test solution	Not appropriate	
3.3.5	Initial TS concentration	0.83 mg/L and 2.0 mg/L	
3.3.6	Duration of test	28 days	
3.3.7	Analytical parameter	Closed Bottle: measurement of oxygen depletion	
3.3.8	Sampling	Samples were taken on days 0, 7, 14, 21 and 28. 4 replicate bottles were taken at each sample point	
3.3.9	Intermediates/ degradation products	Not identified	
3.3.10	Nitrate/nitrite measurement	Not applicable	
3.3.11	Controls	Inoculum activity control – 4.04 mg/L sodium acetate was prepared by dissolving 0.4045 g of the reference substance in 100 mL of ultrapure water. From this stock solution a dilution was made in inoculated mineral medium. Toxicity control – Dilutions of the test substance and the reference substance stock solutions were prepared in inoculated mineral	

Section A7 Subsection A7.1.1.2.1/1 Annex Point IIA7.6.1.1		Ecotoxicological Profile Including Environmental Fate and Behaviour BIOTIC Biodegradability (ready) (01)	
		medium. The toxicity control contained 2.0 mg/L 1,2-benzisothiazolin-3-one and 4.04 mg/L sodium acetate. Blank – Inoculated mineral medium	
3.3.12	Statistics	The oxygen depletion, due to the test or control substance, at each time was calculated by subtracting the mean oxygen consumptions in the blanks from that in the bottle under consideration. These crude values were then converted to values per mg substance (Biological Oxygen Demand, BOD). The percentage biodegradation of the test substance was calculated as BOD/ThOD x 100.	
		4 RESULTS	
4.1	**Degradation of test substance**		
4.1.1	Graph	Figure A7.1.1.2.1/1-1	
4.1.2	Degradation	The BOD of the test substance never reached a positive value indicating that no degradation occurred. Please refer to Tables A7.1.1.2.1/1-4 - A7.1.1.2.1/1-6.	
4.1.3	Other observations	Not applicable	
4.1.4	Degradation of TS in abiotic control	No abiotic control	
4.1.5	Degradation of reference substance	The reference substance reached the 60 % pass level of degradation within 14 days. In the toxicity control, the test substance was observed to inhibit the biodegradation of the reference substance.	
4.1.6	Intermediates/ degradation products	Intermediates were not identified	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The ready biodegradability of 1,2-benzisothaizolin-3-one was assessed by measuring the test substance related increase in oxygen consumption in inoculated nutrient solution during 28 days of incubation. The test substance was added to four vessels containing mineral medium inoculated with activated sludge at a nominal test concentrations of 0.83 mg/L and 2.0 mg/L. Three controls were also investigated: activity control (reference substrate and mineral medium), toxicity control (test substance, mineral medium and reference substance) and a blank control. This study was conducted according to OECD guideline 301D and is described under point 3 with the following deviation:	**X**

Section A7 Subsection A7.1.1.2.1/1 Annex Point IIA7.6.1.1	Ecotoxicological Profile Including Environmental Fate and Behaviour BIOTIC Biodegradability (ready) (01)	
	1. The amount of NH_4Cl in nutrient stock solution A2 was 1.5 g instead of 0.5 g. However, this deviation is not considered to compromise the scientific validity of this study.	
5.2 Results and discussion	The oxygen depletion in the inoculum blank was 3.03 mg O_2/L after 28 days of incubation. This is higher than the limit given in the guideline of 1.5 mg O_2/L. However, this is not considered to have influenced the degradation of the test substance. The reference substance, sodium acetate was biodegraded by more than 60 % within 14 days. The calculated BOD of sodium acetate after 7 and 14 days was 0.77 and 0.65 mg O_2/mg respectively, which corresponds with its Theoretical Oxygen Demand (ThOD) of 0.68 mg O_2/mg. The oxygen depletion in the toxicity control (sodium acetate and test substance) was 2.84 mg O_2/L after 14 days, which was lower than that of the inoculum control with sodium acetate only (4.82 mg O_2/L). This indicated that the test substance inhibited the biodegradation of sodium acetate. After 14 days of incubation 30 % degradation was found in the toxicity control. The BOD of the test substance never reached a positive value, indicating that no degradation occurred. During the test the oxygen depletion was more inhibited at the higher concentration, an effect expected with a toxic substance. For these reasons, the biodegradability of 1,2-benzisothiazolin-3-one could not be established on the basis of this test.	**X**
5.3 Conclusion	The test substance clearly inhibited the oxygen depletion both in the toxicity control and the test bottles. Therefore, the biodegradability of 1,2-benzisothiazolin-3-one could not be established by this test due to its toxicity to the inoculum organisms. As per the guideline, the substance was tested at the lowest possible concentration, which could still allow for a reliable determination of the biodegradability. However, in this case the concentrations required will be so low that the use of radiolabelled test substance would be required to establish its biodegradability. See pass levels in Table A7.1.1.2.1/2-7.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	Yes, One deficiency was noted and is outlined under points 2.3 and 5.1. However, it does not compromise the scientific validity of this study.	

Evaluation by Competent Authorities

Section A7

Subsection A7.1.1.2.1/1

Annex Point IIA7.6.1.1

Ecotoxicological Profile Including Environmental Fate and Behaviour

BIOTIC

Biodegradability (ready) (01)

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*November 2009*
Materials and Methods	*Applicant's version is accepted with the following comment:* *5.1. The amount of NH_4Cl in nutrient stock solution A2 was 1.5 g instead of 0.5 g.*
Results and discussion	*Applicant's version is accepted with the following comments:* *5.2. The oxygen depletion in the inoculum blank was 3.03 mg O_2/L after 28 days of incubation. This is higher than the limit given in the guideline of 1.5 mg O_2/L. According to the guidelines, values higher than 1.5 mg require investigation of the experimental techniques.* *5.2. Theoretical Oxygen Demand (ThOD) of sodium acetate is 0,78 instead of 0,68.*
Conclusion	*BIT inhibited the oxygen depletion both in the toxicity control and the test bottles. Therefore, the biodegradability of 1,2-benzisothiazolin-3-one could not be established by this test due to its toxicity to the inoculum organisms.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.1.1.2.1/1-1: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Source	Oxidation ditch used to treat domestic wastewater
Sampling site	Hazerswoude, the Netherlands
Laboratory culture	Yes
Preparation of inoculum for exposure	The inoculum will be prepared by allowing the sludge (about 3-4 g of dry solids/L) to settle, followed by decanting of the liquid phase. A 25 to 40 mL aliquot of the supernatant will be used to inoculate one litre of nutrient medium.
Pretreatment	No

Table A7.1.1.2.1/1-2: Test system

Criteria	Details
Culturing apparatus	Not documented
Number of culture flasks/concentration	4 biodegradation flasks containing 0.83 mg/L test substance 4 biodegradation flasks containing 2.0 mg/L test substance 4 inoculum activity control flasks containing 4.04 mg/L reference substance 4 toxicity control flasks containing 4.04 mg/L reference substance and 2 mg/L test substance 4 blank control flasks containing 0 mg/L test substance
Aeration device	Not documented
Measuring equipment	The oxygen concentrations were measured using an oxygen electrode.
Test performed in closed vessels due to significant volatility of TS	No

Table A7.1.1.2.1/1-3: Test conditions

Criteria	Details
Composition of medium	Solution a: KH_2PO_4 8.5 g/L K_2HPO_4 21.8 g/L $Na_2HPO_4.7H_2O$ 33.4 g/L NH_4Cl 1.5 g/L Solution b: $MgSO_4.7H_2O$ 22.5 g/L Solution c: $CaCl_2.2H_2O$ 36.4 g/L Solution d: $FeCl_3.6H_2O$ 0.25 g/L Nutrient stock solutions were dissolved in and made up to 1000 ml with ultrapure water.
Additional substrate	No
Test temperature	19.5 °C – 20.4 °C
pH	pH 6.9- 7.3
Aeration of dilution water	Yes, method not documented
Suspended solids concentration	3 kg dry solids/L
Other relevant criteria	Not relevant

Table A7.1.1.2.1/1-4: Biodegradation of 1,2-benzisothiazolin-3-one (0.83 mg/L) expressed as the BOD (mg O_2/mg) and as a percentage of its ThOD

Time (days)	BOD (mg O_2/mg)	Biodegradation ThOD (%)
7	-0.03	-1
14	-1.04	-58
21	-0.09	-5
28	-0.74	-41

Table A7.1.1.2.1/1-5: Biodegradation of 1,2-benzisothiazolin-3-one (2.00 mg/L) expressed as the BOD (mg O_2/mg) and as a percentage of its ThOD

Time (days)	BOD (mg O_2/mg)	Biodegradation ThOD (%)
7	-0.17	-9
14	-0.61	-34
21	-0.51	-28
28	-0.73	-41

Table A7.1.1.2.1/1-6: Incoculum activity and toxicity control tests: mean values of oxygen depletion and biodegradation as a percentage of the ThOD

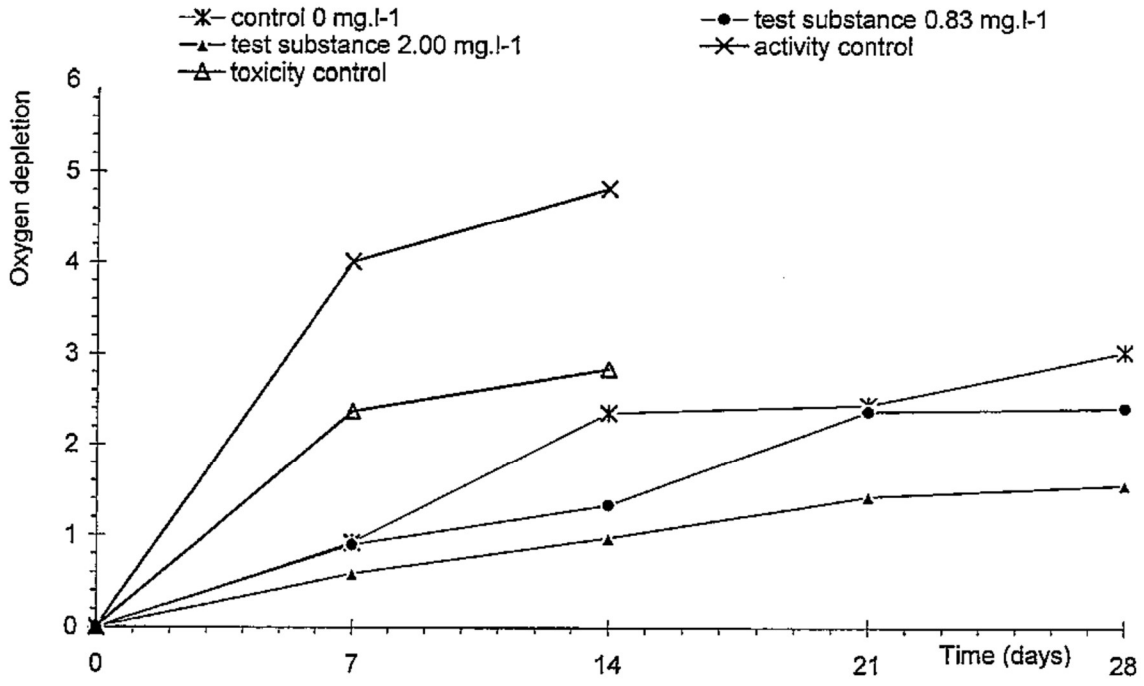
Time (days)	Inoculum blank (mg O_2/L)	Inoculum activity control [1]		Toxicity control [2]	
		mg O_2/L	Biodegradation ThOD %	mg O_2/L	Biodegradation ThOD %
7	0.92 (0.47-1.31)	4.01	113	2.37	23
14	2.19	4.82 (4.45-5.24)	96 (82-111)	2.84	30
21	2.44	n.d.	n.d.	n.d.	n.d.
28	3.03	n.d.	n.d.	n.d.	n.d.

[1] 4.04 mg/L sodium acetate
[2] 4.04 mg/L sodium acetate and 2.00 mg/L 1,2-benzisothiazol-3-one

Table A7.1.1.2.1/1-7: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$		Not fulfilled
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		Not fulfilled
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	Fulfilled	
Percentage of removal of reference substance reaches pass level by day 14	Fulfilled	

Figure A7.1.1.2.1/1-1: Oxygen depletion (mg O_2/L) in the inoculated mineral medium biodegradation test with two concentrations of 1,2-benzisothiazolin-3-one



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Ecotoxicological Profile Including Environmental Fate and Behaviourç

BIOTIC

Biodegradability (ready) (02)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: May 10, 2002 – June 07, 2002.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on OECD guideline 301D and EC method C. 4E	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes this study deviates from OECD guideline 301D in the following respects: 1. The amount of chemicals used to make up the stock solutions of mineral medium were 1/10 of that recommended in the Guideline and were made up to 100 ml rather than 1 L. 2. 4 ml of mineral medium stock solutions a, b, c and d were added to 3984 mL of double distilled water as opposed to 1 ml of solutions a, b, c and d to 800 mL of water and made up to 1 litre. 3. 80 mg of test substance and reference substance were made up to 100 ml and 10 mL respectively with mineral medium as opposed to made up to 1 litre. However, these deviations are not considered to compromise the scientific validity of this study.	**X**
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazol-3-(2*H*)-one	
3.1.1	Lot/Batch number	BT17301	
3.1.2	Specification	Please refer to Doc. III-A, 2/1	

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3.1.3	Purity	97.42 %	
3.1.4	Further relevant properties	Not relevant	
3.1.5	Composition of Product	Not documented	
3.1.6	TS inhibitory to microorganisms	Not documented	**X**
3.1.7	Specific chemical analysis	Not applicable	
3.2	**Reference substance**	Yes, potassium hydrogen phthalate	
3.2.1	Initial concentration of reference substance	2 mg/L	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	See Table A7.1.1.2.1/2-1	
3.3.2	Test system	See Table A7.1.1.2.1/2-2	
3.3.3	Test conditions	See Table A7.1.1.2.1/2-3	
3.3.4	Method of preparation of test solution	A stock solution was made by transferring 80 mg of BIT to a 100 mL volumetric flask and dissolving in mineral medium. Bulk solution of test substance was prepared by mixing 10 mL of BIT stock solution and 1 mL mixed inoculum to 3989 mL of mineral medium in a 5 L conical flask. The solution was mixed thoroughly. The final concentration of BIT in mineral medium was 2 mg/L.	
3.3.5	Initial TS concentration	2.0 mg/L	
3.3.6	Duration of test	28 days	
3.3.7	Analytical parameter	Dissolved oxygen	
3.3.8	Sampling	Duplicate samples were taken on days 0, 7, 14, 21 and 28.	
3.3.9	Intermediates/ degradation products	Not identified	
3.3.10	Nitrate/nitrite measurement	Yes	

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3.3.11	Controls	Mineral medium inoculated with inoculum only.	
3.3.12	Statistics	The Chemical Oxygen Demand (COD) was calculated as follows: COD = FAS used for blank – FAS used for sample/Volume of sample where FAS = Ferrous Ammonium Sulfate Solution The Theoretical Oxygen Demand (ThOD) was calculated from the elemental composition of the test substance and the reference substance. The Biological Oxygen Demand (BOD) was calculated as follows: BOD = ((mgO_2/L by test substance – mgO_2/L by blank) – (Total oxygen equivalent due to nitrate and nitrite))/mg test substance/L in vessel The percentage biodegradation of the test substance was calculated as BOD/ThOD or COD x 100.	
		4 RESULTS	
4.1	**Degradation of test substance**		
4.1.1	Graph	Figures A7.1.1.2.1/2-1 and A7.1.1.2.1/2-2	
4.1.2	Degradation	The percent degradation of the test substance on days 7, 14, 21 and 28 were 2.12%, 1.73%, 1.34% and 7.79%, respectively (based on COD), and 1.35%, 1.10%, 0.85% and 4.94%, respectively (based on ThOD).	
4.1.3	Other observations	Not applicable	
4.1.4	Degradation of TS in abiotic control	No abiotic control	
4.1.5	Degradation of reference substance	The percent degradation of the reference substance on days 7, 14, 21 and 28 were 45.49%, 68.45%, 81.63% and 86.31%, respectively (based on COD) and 45.53%, 68.51%, 81.70% and 86.38%, respectively (based on ThOD).	
4.1.6	Intermediates/ degradation products	Intermediates were not identified	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The biodegradability of BIT was determined at a concentration of 2 mg/L of test substance in mineral medium inoculated with a mixture of river water, garden soil extract and supernatant of septic tank (0.25 mL/L). Potassium hydrogen phthalate was used as a reference substance to check the validity of the method. Mineral medium inoculated with mix inoculum (0.25 mL/L) without BIT and potassium hydrogen phthalate served as a control. Seperate test solutions were maintained contemporaneously (in duplicate) for test, refereence and control samples, for observattion on day 0, 7, 14, 21	

Section A7 Subsection A7.1.1.2.1/2 Annex Point IIA7.6.1.1	Ecotoxicological Profile Including Environmental Fate and Behaviourç BIOTIC Biodegradability (ready) (02)	
	and 28. The Chemical Oxygen Demand of BIT and potassium hydrogen phthalate was determined by the open reflux method. This study was conducted according to OECD guideline 301D and is described under point 3 with the following deviations: 1. The amount of chemicals used to make up the stock solutions of mineral medium were 1/10 of that recommended in the Guideline and were made up to 100 ml rather than 1 L. 2. 4 ml of mineral medium stock solutions a, b, c and d were added to 3984 mL of double distilled water. 3. 80 mg of test substance and reference substance were made up to 100 mL and 10 mL respectively with mineral medium. These deviations are not considered to compromise the scientific validity of this study.	
5.2 Results and discussion	The biodegradability of BIT was 2.12%, 1.73%, 1.34%, and 7.79% (based on COD i.e. 1.413 mg O_2/mg of test substance) and 1.35%, 1.10%, 0.85% and 4.94% (based on ThOD i.e. 2.225 mg O_2/mg of test substance) on days 7, 14, 21 and 28. Please refer to Tables A7.1.1.2.1/2-4 to A7.1.1.2.1/2-7. The biodegradability of potassium hydrogen phthalate was 45.49, 68.45, 81.63 and 86.31 % (based on COD i.e. 1.176 mg/O_2 of reference substance) and 45.53, 68.51, 81.70 and 86.38 % (based on ThOD i.e. 1.175 mg O_2/mg of reference substance) on day 7, 14, 21 and 28 respectively. Please refer to Tables A7.1.1.2.1/2-4 to A7.1.1.2.1/2-5 and Table A7.1.1.2.1/2-8.	
5.3 Conclusion	The percentage biodegradation data revealed that the test substance was not readily biodegradable. Even though the maximum degradation was below 10 %, it was not observed in a time related manner. Therefore, it can be inferred that BIT is not readily biodegradable. See pass levels in Table A7.1.1.2.1/2-9	
5.3.1 Reliability	1	
5.3.2 Deficiencies	Yes, Three deviations were noted and are outlined under points 2.3 and 5.1. However, they do not compromise the scientific validity of this study.	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2012.*

Section A7

Subsection A7.1.1.2.1/2

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Biodegradability (ready) (02)

Materials and Methods	*Applicant's version is accepted despite the following deviations from the OECD guideline 301D:* ▪ *The amount of chemicals used to make up the stock solutions of mineral medium were 1/10 of that recommended in the Guideline and were made up to 100 mL rather than 1 L.* ▪ *4 mL of mineral medium stock solutions a, b, c and d were added to 3984 ml of double distilled water.* ▪ *80 mg of test substance and reference substance were made up to 100 mL and 10 mL respectively with mineral medium.* ▪ *TS inhibitory to microorganisms: There is no toxicity control to provide evidence of this inhibition.*
Results and discussion	*Applicant's version is accepted*
Conclusion	*The percentage biodegradation data revealed that the test substance was not readily biodegradable. Even though the maximum degradation was below 10 %, it was not observed in a time related manner. Therefore, it can be inferred that BIT is not readily biodegradable.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*Key Study.*

Table A7.1.1.2.1/2-1: Inoculum / Test organism

Criteria	Details
Nature	Garden soil extract and supernatant of septic tank
Species	Not documented
Strain	Not documented
Source	River water + garden soil + supernatant of septic tank mix
Sampling site	River water: upstream of Daman Ganga River, Vapi, Gujarat, India Garden soil: Jai Research Foundation
Laboratory culture	Yes
Method of cultivation	Stirring for 20 minutes
Preparation of inoculum for exposure	The inoculum was filtered through Whatman filter paper No1 and aerated for 2 hours at 20 ± 1°C prior to use.
Pretreatment	Not documented
Initial cell concentration	Not documented

Table A7.1.1.2.1/2-2: Test system

Criteria	Details
Culturing apparatus	BOD bottles
Number of culture flasks/concentration	Test solution – 2 mg/L (10 mL of stock test solution + 1 mL of mixed inoculum + 3989 mL mineral medium Reference solution – 2 mg/L (1 mL stock reference solution + 1 mL of inoculum + 3998 mL mineral medium) Control – 1 mL of inoculum + 3999 mL of mineral medium
Aeration device	Not documented
Measuring equipment	Dissolved oxygen meter (YSI 5100)
Test performed in closed vessels due to significant volatility of TS	No

Table A7.1.1.2.1/2-3: Test conditions

Criteria	Details
Composition of medium	Solution a: KH_2PO_4 0.85 g/100mL K_2HPO_4 2.175 g/100mL $Na_2HPO_4.7H_2O$ 3.34 g/100mL NH4Cl 0.05 g/100mL Solution b: $CaCl_2.2H_2O$ 3.64 g/100mL Solution c: $MgSO_4.7H_2O$ 2.25 g/100mL Solution d: $FeCl_3.6H_2O$ 0.025 g/100mL Three conical flasks (5 L capacity) each containing 3984 mL of double distilled water were taken. To each conical flask 4 mL of stock solutions A, B, C and D were added. The mineral medium was strongly aerated for 20 minutes using an aerator and allowed to stand for 20 h at 20 ± 1°C. All operations were performed in a horizontal laminar flow under aseptic conditions.
Additional substrate	No
Test temperature	20 ± 1°C
pH	pH 7.4 (solution a)
Aeration of dilution water	Not documented
Suspended solids concentration	Not documented

Table A7.1.1.2.1/2-4: Dissolved oxygen values for the control, reference substance and test substance

Treatment		Dissolved oxygen (mg/L) on day				
		0	7	14	21	28
Control	Replicate - 1	9.06	8.33	8.05	7.95	7.79
	Replicate - 2	9.09	8.35	8.09	7.98	7.87
	Mean	9.08	8.34	8.07	7.97	7.83
Potassium hydrogen phthalate (2 mg/L)	Replicate - 1	9.08	7.27	6.46	5.96	5.80
	Replicate - 2	9.11	7.31	6.50	6.17	5.84
	Mean	9.10	7.29	6.48	6.07	5.82
BIT (2 mg/L)	Replicate - 1	9.12	8.34	8.07	8.00	7.62
	Replicate - 2	9.19	8.38	8.13	8.02	7.76
	Mean	9.16	8.36	8.10	8.01	7.69

Table A7.1.1.2.1/2-5: Oxygen consumption for degradation of reference and test substance

Treatments	Dissolved oxygen (mg/L) on day			
	7	14	21	28
Potassium hydrogen phthalate (2 mg/L)	1.07	1.61	1.92	2.03
BIT (2 mg/L)	0.06	0.05	0.04	0.22

Table A7.1.1.2.1/2-6: Oxygen consumption for degradation of test substance after correction of total oxygen equivalent due to nitrate and nitrite

Oxygen consumption (corrected) on day (mg/L)			
7	14	21	28
0.060	0.0489	0.0379	0.22

Table A7.1.1.2.1/2-7: BOD and percentage degradation of BIT

Day	BOD (mg O_2/mg test substance)	Percentage of degradation	
		Based on COD	Based on ThOD
7	0.0300	2.12	1.35
14	0.0245	1.73	1.10
21	0.0190	1.34	0.85
28	0.1100	7.79	4.94

Table A7.1.1.2.1/2-8: BOD and percentage degradation of Potassium Hydrogen Phthalate

Day	BOD (mg O2/mg test substance)	Percentage of degradation	
		Based on COD	Based on ThOD
7	0.535	45.49	45.53
14	0.805	68.45	68.51
21	0.960	81.63	81.70
28	1.015	86.31	86.38

Table A7.1.1.2.1/2-9: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$		Not fulfilled
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		Not fulfilled
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	Fulfilled	
Percentage of removal of reference substance reaches pass level by day 14	Fulfilled	

Figure A7.1.1.2.1/2-1: Degradation curve of BIT

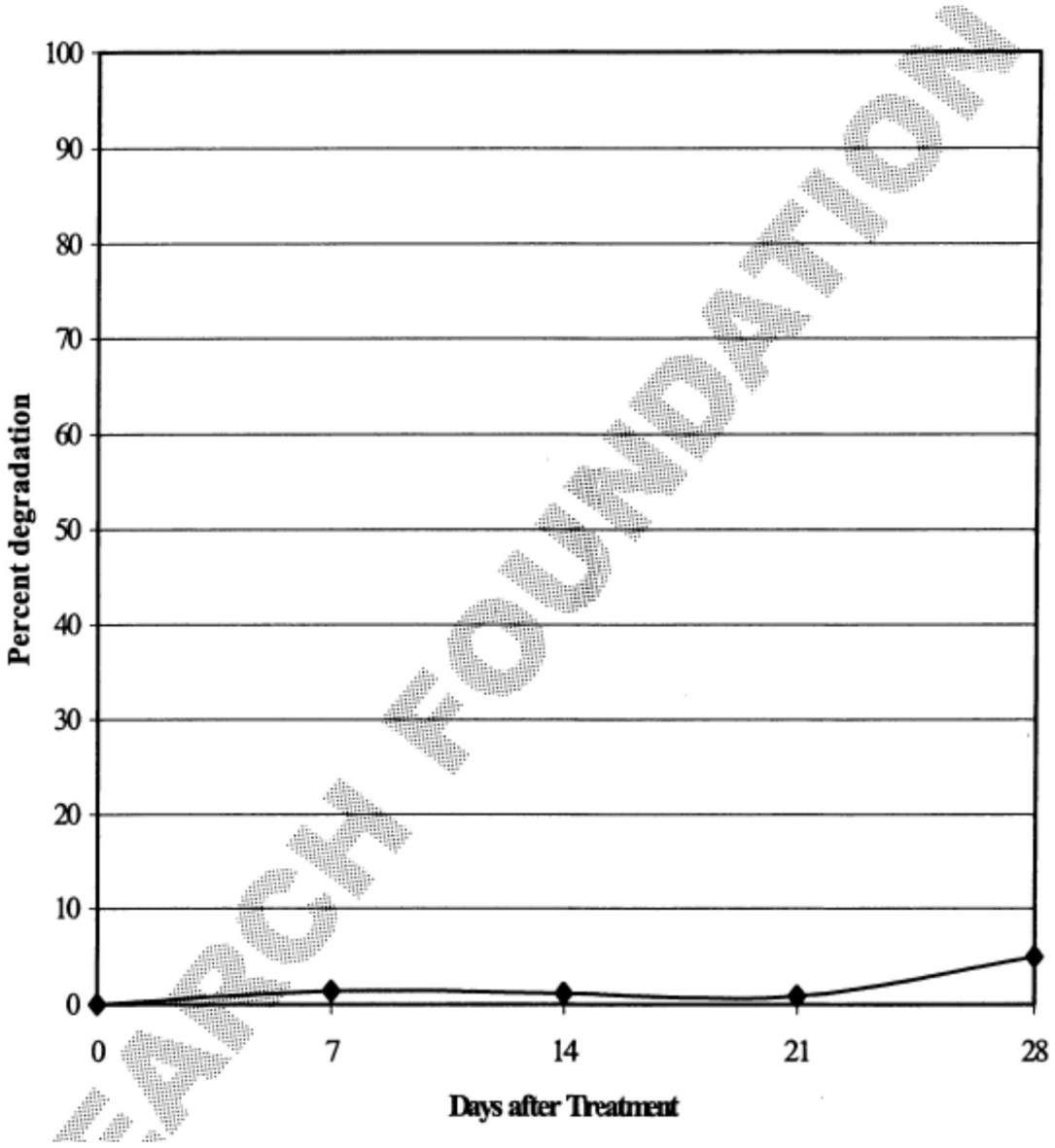
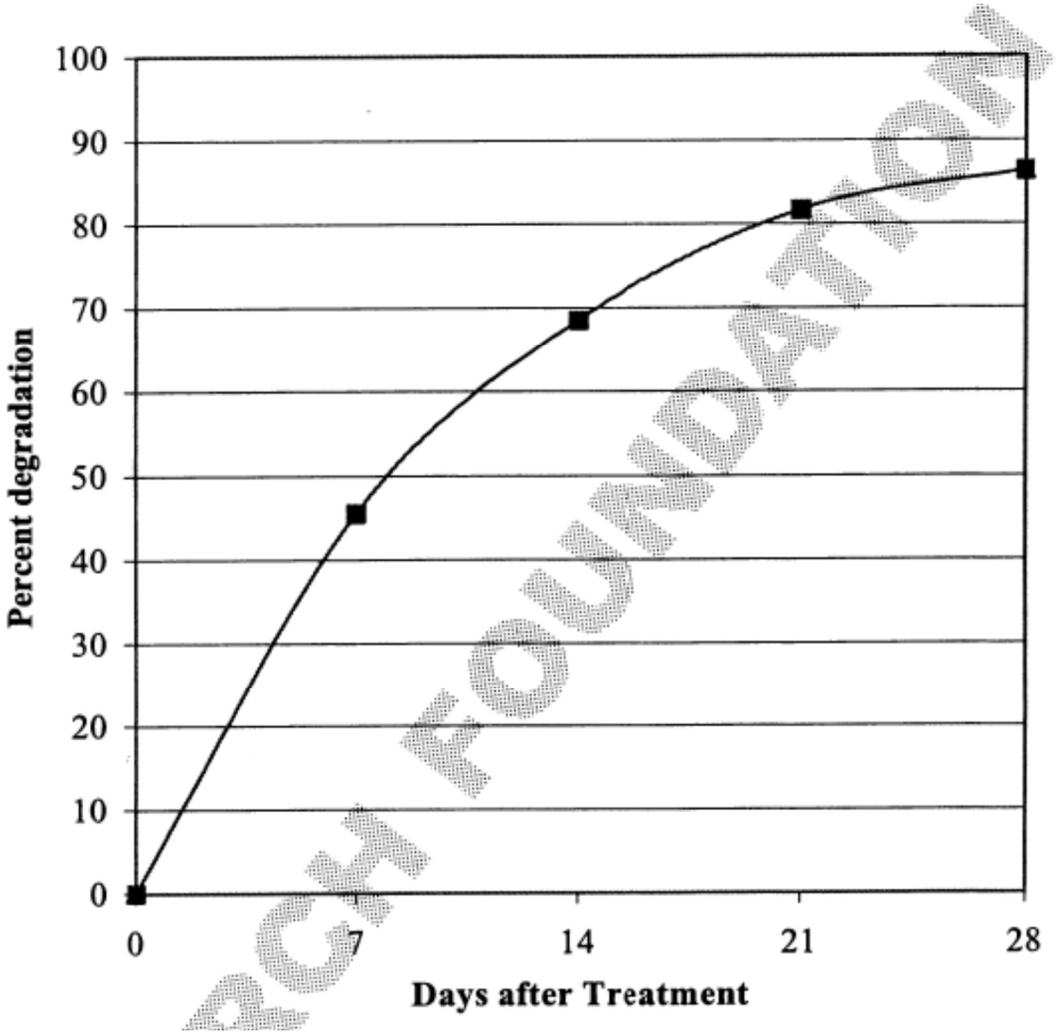


Figure A7.1.1.2.1/2-2: Degradation curve of potassium hydrogen phthalate



Section A7
Subsection A7.1.1.2.1/3
Annex Point IIA7.6.1.1

Ecotoxicological Profile Including Environmental Fate and Behaviourç
BIOTIC

Biodegradability (ready) (03)

		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] Dates of experimental work: December 19, 2005 – January 19, 2006.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Rohm and Hass	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on OECD guideline 301B, CO_2 evolution test.	
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	220904	
3.1.2	Specification	Please refer to point 3.1.3	
3.1.3	Purity	100 %	
3.1.4	Further relevant properties	Not applicable	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	No	**X**

Section A7 Subsection A7.1.1.2.1/3 Annex Point IIA7.6.1.1	Ecotoxicological Profile Including Environmental Fate and Behaviourç BIOTIC Biodegradability (ready) (03)	
3.1.7 Specific chemical analysis	Not applicable	
3.2 Reference substance	Yes, sodium benzoate	
3.2.1 Initial concentration of reference substance	7.7 g/L	
3.3 Testing procedure		
3.3.1 Inoculum / test species	Please refer to Table A7.1.1.2.1/3-1	
3.3.2 Test system	Please refer to Table A7.1.1.2.1/3-2	
3.3.3 Test conditions	Please refer to Table A7.1.1.2.1/3-3	
3.3.4 Method of preparation of test solution	Not appropriate	
3.3.5 Initial TS concentration	17.9 mg/L – 18.0 mg/L	
3.3.6 Duration of test	28 days	
3.3.7 Analytical parameter	Carbon dioxide evolution	
3.3.8 Sampling	Samples were taken on days 2, 6, 9, 12, 14, 19, 23, 27, 28 and 29.	
3.3.9 Intermediates/ degradation products	Not identified	
3.3.10 Nitrate/nitrite measurement	Not applicable	
3.3.11 Controls	Abiotic control: 18.2 mg/L test substance + 10 mg/L mercury dichloride Toxicity control: 18.2 mg/L test substance + 25.7 mg/L reference substance + inoculum Procedure control: 25.7 mg/L reference substance + inoculum Inoculum control: Inoculum Abiotic control blank: 10 mg/L mercury dichloride	

Section A7 Subsection A7.1.1.2.1/3 Annex Point IIA7.6.1.1	Ecotoxicological Profile Including Environmental Fate and Behaviourç BIOTIC Biodegradability (ready) (03)	
3.3.12 Statistics	The percent degradation was calculated from: % degradation n = (mg IC_{prod} in test flask – mg IC_{prod} in blank/mg TOC) x 100 % Where, Test flask = flasks containing test item and or reference item Blank = flasks containing neither test item nor reference item TOC = mg TOC added as test and or reference item The conversion factor of carbon to carbon dioxide is 3.67.	
	4 RESULTS	
4.1 Degradation of test substance		
4.1.1 Graph	Figure A7.1.1.2.1/3-1	
4.1.2 Degradation	The CO_2 production of 1,2-Benzisothiazolin-3-one in the test media was slightly lower than the CO_2 production of the inoculum controls. Please refer to Tables A7.1.1.2.1/3-4 - A7.1.1.2.1/3-6.	
4.1.3 Other observations	Not applicable	
4.1.4 Degradation of TS in abiotic control	In the abiotic control, containing 1,2-Benzisothiazolin-3-one and poisoned test medium, no significant degradation was noted at the end of the 28 day exposure period (< 10 % of the TOC).	
4.1.5 Degradation of reference substance	In the procedure controls, the reference item was degraded to an average extent of 78 % by day 14, thus confirming suitability of the activated sludge (> 60 % degradation by day 14). By the end of the test (day 28), the reference item was degraded to an average extent of 85 %. Please refer to table A7.1.1.2.1/3-6.	
4.1.6 Intermediates/ degradation products	Intermediates were not identified	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The percent biodegradation of 1,2-Benzisothiazolin-3-one was calculated based on a Total Organic Carbon (TOC) content of 0.56 mg C/mg test item. One day before the test start, between 2400 and 3000 mL of untreated test medium was placed into 5 L flasks. To each flask (except for the abiotic control and the abiotic control blank), 90 mL of activated sludge inoculum was added. For the abiotic control and the abiotic control blank, the untreated test medium was poisoned with 10 mg/L mercury dichloride. The test media were aerated overnight with CO_2 free air. On day 0, defined amounts of the test item were weighed and transferred to the designated flasks with test water. Sodium benzoate was tested	

Section A7 Subsection A7.1.1.2.1/3 Annex Point IIA7.6.1.1	Ecotoxicological Profile Including Environmental Fate and Behaviourç BIOTIC Biodegradability (ready) (03)	
	simultaneously under the same conditions as 1,2-Benzisothiazolin-3-one and functioned as a procedure control. A stock solution containing 771 mg sodium benzoate per 100 mL test water was prepared. From this, 10 mL aliquots were added to the corresponding test flasks. The test flasks were made up to a volume of three litres with test water. Samples were then taken on days 0, 2, 6, 9, 12, 14, 19, 23, 27, 28 and 29 for analysis of CO_2 content.	
5.2 Results and discussion	The CO_2 production of the test item in the test media was slightly below the CO_2 production of the inoculum controls. In the abiotic control, containing 1,2-Benzisothiazolin-3-one and poisoned test medium, no significant degradation was noted at the end of the 28-day exposure period (< 10 % of the TOC content). In the procedure controls, the reference item was degraded to an average extent of 78 % by day 14, thus confirming suitability of the activated sludge (> 60 % degradation by day 14). By the end of the test (day 28), the reference item was degraded to an average extent of 85 %.	
5.3 Conclusion	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 no inhibitory effect on the activity of activated sludge microorganisms at the tested concentration of 18 mg/l. 1,2-Benzisothiazolin-3-one was not biodegradable under the test conditions within 28 days.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*March2013*
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.*

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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 no inhibitory effect on the activity of activated sludge microorganisms at the tested concentration of 18 mg/L.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.1.1.2.1/3-1: Inoculum / Test organism

Criteria	Details
Nature	Aerobic activated sludge
Source	Wastewater treatment plant treating predominantly domestic wastewater
Sampling site	ARA Ergolz II, Füllinsdorf, Switzerland
Laboratory culture	Yes
Preparation of inoculum for exposure	The sludge was washed twice with tap water by centrifugation and the supernatant liquid 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. Calculated amounts of wet sludge were suspended in test water to obtain a concentration of 4 mg dry material/L. During holding, the sludge was aerated at room temperature until use. Prior to use, the sludge was diluted with test water the solids level was then determined and an appropriate volume used to inoculate test vessels.
Pretreatment	No
Initial cell concentration	30 mg dry material/L

Table A7.1.1.2.1/3-2: Test system

Criteria	Details
Culturing apparatus	5 L Amber glass flasks
Number of culture flasks/concentration	9
Aeration device	Air was led through a bottle containing about 750 mL of a 2 M NaOH solution to trap CO_2. The CO_2-free air was passed through the test solutions at a rate corresponding to about 30 – 100 mL/min.
Measuring equipment	The samples were analysed for inorganic carbon using a TOC analyser (Shimadzu TOC-5000A) equipped with an automatic sampler.
Test performed in closed vessels due to significant volatility of TS	No

Table A7.1.1.2.1/3-3: Test conditions

Criteria	Details
Composition of medium	Solution a: KH_2PO_4 8.5 g/L K_2HPO_4 21.75 g/L $Na_2HPO_4.2H_2O$ 33.4 g/L NH_4Cl 0.5 g/L Solution b: $MgSO_4.7H_2O$ 22.5 g/L Solution c: $CaCl_2.2H_2O$ 36.4 g/L Solution d: $FeCl_3.6H_2O$ 0.25 g/L 10 mL of stock solution a and 1 mL of stock solutions b, c and d were added to approximately 800 mL of purified water and made up to 1000 mL with purified water. The pH was adjusted from 7.7 to 7.4 with a diluted hydrochloric acid solution.
Additional substrate	No
Test temperature	20 °C – 22 °C
pH	pH 7.6 - 7.7
Aeration of dilution water	Purged with CO_2 free air
Suspended solids concentration	30 mg dry material/L
Other relevant criteria	Not relevant

Table A7.1.1.2.1/3-4: Inorganic carbon (IC concentrations) measured in the absorber flasks

	IC found in absorber flasks (IC_{abs}, mg C/L)

Time (days)*	Test item		Sodium benzoate		Toxicity control	Abiotic control	Abiotic blank	Inoculum control		Volume (mL) NaOH
	Replicate number:									
	1	2	1	2	1	1	1	1	2	
2/1	12.4	10.2	32.3	64.2	8.7	7.0	5.6	11.6	12.3	300
6/1	19.4	17.8	51.9	111.6	79.5	10.6	8.8	28.1	31.0	295
9/1	25.9	23.0	66.9	132.9	93.3	14.3	11.6	34.9	39.4	290
12/1	30.1	27.1	74.8	142.6	100.7	16.2	13.8	39.4	44.8	285
14/1	32.2	33.6	81.7	149.1	106.6	18.5	16.4	43.6	48.5	280
14/2	11.3	10.3	133.0	56.3	55.8	4.6	6.8	22.8	20.5	200
19/1	40.3	37.8	97.0	162.9	115.8	22.5	19.6	50.1	56.0	275
23/1	46.3	45.2	104.8	171.8	121.8	26.3	23.2	52.8	62.6	270
27/1	53.2	50.7	111.8	182.4	124.4	33.3	28.2	58.5	67.8	265
28/1	54.1	50.4	108.4	174.1	128.1	32.9	28.6	58.6	70.8	260
28/2	15.6	13.3	144.6	62.6	66.9	7.3	9.8	25.7	23.2	195
29/1	58.1	55.9	115.2	185.9	133.2	35.0	31.3	69.4	73.3	255
29/2	18.7	16.5	149.1	67.9	68.5	5.5	10.9	30.0	23.8	190

* Absorber flask 1 or 2

Table A7.1.1.2.1/3-5: Absolute amount of inorganic carbon (IC) produced

Time (days)	IC production per test flask (ICprod, mg C/3L)								
	Test item		Sodium benzoate		Toxicity control	Abiotic control	Abiotic blank	Inoculum control	
	Replicate number:								
	1	2	1	2	1	1	1	1	2
2	4.0	3.4	13.5	20.9	4.2	2.2	1.9	4.1	4.3
6	6.8	6.2	26.9	38.1	28.3	3.6	3.2	10.3	11.0
9	9.1	8.1	36.9	46.7	34.7	4.8	4.3	13.3	14.3
12	10.8	9.7	44.9	51.8	39.2	5.6	5.2	15.5	16.7
14	11.7	11.9	50.6	55.3	42.4	6.3	6.2	17.3	18.3
19	14.2	13.2	55.6	59.5	45.7	7.6	7.2	19.3	20.6
23	16.1	15.4	58.4	62.3	48.0	8.8	8.4	20.2	22.5
27	18.2	17.0	60.9	65.4	49.3	10.8	9.9	21.9	24.0
28	18.5	17.0	60.2	63.3	50.4	10.7	10.0	22.0	24.8
29	20.1	19.0	62.7	67.4	52.0	10.9	10.9	25.5	25.6

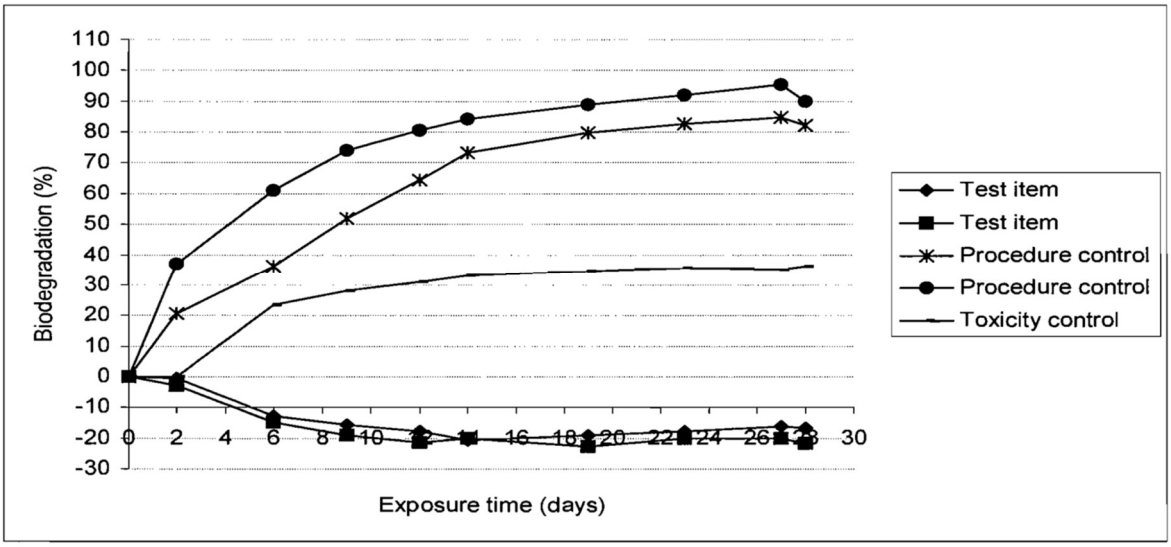
Table A7.1.1.2.1/3-6: Biodegradation of 1,2-Benzisothiazolin-3-one and sodium benzoate

Time (days)	% Degradation							
	1,2-Benzisothiazolin-3-one			Sodium benzoate			Toxicity control	Abiotic control
	Replicate number:			Replicate number:			Replicate number:	
	1	2	Mean	1	2	Mean	1	1
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	81.8	88.9	85.4	35.8	2.4

Table A7.1.1.2.1/3-7: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$		Not fulfilled
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		Not fulfilled
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	Fulfilled	
Percentage of removal of reference substance reaches pass level by day 14	Fulfilled	

Figure A7.1.1.2.1/3-1: Biodegradation of 1,2-Benzisothiazolin-3-one and sodium benzoate during the incubation period



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			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: 31 October, 2006 – 15th February, 2007	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Rohm and Haas	
1.2.2	Companies with letter of access	Troy Chemical Company B.V.	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on a modified OECD guideline 301B, CO_2 evolution test and the US EPA method 835.3110 (m)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No. Please note that as the test substance is known to be inhibitory to the test systems routinely employed to assess biodegradation. Consequently, in order to assess the intrinsic biodegradability of the test substance a modified test regime was employed. The test substance was tested at a concentration below the limit of inhibition. At low-test concentrations, the standard method of analysis would not be able to distinguish between the low levels of carbon dioxide evolved from biodegradation of the test compound because this would be indistinguishable from the relatively high background level of carbon dioxide evolution from the culture. Consequently, a radiolabelled test substance was used to lower the limit of evolved CO_2 (i.e., $^{14}CO_2$) detection.	**X**
		3 MATERIALS AND METHODS	
3.1	**Test material**	Radiolabelled and non-radiolabelled 1,2-Benzisothiazol-3-(2*H*)-one (BIT)	
3.1.1	Lot/Batch number	Radiolabelled test material: 1077.00 Non-radiolabelled test material: 060309/1	

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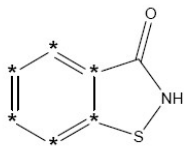
3.1.2	Specification	Please refer to point 3.1.3	
3.1.3	Purity	Radiolabelled test material: 97.7 % Non-radiolabelled test material: 99.8 %	
3.1.4	Specific Activity	163.79 mCi/g	
3.1.5	Radiolabelling	 * site of ^{14}C label	
3.1.6	Further relevant properties	Not applicable	
3.1.7	Composition of Product	Not applicable	
3.1.8	TS inhibitory to microorganisms	Yes	
3.1.9	Specific chemical analysis	Not applicable	
3.2	**Reference substance**	Yes, sodium benzoate	
3.2.1	Initial concentration of reference substance	15 mgCarbon/L (mgC/L)	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	Please refer to Table A7.1.1.2.1/4-1	
3.3.2	Test system	Please refer to Table A7.1.1.2.1/4-2	
3.3.3	Test conditions	Please refer to Table A7.1.1.2.1/4-3	
3.3.4	Method of preparation of test solution	Not appropriate	
3.3.5	Initial TS concentration	5, 2.5, 1.25, 0.625 and 0.313 mg/L for both preliminary tests and 0.313 mg/L for the main study.	**X**

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3.3.6	Duration of test	15 days for the first preliminary, 10 days for the second preliminary test and 29 days for the main test.	
3.3.7	Analytical parameter	Carbon dioxide evolution	
3.3.8	Sampling	First preliminary test: days 1, 2, 3, 6, 8, 10, 14 and 15 Second preliminary test: days 1, 3, 6, 8, 9 and 10 Main test: days 1, 3, 6, 8, 10, 13, 15, 16, 20, 22, 24, 28 and 29	
3.3.9	Intermediates/ degradation products	HPLC analysis of samples from the two main test cultures showed that there were two metabolites at retention times of 18 minutes and 20 minutes constituting 22.6 % and 49.19 % of the detected compounds (by area).	
3.3.10	Nitrate/nitrite measurement	Not applicable	
3.3.11	Controls	Control: inoculated mineral salts medium Toxicity control: inoculated mineral salts medium + 15 mgC/L sodium benzoate + 0.313 mg/L BIT	
3.3.12	Statistics	The extent of biodegradation in vessels containing the test substance, the reference substance or both, expressed as a percentage, was calculated as: % biodegradation = $\frac{cumulative\ CO_2}{theoretical CO_2}$ x 100	
		4 RESULTS	
4.1	**Degradation of test substance**		
4.1.1	Graph	Figures A7.1.1.2.1/4-1 - A7.1.1.2.1/4-5	
4.1.2	Degradation	In the main test, after an initial lag phase of 8 days, biodegradation of ^{14}C-BIT progressed steadily and achieved 10 % by day 11 of the test. From day 13 onward the rate of degradation slowed reaching 20.1 % on day 16 and 23.8 % by the end of the test on day 28. Maximum divergence between replicates was 1 % and was observed on day 10 of the test. Please refer to Table A7.1.1.2.1/4-7.	
4.1.3	Other observations	Preliminary test 1: Total viable cell count data at day 7 and day 14 show that relative to the control cultures, the microbial population was not depleted in the presence of the test substance. The results clearly demonstrated that the viable cell density increased with increasing concentrations of BIT. The biodegradation of sodium benzoate was only suppressed at a BIT concentration of 5 mg/L. At all other dose concentrations the biodegradation of sodium benzoate was either comparable with or	

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		higher than that seen in the vessels containing sodium benzoate. Please refer to Tables A7.1.1.2.1/4-4 and A7.1.1.2.1/4-5 and Figure A7.1.1.2.1/4-1. Preliminary test 2: Carbon dioxide data show that at BIT concentrations of 1.25 mg/L and below, the carbon dioxide evolution of the cultures was comparable to that of a standard culture with no BIT. Please refer to Table A7.1.1.2.1/4-6 and Figure A7.1.1.2.1/4-2.	
4.1.4	Degradation of TS in abiotic control	Not applicable	
4.1.5	Degradation of reference substance	In the main test, rapid CO_2 generation commenced immediately in the reference controls and declined to a more gradual rate around day 6. The rate of biodegradation began to plateau around day 16. Biodegradation exceeded 60 % in both vessels by day 14 and the mean level of degradation on day 28 was 88 %. Please refer to Table A7.1.1.2.1/4-9 and Figure A7.1.1.2.1/4-4.	
4.1.6	Intermediates/ degradation products	HPLC analysis of samples from the two main test cultures showed that there were two major metabolites at retention times of 18 minutes and 20 minutes constituting 22.6 % and 49.19 % of the detected compounds (by area).	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Preliminary test 1: BIT was added in its non-radiolabelled form. Three treatment groups were established: Control: Inoculated mineral salts medium (two control vessels) Reference: Inoculated mineral salts medium + 15 mgC/L sodium benzoate (two reference vessels) Toxicity control: Inoculated mineral salts medium + 15 mgC/L sodium benzoate + BIT at the following concentrations; 5 mg/L, 2.5 mg/L, 1.25 mg/L, 0.625 mg/L and 0.313 mg/L (five toxicity control vessels) All vessels were fitted with a series of three barium hydroxide traps (nominal 0.0125 M). Biodegradation was of the reference substance monitored by titration of the trap. Total viable cells were undertaken on day 7 and and day 14 in order to monitor the microbial content of the cultures. Preliminary test 2: BIT was added in its non-radiolabelled form. Two treatment groups were estabished: Control: Inoculated mineral salts medium (two control vessels)	

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Test: Inoculated mineral salts medium + BIT at the following concentrations; 5 mg/L, 2.5 mg/L, 1.25 mg/L, 0.625 mg/L and 0.313 mg/L (five test vessels)

All vessels were fitted with a series of 3 barium hydroxide traps (nominal 0.0125 M). Carbon dioxide evolution from each vessel was monitored by titration.

Main test:

Four treatment groups were established:

Control: Inoculated mineral salts medium (two control vessels using barium hydroxide traps and two control vessels using sodium hydroxide traps)

Reference: Inoculated mineral salts medium + 15 mgC/L sodium benzoate (two reference vessels)

Toxicity control: Inoculated mineral salts medium + 15 mgC/L sodium benzoate + 0.313 mg/L BIT (one toxicity control vessel)

Test: Inoculated mineral salts medium + 0.313 mg/L ^{14}C-BIT (two test vessels)

The test substance was sufficiently soluble to allow the preparation of aqueous stock solutions for dosing. In both preliminary tests, an aqueous stock solution was prepared by accurately weighing 37.52 mg of non-radiolabelled BIT into a glass weighing boat and rinsing this into a 250 mL volumetric flask with ultrapure water. The solution was made up to the mark with ultrapure water to provide a stock solution at 150 mg/L. Test concentrations of 5, 2.5, 1.25, 0.625 and 0.313 mg/L were acheived by addition to individual vessels of aliquots of 100, 50, 25, 12.5 and 6.25 mL, respectively.

In the main biodegradation test, an aqueous stock solution of ^{14}C-BIT was prepared by accurately weighing 4.580 mg ^{14}C-BIT and adding approximately 50 ml ultrapure water. The radiochemical purity of the solution was determined using HPLC and TLC. The specific activity of the dosing solution was determined using LSC before and after dosing was performed. Addition of 9.8 mL of this stock solution to the two test vessels delivered 0.971043 mg ^{14}C-BIT to give a nominal concentration of 0.3237 mg/L. The toxicity control vessel was dosed with non-radiolabelled BIT by additon of 6.25 mL of an aqueous solution at 37.48 mg/L.

A stock solution at 2.25 gC/L was prepared by dissolving approximately 3.859 g sodium benzoate in 1000 mL reverse-osmosis water. Reference and toxicity control vessels were dosed by addition of 20 mL of the stock solution, to give a nominal sodium benzoate concentration corresponding to 15 mg C/L.

Once all the additions were complete, the volume in each vessel was raised to 3 L by additon of ultra-pure water. The pH of each culture was measured. Each vessel was then sealed, connected to a dedicated series of three traps and the air supply restored.

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5.2 Results and discussion	Total viable cell count data at day 7 and day 14 show that the microbial population was not depleted in the presence of the test substance. The results clearly demonstrated that the viable cell density increased with increasing concentrations of BIT. Carbon dioxide data show that at BIT concentrations of 1.25 mg/L and below, the carbon dioxide evolution of the cultures was comparable to that of a standard culture with no BIT. Mean CO_2 production in the control cultures was 81.4 mg at the end of the main test. This was considered acceptable for this test system. After an initial lag phase of 8 days, biodegradation of ^{14}C-BIT progressed steadily and achieved 10 % by day 11 of the test. From day 13 onward the rate of degradation slowed reaching 20.1 % on day 16 and 23.8 % by the end of the test on day 28. Maximum divergence between replicates was 1 % and was observed on day 10 of the test. In the reference controls containing only sodium benzoate, rapid CO_2 generation commenced immediately and declined to a more gradual rate around day 6. The rate of biodegradation began to plateau around day 16. Biodegradation exceeded 60 % in both vessels by day 14, satisfying the validity criterion and the mean level of degradation on day 28 was 88 %. This data indicated that the sample of activated sludge used to inoculate cultures was viable. Assessment of degradation in the toxicity control was confined to measuring the evolution of $^{12}CO_2$ from sodium benzoate. The rate of CO_2 production in this vessel generally kept pace with that observed in the two reference vessels containing sodium benzoate alone. The level of biodegradation on day 28 was 88 %. The absence of any suppression with respect to the performance of sodium benzoate alone demonstrates that the ^{14}C-BIT present in the toxicity control did not initially inhibit the microbial degradation of the reference substance. HPLC analysis of samples from the two main test cultures showed that there was no BIT present at the end of the test and that there were two major metabolites at retention times of 18 minutes and 20 minutes constituting 22.6 % and 49.19 % of the detected compounds (by area).	**X**
5.3 Conclusion	^{14}C-BIT cannot be considered to be readily biodegradable. Although ^{14}C-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 and would not persist in an aerobic aquatic environment.	**X**
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

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Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*November 2009*
Materials and Methods	*Applicant's version is accepted with the following comments:* *3.3.5. 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.*
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	*^{14}C-BIT cannot be considered to be readily biodegradable. Although ^{14}C-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. However, in this study, BIT was tested at lower concentration than expected in the environment.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.1.1.2.1/4-1: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Source	Return line at a sewage treatment works with a waste water catchment that is predominantly domestic.
Sampling site	Burley Menston sewage treatment works, West Yorkshire, UK
Laboratory culture	Yes
Preparation of inoculum for exposure	On arrival at the laboratory, the sample was blended and aerated by means of a compressed air supply delivered through a diffuser block. The suspended solids concentration was determined by filtering a 25 mL subsample through a pre-dried and pre-weighed glass microfibre filter (Whatman GF/C). The filter and retained solids were then dried in an oven and re-weighed. The contribution made by the sludge solids was determined by difference.
Pretreatment	No
Initial cell concentration	90 mg suspended solids /L, each test vessel had a nominal final solids concentration of 30 mg/L in each test vessel.

Table A7.1.1.2.1/4-2: Test system

Criteria	Details
Culturing apparatus	Not described
Number of culture flasks/concentration	Preliminary test 1: 9 Preliminary test 2: 7 Main test: 9
Aeration device	The air used in this study was a cylinder supply of nominally CO_2-free air (Air Products). The air flow in this study was regulated in two stages. Initial control was provided by a gas regulator and the air flow to each vessel controlled by individual needle valves. During the main test, measurements were made on four occasions (weekly), with a bubble flow meter and stopwatch, of the flow rate exiting each test vessel. Adjustments were made necessary to maintain a flow rate of approximately 50 mL per minute.
Measuring equipment	The inorganic carbon concentration was determined using an Apollo 9000 carbon analyser. In this analysis the sample was acidified with phosphoric acid to convert inorganic carbon to CO_2. CO_2 free air was then passed through the sample and the generated CO_2 carried to a non-dispersive infra-red (NDIR) detector. The concentration of CO_2 was determined in the NDIR detector, by measuring the amount of infra-red energy absorbed by the sample. A calibration curve was produced by injecting a series of sodium hydrogen carbonate standards and this used to quantify the inorganic carbon present in the samples.
Test performed in closed vessels due to significant volatility of TS	No

Table A7.1.1.2.1/4-3: Test conditions

Criteria	Details
Composition of medium	Solution a: KH_2PO_4 8.5 g/L K_2HPO_4 21.75 g/L $Na_2HPO_4.2H_2O$ 33.4 g/L NH_4Cl 0.5 g/L Solution b: $CaCl_2.2H_2O$ 36.4 g/L Solution c: $MgSO_4.7H_2O$ 22.5 g/L Solution d: $FeCl_3.6H_2O$ 0.25 g/L Solutions a-d were dissolved in and made up to 1 L with reverse-osmosis water. A test medium concentrate was prepared containing 30 mL/L solution a and 3 mL/L of each of solutions b, c and d.
Additional substrate	No
Test temperature	21 ± 1 °C
pH	Please refer to Table A7.1.1.2.1/4-10.
Aeration of dilution water	Not documented
Suspended solids concentration	30 mg suspended solids/L
Other relevant criteria	None

Table A7.1.1.2.1/4-4: Total viable cell count data in preliminary test 1

	Mean total Viable Cells (cells/mL)	
	Day 7	Day 14
Control 1	1042.5	647.5
Control 2	840	2150
Reference 1	720	2717.5
Reference 2	2775	1420
BIT 5 mg/L	670250	100625
BIT 2.5 mg/L	14225	3285
BIT 1.25 mg/L	3525	2482.5
BIT 0.625 mg/L	1752.5	427.5
BIT 0.313 mg/L	1595	505

Table A7.1.1.2.1/4-5: Percentage biodegradation of sodium benzoate in preliminary test 1

BIT concentration (mg/L)	Percentage Biodegradation of Sodium Benzoate (%)							
	Day 1	Day 2	Day 3	Day 6	Day 8	Day 10	Day 14	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 A7.1.1.2.1/4-6: CO_2 evolution in preliminary test 2

BIT concentration (mg/L)	CO_2 Evolution in Vessels (mg)					
	Day 1	Day 3	Day 6	Day 8	Day 9	Day 10
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 A7.1.1.2.1/4-7: Cumulative percentage biodegradation of ^{14}C-BIT in the main test

	Percentage Biodegradation1 (%)											
	Day 1	Day 3	Day 6	Day 8	Day 10	Day 13	Day 15	Day 16	Day 20	Day 22	Day 24	Day 28
Test replicate 1	0	0	0.2	0.6	6.6	16.0	18.8	19.9	21.8	22.6	23.0	23.7
Test replicate 2	0	0	0.2	0.6	7.6	16.3	19.2	20.3	22.3	22.8	23.2	23.8
Mean	0	0	0.2	0.6	7.1	16.2	19.0	20.1	22.1	22.7	23.1	23.8

[1] Equivalent to cumulative % recovered radioactivity

Table A7.1.1.2.1/4-8: Cumulative carbon dioxide evolution in control, reference and toxicity control cultures in the main test

	Evolved Carbon Dioxide ($mgCO_2$)												
	Day 1	Day 3	Day 6	Day 8	Day 10	Day 13	Day 15	Day 16	Day 20	Day 22	Day 24	Day 28	Day 29
Control replicate 1	2.8	15.0	26.3	35.1	42.6	51.5	58.2	63.9	69.1	72.8	75.4	78.7	81.6
Control replicate 2	3.6	15.2	26.8	35.4	42.7	51.3	58.1	63.7	69.5	73.4	75.5	78.7	81.2
Mean	3.2	15.1	26.6	35.3	42.7	51.4	58.1	63.8	69.3	73.1	75.5	78.7	81.4
Reference replicate 1	14.7	87.4	121.7	142.1	157.5	176.4	189.2	200.2	208.3	214.3	218.3	223.0	227.5
Reference replicate 2	15.5	88.4	121.3	140.3	155.4	173ft5.0	185.8	197.1	205.4	211.5	216.0	221.0	226.4
Toxicity control	5.7	69.9	96.3	110.4	119.6	128.3	134.1	137.9	139.0	141.0	142.3	143.5	145.0

Table A7.1.1.2.1/4-9: Biodegradation of sodium benzoate as a percentage of theoretical CO_2 yield in the main test

	Biodegradation (%)												
	Day 1	Day 3	Day 6	Day 8	Day 10	Day 13	Day 15	Day 16	Day 20	Day 22	Day 24	Day 28	Day 29
Reference replicate 1	7	44	58	65	70	76	79	83	84	86	87	87	89
Reference replicate 2	7	44	57	64	68	74	77	81	82	84	85	86	88
Mean	7	44	58	64	69	75	78	82	83	85	86	87	88
Toxicity control	3	42	58	67	72	78	81	84	84	85	86	87	88

Table A7.1.1.2.1/4-10: pH measurements on day 0 and day 28 in the main biodegradation test

	pH	
	Day 0	**Day 28**
Control replicate 1	7.42	7.26
Control replicate 2	7.43	7.24
Control replicate 3	7.41	7.22
Control replicate 4	7.41	7.26
^{14}C-BIT replicate 1	7.40	7.31
^{14}C-BIT replicate 2	7.41	7.36
Reference replicate 1	7.56	7.38
Reference replicate 2	7.51	7.40
Toxicity control: ^{14}C-BIT plus sodium benzoate	7.40	7.37

Table A7.1.1.2.1/4-11: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	**not fulfilled**
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$		Not fulfilled
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		Not fulfilled
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	Fulfilled	
Percentage of removal of reference substance reaches pass level by day 14	Fulfilled	

Figure A7.1.1.2.1/4-1: Percentage biodegradation of sodium benzoate in preliminary test 1

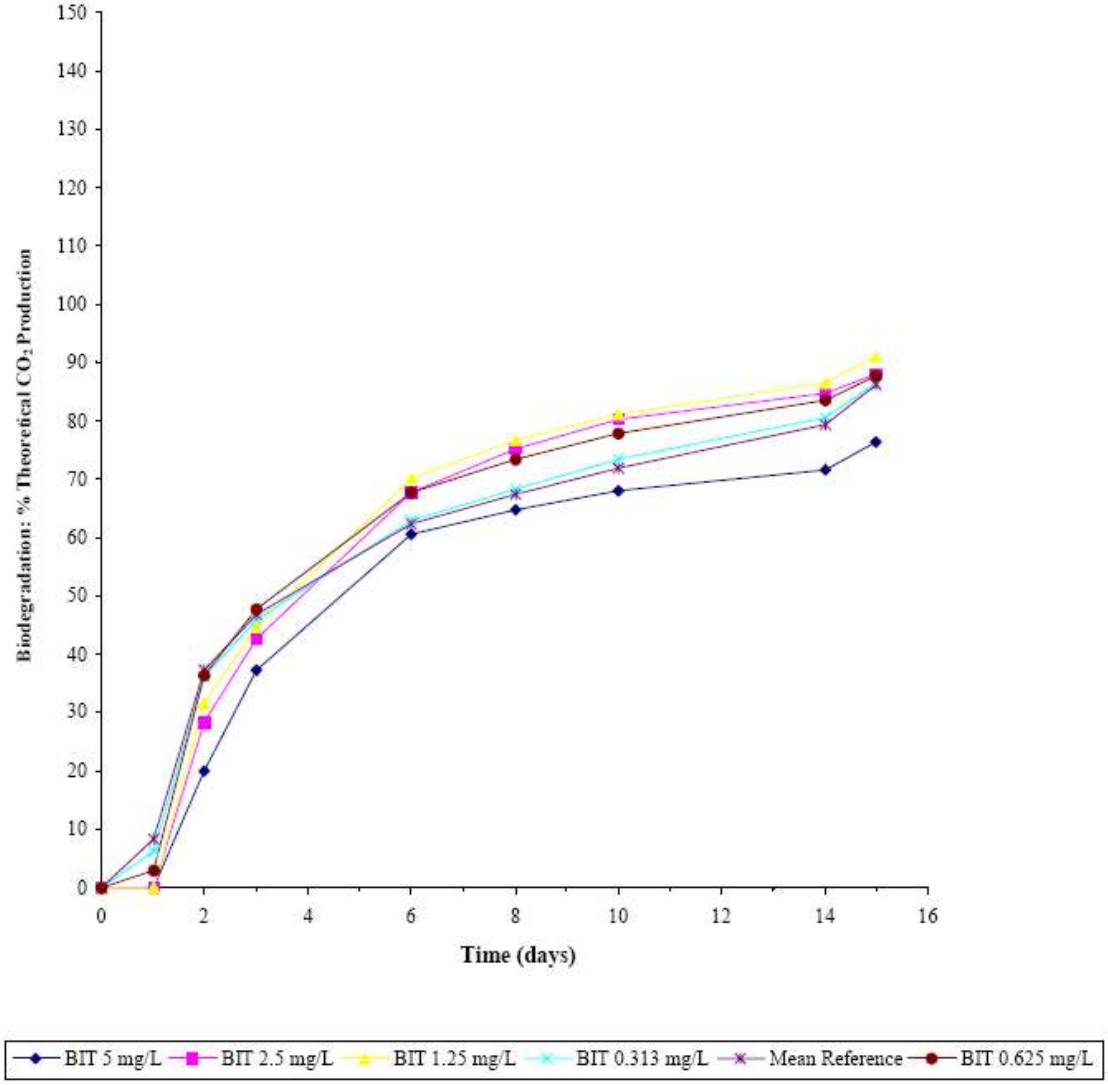


Figure A7.1.1.2.1/4-2: Carbon dioxide evolution in preliminary test 2

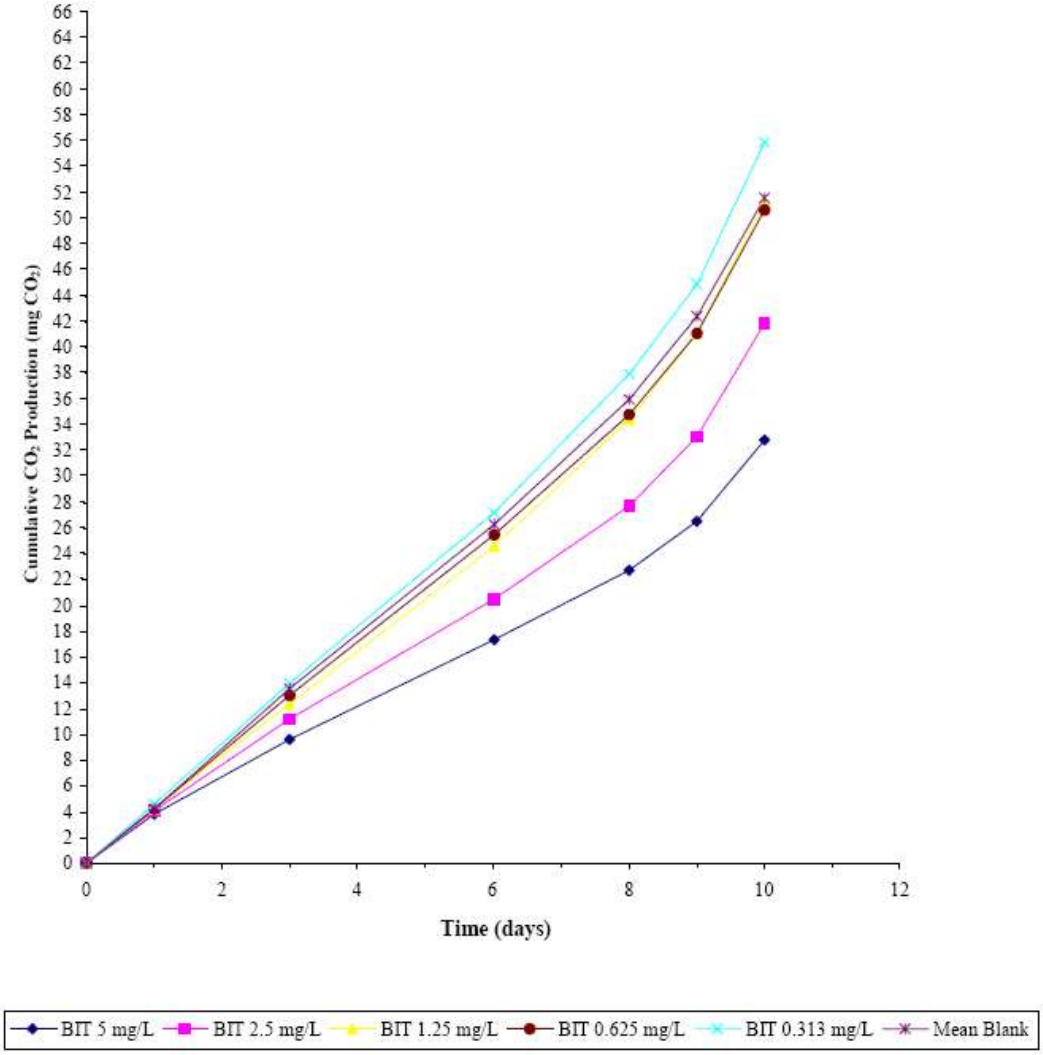


Figure A7.1.1.2.1/4-3: Percentage biodegradation of ^{14}C-BIT in the main biodegradation test

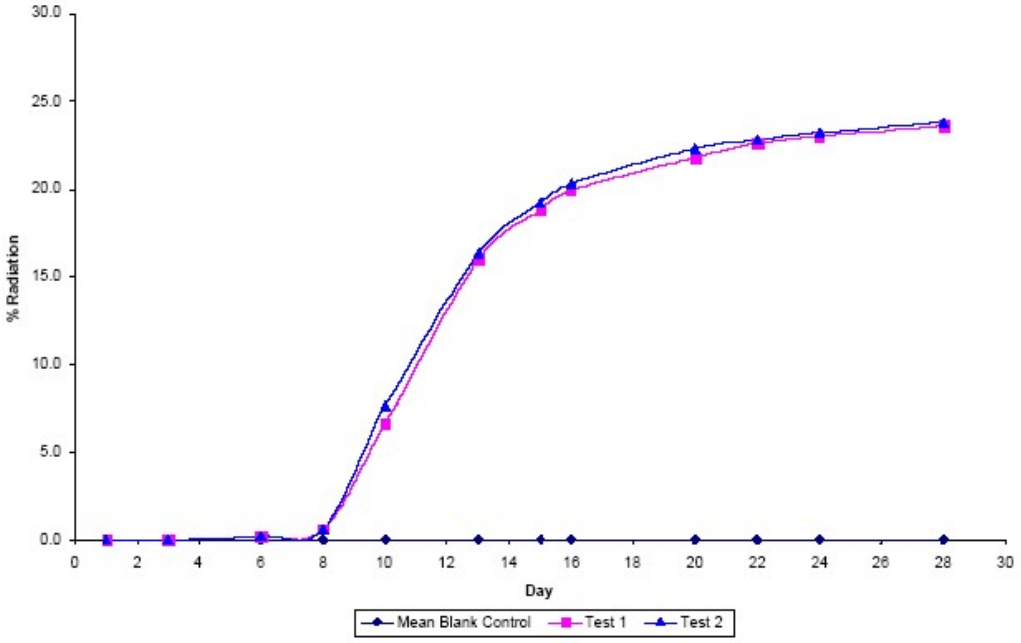


Figure A7.1.1.2.1/4-4: Percentage biodegradation of sodium benzoate in the main biodegradation test

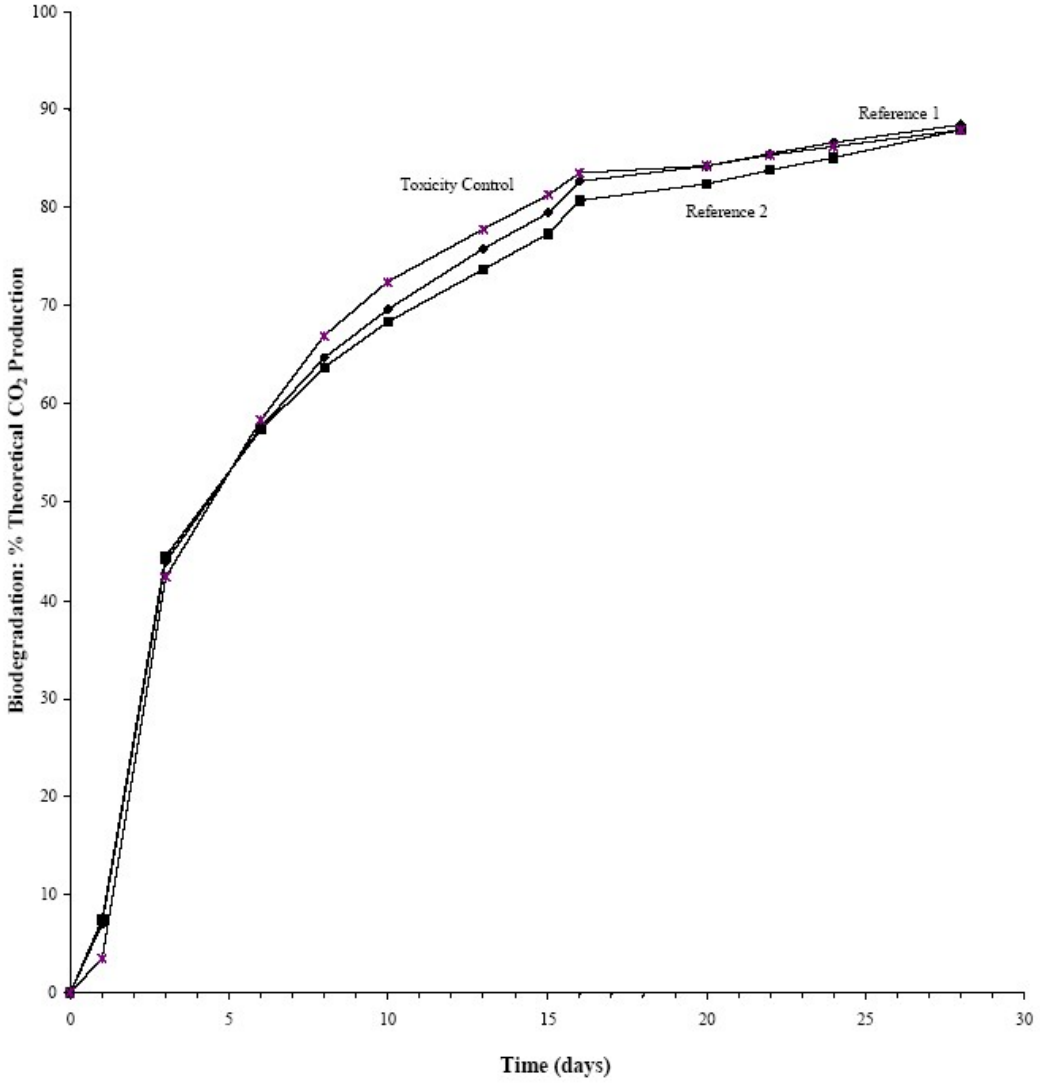
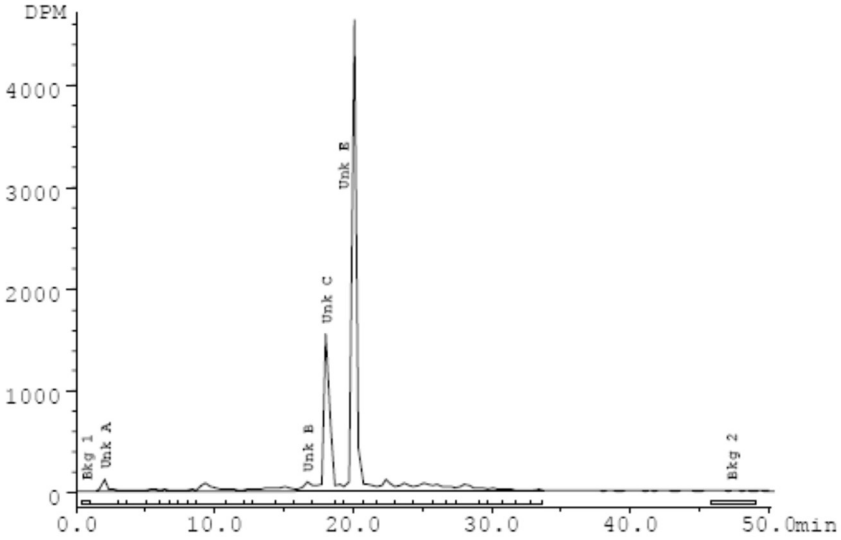


Figure A7.1.1.2.1/4-5: Example chromatogram



Method: METHOD1 Filename: 1CHR011.RFC Evaluation: A User: A.Scholey
Channel: PACKARD Detector: LSC

Name	Start	End (m)	RT (m)	Height (DPM)	Area (DPM)	%Total (%)	%ROI (%)
Bkg 1	0.3-	1.0	0.7	18.5			
Unk A	1.0-	3.0	2.0	110.6	187.4	1.78	1.79
	3.0-	3.7	3.3	15.0	23.3	0.22	0.22
	3.7-	5.0	4.3	11.8	41.8	0.40	0.40
	5.0-	6.0	5.7	25.3	48.9	0.47	0.47
	6.0-	6.7	6.3	21.6	33.5	0.32	0.32
	6.7-	7.3	7.0	12.3	23.1	0.22	0.22
	7.3-	8.0	7.7	14.0	23.7	0.23	0.23
	8.0-	8.7	8.3	16.4	27.7	0.26	0.26
	8.7-	10.7	9.3	74.1	243.8	2.32	2.33
	10.7-	12.0	11.0	21.4	73.0	0.69	0.70
	12.0-	12.7	12.3	16.1	27.1	0.26	0.26
	12.7-	14.3	14.0	29.7	116.0	1.10	1.11
	14.3-	15.7	15.0	47.7	141.9	1.35	1.36
Unk B	15.7-	17.0	16.7	89.7	171.5	1.63	1.64
Unk C	17.0-	18.7	18.0	1555.2	2339.6	22.26	22.37
	18.7-	19.3	19.0	66.8	118.3	1.13	1.13
Unk E	19.3-	20.7	20.0	4619.8	5170.9	49.19	49.43
	20.7-	21.7	21.0	64.3	179.7	1.71	1.72
	21.7-	23.0	22.3	108.9	276.7	2.63	2.65
	23.0-	24.3	23.7	74.5	227.6	2.17	2.18
	24.3-	25.7	25.0	73.4	236.7	2.25	2.26
	25.7-	26.7	26.0	60.4	162.7	1.55	1.56
	26.7-	27.3	27.0	43.0	83.6	0.80	0.80
	27.3-	29.0	28.0	60.9	229.1	2.18	2.19
	29.0-	29.7	29.3	33.0	64.8	0.62	0.62
	29.7-	30.7	30.0	25.2	70.2	0.67	0.67
	30.7-	31.7	31.0	23.8	53.7	0.51	0.51
	31.7-	32.7	32.3	12.2	32.4	0.31	0.31
	32.7-	33.7	33.3	16.2	31.5	0.30	0.30
Bkg 2	45.7-	49.0	47.3	24.6			
29 Peaks					10460.1	99.50	100.00

Bkg Area 3250.0 DPM
Total Area 10512.3 DPM
Unallocated 52.2 DPM (0.50 %)

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2/1 BIODEGRADABILITY (INHERENT) (01)

Annex Point IIA7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: June 26, 2006 – August 03, 2006	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Rohm and Haas	
1.2.2	Companies with letter of access	Troy Chemical Company B.V.	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on a modified OECD guideline 302C (modified)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, this study deviates from OECD guideline 302C in the following respects: 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 2. The sludge sampling did not take place in at least 10 places throughout the country 3. No reference is made to the mixing of old and new activated sludge samples 4. The number and type of test flasks prepared differed to that of the guideline However, these deviations are not considered to compromise the scientific validity of this study.	**X**
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	220904	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2/1 BIODEGRADABILITY (INHERENT) (01)

Annex Point IIA7.6.1.1

3.1.2	Specification	Please refer to point 3.1.3	
3.1.3	Purity	100 %	
3.1.4	Further relevant properties	Not applicable	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to micro-organisms	No	**X**
3.1.7	Specific chemical analysis	Not applicable	
3.2	**Reference substance**	Yes. Sodium Benzoate.	
3.2.1	Initial concentration of reference substance	100 g/L	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	Please refer to Table A7.1.1.2.2-1	
3.3.2	Test system	Please refer to Table A7.1.1.2.2-2	
3.3.3	Test conditions	Please refer to Table A7.1.1.2.2-3	
3.3.4	Method of preparation of test solution	The test item was weighed by means of an analytical balance and transferred to the test flasks with test water. No emulsifiers or solvents were used.	
3.3.5	Initial TS concentration	30 mg/L, 31 mg/L and 32 mg/L	
3.3.6	Duration of test	28 days	
3.3.7	Analytical parameter	Oxygen consumption	
3.3.8	Sampling	Samples were taken on days 1 – 28 inclusive	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2/1 BIODEGRADABILITY (INHERENT) (01)

Annex Point IIA7.6.1.1

3.3.9	Intermediates/ degradation products	Not applicable
3.3.10	Nitrate/nitrite measurement	Not applicable
3.3.11	Controls	Inoculum control Procedure control Abiotic control Toxicity control
3.3.12	Statistics	The percentage biodegradation was calculated according to the following formula: % biodegradation = $\frac{BOD}{ThOD}$x 100 BOD = Biological Oxygen Demand ThOD = Theroretical Oxygen Demand
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	Graph	Figures A7.1.1.2.2-1 - A7.1.1.2.2-3
4.1.2	Degradation	The percent biodegradation of BIT was calculated based on the ThOD of 1.80 mg O_2/mg BIT without nitrification ($ThODNH_4$) and 2.22 mg O_2/mg BIT with nitrification ($ThODNO_3$). During the study period of 28 days, the BOD of BIT in the test media was lower than the normal range found for the inoculum controls. Consequently, BIT was not biodegradable under the test conditions within 28 days. Please refer to Table A7.1.1.2.2-4 and A7.1.1.2.2-1.
4.1.3	Other observations	Not applicable
4.1.4	Degradation of TS in abiotic control	No degradation of the test item occurred in the abiotic control under the test conditions within 28 days.
4.1.5	Degradation of reference substance	The percent biodegradation of the reference item, sodium benzoate, was calculated based on the ThOD of 1.67 mg O_2/mg. In the procedure controls, the reference item was degraded by an average of 72% and 81% by Exposure Days 7 and 14, respectively; thus confirming suitability of the activated sludge. By the end of the test (Exposure Day 28), the reference item was degraded by an average of 86%. Please refer to Tables A7.1.1.2.2-4 and A7.1.1.2.2-5 and Figures A7.1.1.2.2-1 to A7.1.1.2.2-3.

Section A7
Subsection A7.1.1.2.2/1
Annex Point IIA7.6.1.1

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

4.1.6	Intermediates/ degradation products	Not applicable
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test item was weighed by means of an analytical balance and transferred to the test flasks with test water. No emulsifiers or solvents were used. The reference item sodium benzoate was tested simultaneously under the same conditions as the test item, and functioned as a procedure control. A stock solution containing 2.5 g sodium benzoate per litre test water was prepared by completely dissolving 250 mg sodium benzoate in 100 mL of test water. From this stock solution, 10 mL aliquots were added to the corresponding test flasks containing test water. Finally, with the exception of the abiotic control flask, activated sludge was added to each test flask. The final test volume was 250 mL per test flask.
5.2	**Results and discussion**	During the study period of 28 days, the BOD of BIT in the test media was lower than the normal range found for the inoculum controls. Consequently, BIT was not biodegradable under the test conditions within 28 days. No degradation of the test item occurred in the abiotic control under the test conditions within 28 days. Please refer to Table A7.1.1.2.2-4 and Figure A7.1.1.2.2-1. The percent biodegradation of the reference item, sodium benzoate was calculated based on the ThOD of 1.67 mg O_2/mg. In the procedure controls, the reference item was degraded by an average of 72% and 81% by Exposure Days 7 and 14, respectively; thus confirming suitability of the activated sludge. By the end of the test (Exposure Day 28), the reference item was degraded by an average of 86%. Please refer to Tables A7.1.1.2.2-4 and A7.1.1.2.2-5 and Figures A7.1.1.2.2-1 to A7.1.1.2.2-3. In the toxicity control, the BOD over the 28-day exposure period showed a similar course as the BOD of the two procedure controls, containing only the reference item. However, the BOD in the toxicity control was consistently lower from about Day 5 onwards. This could possibly indicate an inhibitory effect of BIT on the activity of the used sludge, and would also explain the lower BOD found in the two test item flasks. Please refer to Tables A7.1.1.2.2-4 and A7.1.1.2.2-5 and Figures A7.1.1.2.2-1 to A7.1.1.2.2-3. However, according to the test guidelines the test item 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 >25% on Exposure Day 14.
5.3	**Conclusion**	BIT was not biodegradable under the test conditions within 28 days. See pass levels in Table A7.1.1.2.2-6

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2/1 BIODEGRADABILITY (INHERENT) (01)

Annex Point IIA7.6.1.1

5.3.1	Reliability	2	
5.3.2	Deficiencies	Yes, this study deviates from OECD guideline 302C in the following respects: 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 2. The sludge sampling did not take place in at least 10 places throughout the country 3. No reference is made to the mixing of old and new activated sludge samples 4. The number and type of test flasks prepared differed to that of the guideline However, these deviations are not considered to compromise the scientific validity of this study.	**X**

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*March 2013.*
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*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2/1 BIODEGRADABILITY (INHERENT) (01)

Annex Point IIA7.6.1.1

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*	

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*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2/1 BIODEGRADABILITY (INHERENT) (01)

Annex Point IIA7.6.1.1

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 / Test organism

Criteria	Details
Nature	Activated sludge
Source	Wastewater treatment plant treating predominantly domestic wastewater
Sampling site	ARA Ergolz II, Füllinsdorf, Switzerland
Laboratory culture	Yes
Preparation of inoculum for exposure	The sludge was washed twice with tap water by centrifugation and the supernatant liquid phase was decanted.
Pretreatment	No
Initial cell concentration	100 mg dry material /L

Table A7.1.1.2.2-2: Test system

Criteria	Details
Culturing apparatus	500 mL Erlenmeyer flasks
Number of culture flasks/concentration	8 (2 test item flasks, 2 inoculum control flasks, 2 procedure control flasks, 1 abiotic control flask and 1 toxicity control flask)
Aeration device	Not documented
Measuring equipment	The biodegradation process consumes the dissolved oxygen in the liquid and generates CO_2. The CO_2 is adsorbed by soda lime and the total pressure decreases in the airtight test flasks. 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.
Test performed in closed vessels due to significant volatility of TS	No

Table A7.1.1.2.2-3: Test conditions

Criteria	Details
Composition of medium	Solution a: KH_2PO_4 8.5 g/L K_2HPO_4 21.75 g/L $Na_2HPO_4.2H_2O$ 33.4 g/L NH_4Cl 0.5 g/L Solution b: $MgSO_4.7H_2O$ 22.5 g/L Solution c: $CaCl_2.2H_2O$ 36.4 g/L Solution d: $FeCl_3.6H_2O$ 0.25 g/L 10 mL of solution a and 1 mL each of solutions b –d were combined and made up to 1000 mL with purified water. The pH was adjusted with a diluted hydrochloric acid solution.
Additional substrate	No
Test temperature	22 °C
pH	7.7- 7.4
Aeration of dilution water	Not documented
Suspended solids concentration	Not documented
Other relevant criteria	None

Table A7.1.1.2.2-4: Oxygen consumption in the test flasks

Time (days)	Cumulative oxygen consumption (mg/L)							
	Test item		Inoculum control		Procedure control		Abiotic control	Toxicity control
	Replicate No.		Replicate No.		Replicate No.		Replicate No.	Replicate No.
	1	2	1	2	1	2	1	1
0	0	0	0	0	0	0	0	0
1	0	0	3	2	29	27	0	0
2	--	--	--	--	--	--	--	--

Time (days)	Cumulative oxygen consumption (mg/L)							
	Test item		Inoculum control		Procedure control		Abiotic control	Toxicity control
	Replicate No.		Replicate No.		Replicate No.		Replicate No.	Replicate No.
	1	2	1	2	1	2	1	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
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

Time (days)	Cumulative oxygen consumption (mg/L)							
	Test item		Inoculum control		Procedure control		Abiotic control	Toxicity control
	Replicate No.		Replicate No.		Replicate No.		Replicate No.	Replicate No.
	1	2	1	2	1	2	1	1
27	8	10	48	42	189	187	0	132
28	8	10	48	42	190	188	0	132

-- No reading taken

Table A7.1.1.2.2-5: Biodegradation in the test flasks

Time (days)	Percentage Biodegradation							
	Test item based on				Procedure control based on ThOD		Toxicity control based on	
	$ThOD_{NH4}$		$ThOD_{NO3}$				$ThOD_{NH4}$	$ThOD_{NO3}$
	Replicate No.		Replicate No.		Replicate No.		Replicate No.	
	1	2	1	2	1	2	1	
0	0	0	0	0	0	0	0	0
1	*	*	*	*	16	15	-1	-1
2	--	--	--	--	--	--	--	--
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

Time (days)	Percentage Biodegradation							
	Test item based on				Procedure control based on ThOD		Toxicity control based on	
	$ThOD_{NH4}$		$ThOD_{NO3}$				$ThOD_{NH4}$	$ThOD_{NO3}$
	Replicate No.		Replicate No.		Replicate No.		Replicate No.	
	1	2	1	2	1	2	1	
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
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
Mean (Day 28)	*		*		86		Not applicable	

[1] Corrected for the mean oxygen uptake of the inoculum controls
-- Not determined
* Negative value due to higher oxygen consumption in the inoculum controls than in the test flasks with test item

Table A7.1.1.2.2-6: Pass levels and validity criteria for tests on inherent biodegradability

	fulfilled	not fulfilled
Pass levels		

20% removal (DOC or COD);		Not fulfilled
Pass values reached within 10-d window (within 28-d test period)		Not fulfilled
Removal of reference substance (DOC or COD) > 70 % within 14 d	Fulfilled	
Criteria for validity		
Percentage of DOC/COD-removal of reference compound ≥ 70 % within 14 days (OECD 302 B)	Fulfilled	
Percentage of DOC-removal of reference compound ≥ 40 % within 7 days and ≥ 65 % within 14 days Average residual amount of test compound in blank tests ≥ 40 % (OECD 302 C)	Fulfilled	
Removal curve of DOC or COD in the test suspension indicative for biodegradation (gradual elimination over days/weeks)	Fulfilled	

Figure A7.1.1.2.2-1: Oxygen consumption in the test flasks during the incubation period

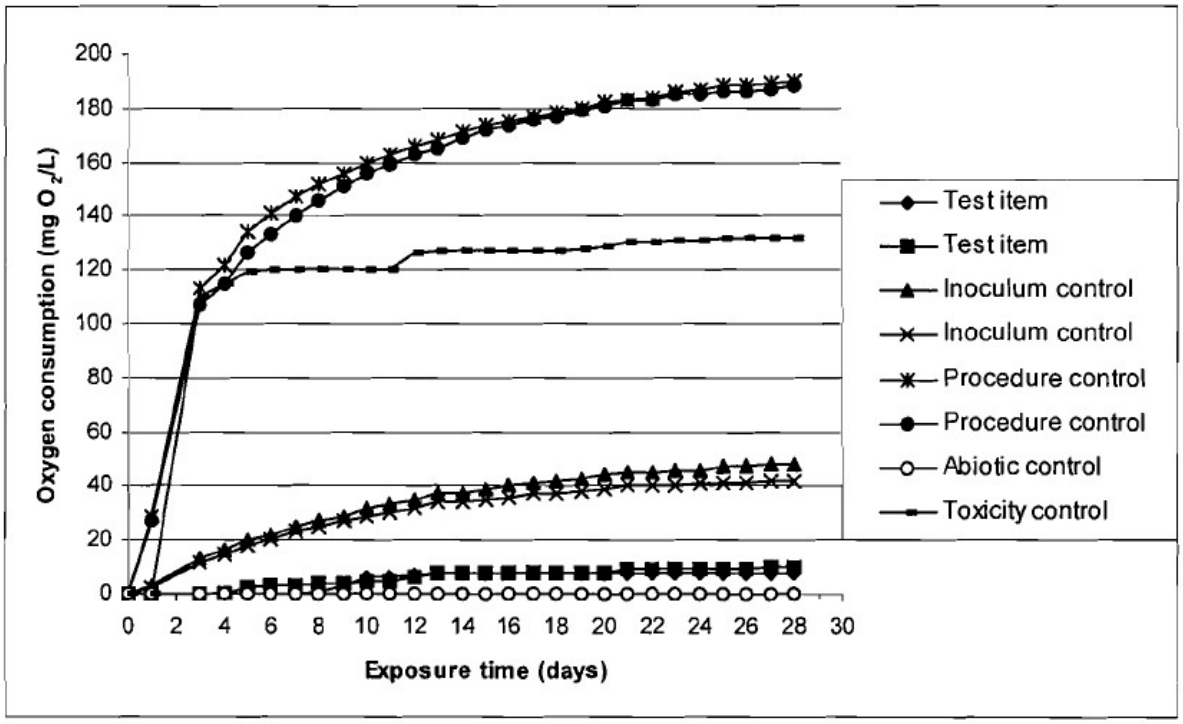


Figure A7.1.1.2.2-2: Biodegradation in the test flasks during the incubation period without nitrification

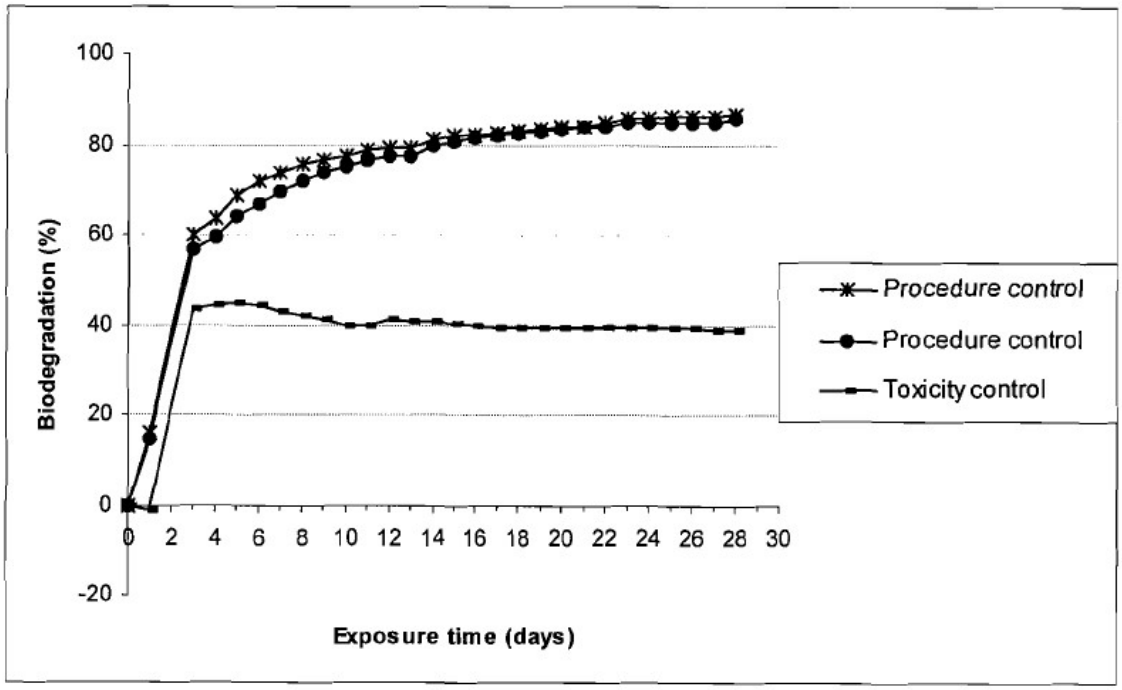
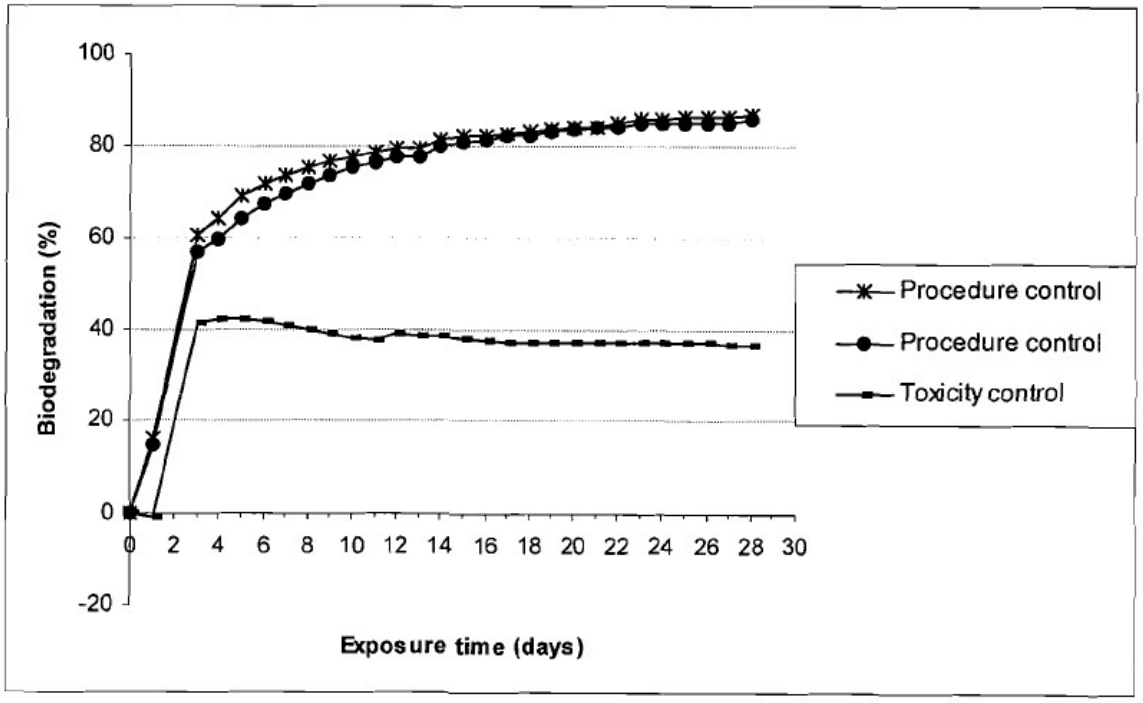


Figure A7.1.1.2.2-3: Biodegradation in the test flasks during the incubation period with nitrification



Section A7
Subsection A7.1.1.2.2/2
Annex Point IIA7.6.1.1

Ecotoxicological Profile Including Environmental Fate and Behaviour

BIODEGRADABILITY (INHERENT) (02)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] [redacted] Study Completion Date: 02 September 2008	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company B.V. and Dow Benelux B.V.	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I/IA.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on OECD guideline 302B (1992)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, this study deviates from OECD guideline 302B in the following way. MLSS concentration in this activated sludge die-away test is higher than the recommended ratio of 2.5/1 to 4/1 for inoculum to test compound (as dissolved organic carbon) in a standard OECD 302B Zahn-Wellens/EMPA Test. This deviation did not affect the scientific validity of the test.	**X**
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	Lot # 2006-00114-15	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99.5 ± 0.1%	
3.1.4	Further relevant properties	Solubility in water is 1.118 g/L at 20 °C. Vapor pressure is 0.0000302 hPa at 20°C. $\log P_{ow}$ is 1.4 at 21°C	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2/2 BIODEGRADABILITY (INHERENT) (02)

Annex Point IIA7.6.1.1

3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to micro-organisms	Yes The EC_{50} for 1,2-benzisothiazolin-3-one was determined to be 3.9 mg/L in an OECD 209 Activated Sludge, Respiration Inhibition test (Patra, 2003b).	**X**
3.1.7	Specific chemical analysis	HPLC with radiochemical detection	
3.2	**Reference substance**	Yes, Aniline.	
3.2.1	Initial concentration of reference substance	100 mg/L	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	See Table A7.1.1.2-2	
3.3.2	Test system	See Table A7.1.1.2-3	
3.3.3	Test conditions	See Table A7.1.1.2-4	
3.3.4	Method of preparation of test solution	Not documented	
3.3.5	Initial TS concentration	0.04 and 0.4 mg/L	**X**
3.3.6	Duration of test	28 days	
3.3.7	Analytical parameter	CO_2 evolution	**X**
3.3.8	Sampling	Initially 0, 1.5, 3, 6, 18 hours and 1, 2, 5, 7, 14, 21 and 28 days.	
3.3.9	Intermediates/ degradation products	Four degradation products were detected and termed products I, II, III and IV. An attempt was made to identify these [^{14}C] compounds using HPLC/MS however the chemical identities of these products could not be determined.	**X**
3.3.10	Nitrate/nitrite measurement	No	
3.3.11	Controls	Toxicity controls amended with 100 mg/L aniline and either 0.4 or 0.04 mg/L 1,2-benzisothiazolin-3-one. Biologically inhibited control (BIC) mixtures were prepared with 1000 mg/L sodium	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2/2 BIODEGRADABILITY (INHERENT) (02)

Annex Point IIA7.6.1.1

		azide were used as abiotic controls. Viability control was Inoculated mineral medium with 100 mg/L aniline.	
3.3.12	Statistics	Descriptive statistics (mean, standard deviation) were used where applicable in this study.	
		4 RESULTS	
4.1	**Degradation of test substance**		
4.1.1	Graph	See Figure A7.1.1.2-1 and Figure A7.1.1.2-2	
4.1.2	Degradation	100% degradation within3 and 24 hours for the 0.04 and 0.4mg/L samples respectively	
4.1.3	Other observations	Mineralization reached 17% after 28 days. Recovery of radioactivity in solution ranged form 72-77% in the viable mixtures and 87-92% in the BIC mixtures.	
4.1.4	Degradation of TS in abiotic control	50% after 24 hours	
4.1.5	Degradation of reference substance	Degradation of aniline in the positive control mixture reached 99% after seven days based on removal of DOC	
4.1.6	Intermediates/ degradation products	Four degradation products were detected and termed products I, II, III and IV. Product I reached a maximum of ~18% of initial radioactivity at 0.4 mg/L [^{14}C]-1,2-benzisothiazolin-3-one after one day, then decreased slightly to ~15% after 28 days. In the corresponding BIC mixture, Product I was not detected through 0.75 days then appeared at 19% of initial radioactivity after one day. Thereafter, the concentration of Product I increased to ~36% after 28 days. Product I was not detected in the reaction mixtures amended with 0.04 mg/L test compound. Definitive LC/MS data was generated for product I. Product II in the reaction mixtures amended with 0.4 mg/L test compound slowly increased during the study, reaching ~ 42% of initial radioactivity after 28 days. Product II was not detected in most samples from the corresponding BIC mixture, indicating that its formation was likely biologically mediated. In reaction mixtures amended with 0.04 mg/L test compound, Product II reached ~59% of initial radioactivity after one day. Definitive LC/MS data was generated for product II. Product III in the viable reaction mixtures amended with 0.4 mg/L test compound reached a maximum of ~13% after seven days, then decreased to ~5% after 28 days. In the corresponding BIC mixture, Product III reached a maximum of ~69% after 0.125 days (*i.e.* 3 hours), then remained between ~7 to 13% for the remainder of the study. In the viable mixtures amended with 0.04 mg/L test compound, Product III was not detected in most samples. No attempt was made to identify Product III because of limited	

Section A7
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Annex Point IIA7.6.1.1

Ecotoxicological Profile Including Environmental Fate and Behaviour
BIODEGRADABILITY (INHERENT) (02)

amounts of the compound present in samples available for LC/MS analysis due to its overall low yield in the experiment.

Product IV in the viable reaction mixtures amended with 0.4 mg/L test compound reached a maximum of ~33% after 0.75 days, then decreased to non-detected levels within five days. In the corresponding BIC mixture, Product IV was detected at 11% or less of initial radioactivity in only selected samples, indicating the formation of this product was biologically mediated. In viable reaction mixtures amended with 0.04 mg/L test compound, Product IV reached a maximum of ~55% after 0.25 days, then decreased to non-detectable levels within 0.75 days. In the corresponding BIC mixture, Product IV was not detected in most samples. Due low levels of this transient Product IV, no definitive MS data could be obtained over background response.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The biodegradability of 1,2-benzisothiazolin-3-one was assessed in an activated sludge die-away study based on a modification of the OECD 302B Zahn-Wellens/EMPA Test for inherent biodegradability in which a carbon-14 labeled test compound was used. The activated sludge die-away study was set with two nominal concentrations of [^{14}C]-1,2-benzisothiazolin-3-one (~0.04 and ~0.4 mg/L) added to municipal activated sludge (500 mg/L mixed liquor suspended solids).

Reaction mixtures (250 mL) were contained in sealed one-liter flasks equipped with caustic traps (1N NaOH) suspended from the caps. Biologically inhibited control (BIC) mixtures were prepared with sodium azide to resolve biological versus abiotic degradation of [^{14}C]-1,2-benzisothiazolin-3-one. Reaction mixtures were incubated on a rotary shaker in the dark at 19.7 ± 0.2°C for 28 days. Reaction mixtures were regularly sampled and analyzed by HPLC with radiochemical detection to determine the concentrations of [^{14}C]-1,2-benzisothiazolin-3-one and [^{14}C] degradation products. In addition, the caustic traps were regularly sampled to measure the amount of $^{14}CO_2$ produced in the reaction mixtures.

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Annex Point IIA7.6.1.1

Ecotoxicological Profile Including Environmental Fate and Behaviour

BIODEGRADABILITY (INHERENT) (02)

5.2	**Results and discussion**	[^{14}C]-1,2-benzisothiazolin-3-one reached non-detected levels within 3 and 24 hours in viable reaction mixtures amended with initial concentrations of 0.04 and 0.4 mg/L of the test compound, respectively. Assuming that pseudo first order kinetics were followed, primary degradation half-lives were determined to be 1.6 and 4.6 hours, respectively. In contrast, levels of [^{14}C]-1,2-benzisothiazolin-3-one remaining in the BIC mixtures were ~32% after 3 hours (0.04 mg/L) and ~50% after 24 hours (0.4 mg/L), corresponding to half-lives of 1.9 and 27 hours, respectively. Since primary degradation of [^{14}C]-1,2-benzisothiazolin-3-one was slower in the BIC mixtures, the results indicate that biological processes were responsible for a substantial portion of the degradation observed, particularly at the higher concentration tested (0.4 mg/L). Mineralization of [^{14}C]-1,2-benzisothiazolin-3-one to $^{14}CO_2$, calculated as a percentage of the radioactivity added, reached 10 and 17% of the initially added radioactivity after 28 days in reaction mixtures with initial concentrations of 0.04 and 0.4 mg/L, respectively. Mineralization in the BIC mixtures was < 0.5%. These results indicate that most of the mineralization of the test compound in the viable mixtures was biologically mediated. The recovery of radioactivity from the test system (*i.e.* mass balance) was greater than 95% in all of the reaction mixtures at the conclusion of the study. Recovery of radioactivity in solution ranged from ~72 to ~77% in the viable mixtures and from ~87 to ~92% in the BIC mixtures. Recovery of radioactivity as $^{14}CO_2$ ranged from ~10 to ~17% in the viable mixtures, and was 0.2% in the BIC mixtures. Radioactivity removed from the reaction mixtures due to sampling ranged from ~7 to ~8%.	
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. 1,2-benzisothiazolin-3-one meets the criteria for classification as inherently biodegradable.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	Yes, please refer to point 2.3.	

Evaluation by Competent Authorities	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*December 2009.*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.1.2.2/2 BIODEGRADABILITY (INHERENT) (02)

Annex Point IIA7.6.1.1

Materials and Methods	*Applicant's version is accepted with the following remarks:* *2.3. MLSS concentration in this activated sludge die-away test is higher than the recommended ratio of 2.5/1 to 4/1 for inoculum to test compound (as dissolved organic carbon) in a standard OECD 302B Zahn-Wellens/EMPA Test.* *3.1.6. Applicant refers to the results of a previous test (Patra, 2003b), which showed an inhibitory effect of test substance on activated sludge. The EC_{50} for 1,2-benzisothiazolin-3-one was determined to be 3.9 mg/L in an OECD 209 Activated Sludge, Respiration Inhibition test (Patra, 2003b).* *3.3. Volume in test vessels is 250 mL, instead of the recommended volume of 500 mL in a standard OECD 302B Zahn-Wellens/EMPA Test.* *3.3.5. Test concentration can be assumed to be lower than biocidal concentration. According to the study report, this low concentration is employed in order to avoid inhibitory effect of BIT. However, according to OECD guidelines, this low concentration can lead to a lower analytical reliability.*
Results and discussion	*Applicant's version is accepted with the following comments:* *4.1.4. Degradation of test substance in abiotic control reaches 50% within 24h. This indicates that a significant part of the degradation observed is due to abiotic processes.* *4.1.6. Degradation products are not identified*
Conclusion	*According to this study, BIT fulfils the requirements to be classified as inherently biodegradable. However, the results of abiotic control show a significant amount of abiotic degradation. In addition, concentration tested is lower than concentration expected in the environment. Thus, RMS considers that BIT cannot be considered inherently biodegradable at biocidal concentrations.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*Results are not applicable for higher environmental concentrations of BIT.*

Table A7.1.1.2-1: Guideline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
DOC Die-Away-Test	C.4-A	301A	ready
CO_2 Evolution-Test (Modified Sturm Test)	C.4-C	301B	ready
Modified OECD-Screening-Test	C.4-B	301E	ready
Manometric Respirometry	C.4-D	301F	ready
MITI-I-Test	C.4-F	301C	ready
Closed-Bottle-Test	C.4-E	301D	ready
Zahn-Wellens-test	C.9	302B	Inherent
Modified MITI-Test (II)	-	302C	Inherent
Modified SCAS-Test	C.12	302A	Inherent
Simulation Test with activated Sewage (Coupled Units-Test)	C.10	302A	Simulation Test[1)]

[1)] Test for the determination of the ultimate degradation of test material under conditions which simulate the treatment in an activated sludge plant

Table A7.1.1.2-2: Inoculum

Criteria	Details
Nature	Activated sludge
Source	Midland Municipal Waste Water Treatment Plant (Midland, Michigan)
Laboratory culture	No
Preparation of inoculum for exposure	Activated sludge was washed twice with mineral medium and aerated until used. Prior to use, the mixed liquor suspended solids (MLSS) was determined using a standard procedure
Initial cell concentration	MLSS concentration of 500 ± 50 mg/L

Table A7.1.1.2-3: Test system

Criteria	Details
Culturing apparatus	1 litre culture flasks on a rotary shaker
Number of culture flasks/concentration	2
Aeration device	none
Measuring equipment	A fiber optic probe (FOXY probe) was inserted through the sampling port and the oxygen concentration in the headspace gas of the flask was measured to confirm that aerobic conditions were maintined..
Test performed in closed vessels due to significant volatility of TS	No

Table A7.1.1.2-4: Test conditions

Criteria	Details
Composition of medium	Stock Solution I: KH_2PO_4 8.5g, K_2HPO_4 21.75 g, Na_2HPO_4•$7H_2O$ 50.4g, NH_4Cl 0.5g. Dissolved in water and made up to 1 liter.The pH of the solution should be a pH of 7.4. Stock Solution II: $CaCl_2$•$2H_2O$ 36.40g. Dissolved in water and made up to 1 liter. Stock Solution III: $MgSO_4$•$7H_2O$ 22.5g. Dissolved in water and made up to 1 liter. Stock Solution IV: $FeCl_3$ 0.15g. Dissolved in water and diluted to 1 liter. One drop of concentrated HCl was added, per liter, to prevent precipitation of the solution. 10 mL of Stock Solution I was mixed with 800 mL Milli-Q water, then 1-mL portions of Stock Solutions II, III, and IV were added, this mixture was diluted to 1 liter with water.
Additional substrate	No
Test temperature	19.7 ± 0.2°C
pH	Initial pH 7.4 ± 0.2. Initial pH 6.8
Aeration of dilution water	No
Suspended solids concentration	500 mg/L mixed liquor suspended solids
Other relevant criteria	Flasks were incubated on a rotary shaker

Table A7.1.1.2-5: Pass levels and validity criteria for inherent biodegradability tests

	fulfilled	not fulfilled
Pass levels		
20% removal (DOC or COD);	Not Applicable[1]	
Pass values reached within 10-d window (within 28-d test period)	Not Applicable[1]	
Removal of reference substance (DOC or COD) > 70 % within 14 d	Yes	
Criteria for validity		
Percentage of DOC/COD-removal of reference compound ≥ 70 % within 14 days (OECD 302 B)	Yes	
Percentage of DOC-removal of reference compound ≥ 40 % within 7 days and ≥ 65 % within 14 days Average residual amount of test compound in blank tests ≥ 40 % (OECD 302 C)	Yes	
Removal curve of DOC or COD in the test suspension indicative for biodegradation (gradual elimination over days/weeks)	Not Applicable[1]	

For this study, the use of the DOC criteria for assessing the biodegradability of BIT does not apply. The use of [^{14}C]-BIT at sub-mg/L concentrations showed the complete primary degradation of the parent compound in the test, coupled with 17% mineralization to $^{14}CO_2$ during the test. These results with the radiotracer indicate that BIT is susceptible to complete degradation (i.e. mineralization) and is thus inherently biodegradable.

Figure A7.1.1.2-1: Plot of Natural Logarithm of [^{14}C]-1,2-benzisothiazolin-3-one Concentration (0.04 mg/L) Versus Time for Degradation in Activated Sludge

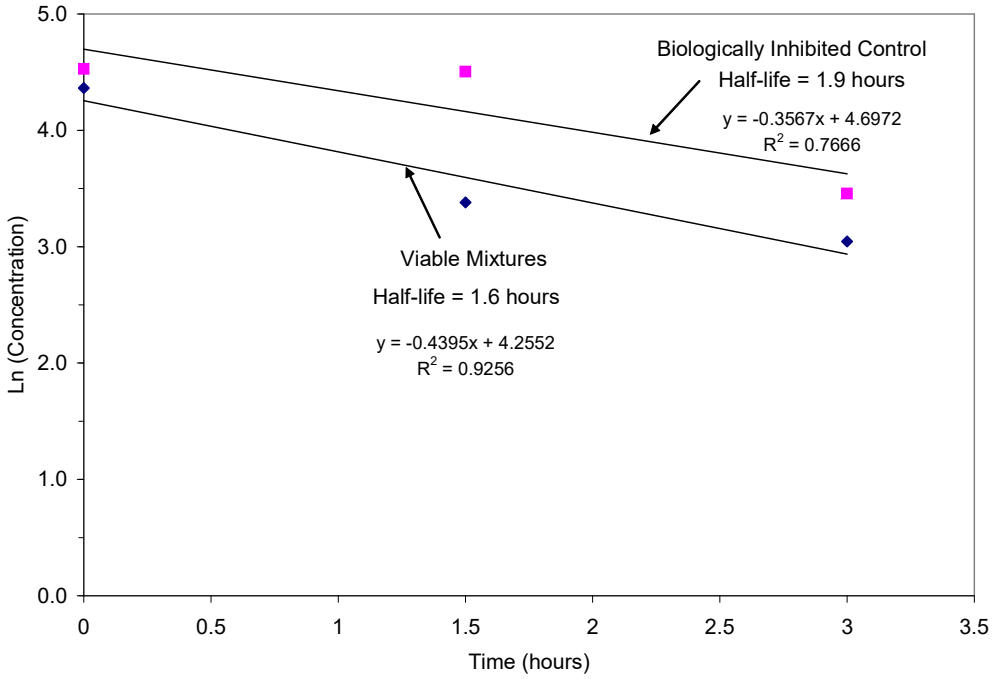
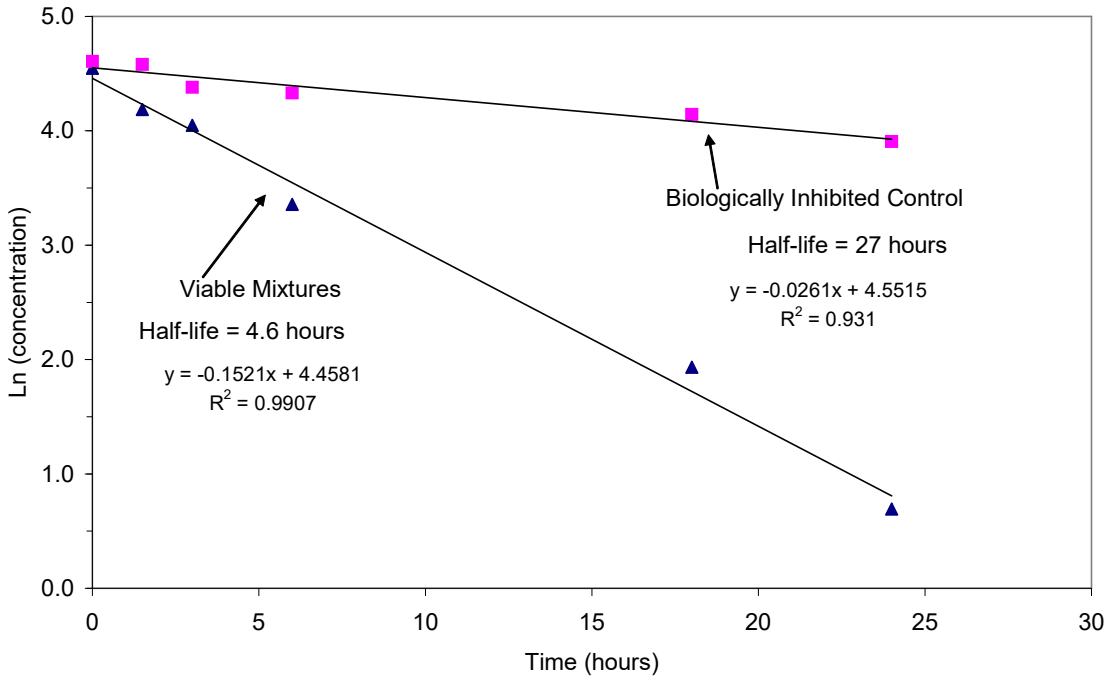


Figure A7.1.1.2-2: Plot of Natural Logarithm of [^{14}C]-1,2-benzisothiazolin-3-one Concentration (0.4 mg/L) Versus Time for Degradation in Activated Sludge



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	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification […].**	
Detailed justification:	According to the TNsG on Data requirements, a biodegradation test in seawater is required if a substance is to be used or released to the marine environments in considerable amounts. Since BIT within PT 2 is intended to be used only indoor and is therefore not directly released to seawater, a study on biodegradation in seawater is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*August 2010.*	
Evaluation of applicant's justification	*Accept applicant's justification.*	
Conclusion	*Accept applicant's justification.*	
Remarks		

Section A7 **Subsection A7.1.2.1.1** **Annex Point IIIA, 6.2.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **BIOLOGICAL SEWAGE TREATMENT—AEROBIC**	
	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 study addressing the aerobic biodegradation of BIT in an activated sludge die-away test (OECD 302b) has already been carried out. Please see study IIIA 7.1.1.2.2-2.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2010*	
Evaluation of applicant's justification	*Applicant's justification is adopted with the following remark:* *The OECD 303 guidance, recommended for this type of study at the 'Data requirements guidance for biocidal product types (October, 2000), is mentioning that those chemicals that fail the inherent biodegradability tests are in principle not to be degraded in the simulation test.* *RMS is therefore considering that a simulation test for Biological Sewage Treatment is not needed.*	
Conclusion	*Acceptable*	
Remarks		

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:	According to the 'Data requirements for biocidal product types, Version 4.3.2' (October, 2000), an anaerobic degradation study is required if exposure to anaerobic conditions is likely. [redacted], according to its recommended use as a preservative, is not to be used in veterinary hygiene products or in animal housing situations. Thus, release into manure storage facilities, where anaerobic conditions might occur, is not possible. Therefore, a study is not presented to address this point.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*August 2010.*	
Evaluation of applicant's justification	*Accept applicant's justification*	
Conclusion	*Accept applicant's justification*	
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	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [...]**	
Detailed justification:	A study to address this data point is not required as the behaviour of BIT in an aerobic aquatic environment has been adequately addressed under the point IIIA, 7.1.1.1.1 "Abiotic degradation of 1,2-benzisothiazolin-3-one according to OECD guideline 111-a preliminary test" [redacted] (2002). Furthermore, a risk assessment carried out on the fate of BIT in surface water following release *via* STP effluent, shows that the risk quotient for BIT in surface water is < 1 indicating no risk to the aquatic compartment. BIT is mainly used indoors and is not applied in considerable amounts. Please be aware that studies investigating the photochemical degradation of BIT are currently underway, the results of which should indicate that the primary route of degradation for BIT in water is photolytically. It is therefore proposed that no testing on the biodegradation of BIT in freshwater is required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2010*	
Evaluation of applicant's justification	*RMS accepts the applicant's justification with the following remark:* *Justification sentence "Furthermore, a risk assessment carried out on the fate of BIT in surface water following release via STP effluent, shows that the risk quotient for BIT in surface water is < 1 indicating no risk to the aquatic compartment. BIT is mainly used indoors and is not applied in considerable amounts" is not applicable for all type of uses in PT 6 (see Doc. II-B).*	
Conclusion	*Acceptable*	
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	**Official use only**
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:	According to the 'Data requirements for biocidal product types, Version 4.3.2' (October, 2000), water-sediment simulation tests are required if the biocide is directly emitted to water or if the solids water equilibrium partition coefficient (K_p) of the substance being investigated is > 2000. BIT, according to its recommended use, does not involve direct application to water. BIT is mainly used indoors and is not applied in considerable amounts. The K_p of BIT, derived from the equation $K_p = F_{oc} \times K_{oc}$, was calculated to be 1.3 and is thus below the threshold value set in the guidance (see above). It is therefore proposed that a study is not required to address this point.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2010*	
Evaluation of applicant's justification	*RMS accepts applicant's justification with the following remark:* *Reference to the outcome of the study 7.1.3 (2) is missing. In this study, a log Koc value of 2.1 was obtained. This will correspond to a Kp value of 12.6. Yet, this value is lower than 2000, and thus applicant's justification is accepted.*	
Conclusion	*Acceptable*	
Remarks		

Section A7
Subsection A7.1.3/1
Annex Point IIA.7.7

Ecotoxicological Profile Including Environmental Fate and Behaviour

ADSORPTION / DESORPTION SCREENING TEST (01)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: February 06, 2002 – February 08, 2002.	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access	not applicable	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on OECD guideline 121.	
2.2	**GLP**	**Yes**	
2.3	**Deviations**	Yes this study deviates from OECD guideline 121 in the following respects: 1. No reference to duplicate determinations was made. However, this deviation is not considered to compromise the scientific validity of this study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-one	
3.1.1	Lot/Batch number	BT 12000	
3.1.2	Specification	Please refer to Doc. III-A, 2/2	
3.1.3	Purity	98 %	
3.1.4	Specific Activity	Soluble in hot water	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.3/1 ADSORPTION / DESORPTION SCREENING TEST (01)

Annex Point IIA.7.7

3.1.5	Further relevant properties	The retention time of the diluted samples was determined by the following HPLC system: Pump: Hewlet Packard Series 1050 Detector: K-2501 Wavelength detector: 254 nm Auto sampler: Spark Marathon Basic Injection volume: 20µL Column: Hypersil ODS 250 x 4.61 x i.d. (Chrompack) Solvent system: 80 % methanol/20 % water Flow eluens: 1.0 mL.min^{-1}
3.2	**Degradation products**	Degradation products tested: No
3.2.1	Method of analysis for degradation products	Not applicable
3.3	**Reference substance**	Yes aniline, benzamide, benzoic acid phenylester, monuron, 4-nitrobenzamide, phenanthrene and 2,5-dichloroaniline
3.3.1	Method of analysis for reference substance	HPLC as decsribed in point 3.1.5
3.4	**Soil types**	Not applicable
3.5	**Test Solutions**	
3.5.1	BIT Test Solutions	The adsorption was determined on a reversed phase HPLC system. The retention time of at least 6 reference substances with known logK_{oc} and the test substance was determined on a reversed phase HPLC system and the capacity factor (k') was calculated. The logK_{oc} of the reference substance versus log k' was plotted and yields a straight line. The logK of the test substance was calculated from the slope and intercept of the curve.
3.5.2	0.01M $CaCl_2$	Approximately 10-40 mg of test and reference substances were weighed and dissolved into 10 mL of methanol. From these solutions, 100 µL was diluted to 2.5 mL methanol and these solutions were injected into the HPLC system.
3.6	**Preliminary Investigations**	
3.2.1	Solubility	Not applicable

Section A7 Subsection A7.1.3/1 Annex Point IIA.7.7	Ecotoxicological Profile Including Environmental Fate and Behaviour ADSORPTION / DESORPTION SCREENING TEST (01)	
3.2.2 Adsorption to containers	Not applicable	
3.2.3 Ratio of soil to solution	Not applicable	
3.2.4 Equilibration time determination	According to (a)" OECD-HPLC-method"[1]: Yes	
3.2.5 Stability test	Not applicable	
	4 RESULTS	
4.1 Preliminary Investigations	Not applicable	
4.2 Screening test: Adsorption	Not applicable	
4.3 Screening test: Desorption	Not applicable	
4.4 Calculations		
4.4.1 K_a , K_d	Not applicable	
4.4.2 Ka_{oc} , Kd_{oc}	A $logK_{oc}$ value of 2.11 was determined for 1,2-benzisothiazolin-3-one	
4.5 Degradation product(s)	Not documented	

1 OECD (1999) OECD-Guidelines for the Testing of Chemicals. Proposal for a new guideline 121: Estimation of the adsorption coefficient (K_{oc}) on soil and on sewage sludge using High Performance Liquid Chromatography (HPLC), Draft Document (August 1999).

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.3/1 ADSORPTION / DESORPTION SCREENING TEST (01)

Annex Point IIA.7.7

	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The adsorption coefficient (K_{oc}) of 1,2-benzisothiazolin-3-one on sewage sludge was estimated using HPLC. Approximately 10-40 mg of test and reference substances were weighed and dissolved into 10 mL of methanol. From these solutions, 100 µL was diluted to 2.5 mL methanol and these solutions were injected into the HPLC system. The retention time of the reference substances with known logK_{oc} and test substance was determined and the capacity factor (k') calculated. This study was conducted according to OECD Guideline 121 and is described under point 3 with the following deviation: 1. No reference to duplicate determinations was made. However, this deviation is not considered to compromise the scientific validity of this study.	
5.2 Results and discussion	logK_{oc} was plotted against logk' for the seven reference substances. The slope and the intercept were determined by linear regression. The logK_{oc} of 1,2-benzisothiazolin-3-one was calculated to be 2.11. Please refer to Table A7.1.3/1-1and Figure A7.1.3/1-1.	
5.2.1 Adsorbed a.s. [%]	Not applicable	
5.2.2 K_a	Not applicable	
5.2.3 K_d	Not applicable	
5.2.4 Ka_{oc}	A log K_{oc} value of 2.11 was determined for 1,2-benzisothiazolin-3-one.	
5.2.5 Ka/Kd	Not applicable	
5.2.6 Degradation products	Not documented	
5.3 Conclusion	A log K_{oc} value of 2.11 was determined for 1,2-benzisothiazolin-3-one.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	Yes, One deviation was noted and is outlined under points 2.3 and 5.1. However, it does not compromise the scientific validity of this study.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE (*)

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.3/1 ADSORPTION / DESORPTION SCREENING TEST (01)

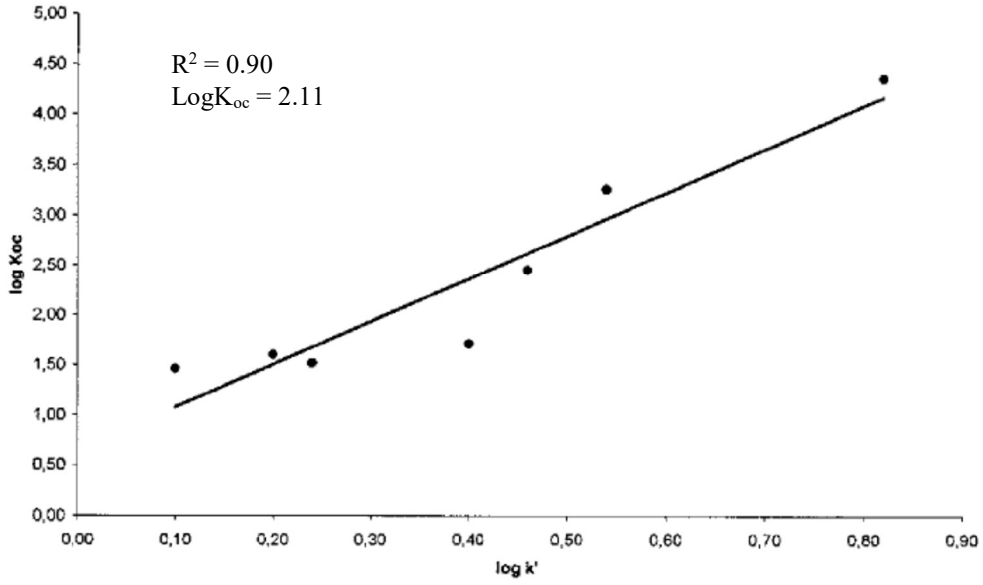
Annex Point IIA.7.7

Date	*November 2012*
Materials and Methods	*Applicant's version is accepted with the following remaks:* ▪ *The used medium, 80:20 Methanol: Water (v:v), is supposed to result in neutral pH, for which an important fraction of the BIT molecules will be present in the ionised form (disassociated), considering the pKa value of 7.2. This study is thus complementing the adsorption study in section 7.1.3 (1), in which the K_{oc} was estimated under acidic conditions, and thus, with most of the BIT molecules in the neutral form.* ▪ *Determinations were not measured in duplicate, and thus, reproducibility and repeatability are not tested.*
Results and discussion	*A logK_{oc} value of 2.1 was determined for 1,2-benzisothiazolin-3-(2H)-one at neutral conditions.*
Conclusion	*Aplicant's version is adopted.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*Key Study.*

Table A7.1.3/1-1: reference substance t_r, t_0, k' and logk' values

Reference Substance	t_r (min)	t_0 (min)	k'	logk'
Monuron	7.28	2.8	1.60	0.20
4-nitrobenzamide	8.70	2.8	2.11	0.32
Benzamide	8.30	2.8	1.96	0.29
Aniline	11.28	2.8	3.03	0.48
2,5-dichloroaniline	12.37	2.8	3.42	0.53
Benzoic acid phenylester	14.28	2.8	4.10	0.61
Phenanthrene	24.59	2.8	7.78	0.89

Figure A7.1.3/1-1: logK_{oc} v logk'


R^2 = 0.90
$LogK_{oc}$ = 2.11
log Koc
log k'
5,00
4,50
4,00
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
0,00
0,10
0,20
0,30
0,40
0,50
0,60
0,70
0,80
0,90

Section A7
Subsection A7.1.3/2
Annex Point IIA.7.7

Ecotoxicological Profile Including Environmental Fate and Behaviour

ADSORPTION / DESORPTION SCREENING TEST (02)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: April 08, 2005 – May 04, 2005.	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access	Dow Benelux BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on OECD guideline 121.	
2.2	**GLP**	**Yes**	
2.3	**Deviations**	Yes this study deviates from OECD guideline 121 in the following respects: 1. No reference to duplicate determinations was made. However, this deviation is not considered to compromise the scientific validity of this study.	**X**
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-(2*H*)-one	
3.1.1	Lot/Batch number	040897-1	
3.1.2	Specification	Please refer to Doc. III-A, 2/2	
3.1.3	Purity	69.6 % (on wet cake), 99.4 % (on dry residue)	
3.1.4	Specific Activity	Not documented	

Section A7 Subsection A7.1.3/2 Annex Point IIA.7.7		Ecotoxicological Profile Including Environmental Fate and Behaviour ADSORPTION / DESORPTION SCREENING TEST (02)	
3.1.5	Further relevant properties	The retention time of the reference substances and the test substance were determined by the following HPLC-With Dode Array Detection (DAD) system. HPLC chromatograph: Waters 2690/5 separation module Column: Platinum CN 100A 5µ, 250 mm x 4.6 mm (Alltech) Column temperature: 30 ± 5°C Injection volume: 10 µL Flow: 0.6 mL/min Run time: 10 min Mobile phase: methanol/0.01 mol/L potassium dihydrogen citrate, 55/45 v/v% adjusted to pH 5.5 using sodium hydroxide. Detection: Diode array from 200-400 nm	
3.2	**Degradation products**	Degradation products tested: No	
3.2.1	Method of analysis for degradation products	Not applicable	
3.3	**Reference substance**	Yes urea, acetanilide, aniline, monuron, pyrazophos, linuron and carbendazim	**X**
3.3.1	Method of analysis for reference substance	HPLC-DAD system as decsribed in point 3.1.5	
3.4	**Soil types**	Not applicable	
3.5	**Test Solutions**		
3.5.1	BIT Test Solutions	The adsorption was determined by HPLC-DAD using a commercial HPLC column of a cyanopropyl resin chemically bound on a silica base with methanol/0.01 mol/L potassium dihydrogen citrate, 55/45 v/v% (pH 5.5) as mobile phase.	
3.5.2	0.01M $CaCl_2$	The test solution was prepared by dissolving 25.26 mg of 1,2-benzisothiazolin-3-(2*H*)-one in 20 mL methanol. This stock solution was diluted by transferring 50 µL in 5 ml methanol/0.01 mol/L potassium dihydrogen citrate 55/45 v/v% (pH 5.5). This test solution was injected into the HPLC system. Reference solutions were prepared by dissolving 5 mg of the reference substance in 5 mL of methanol. For acetonitrile and aniline approximately 10 mg was dissolved in 10 mL methanol. Each stock solution was diluted by transferring 50 µL in 5 mL methanol/0.01 mol/L potassium dihydrogen citrate, 55/45 v/v% (pH 5.5). For the	

Section A7 Subsection A7.1.3/2 Annex Point IIA.7.7	Ecotoxicological Profile Including Environmental Fate and Behaviour ADSORPTION / DESORPTION SCREENING TEST (02)	
	determination of t0 100 mg urea was dissolved in 10 mL methanol/0.01 mol/L potassium dihydrogen citrate, 55/45 v/v% (pH 5.5). These reference solutions were then injected into the HPLC system.	
3.6 Preliminary Investigations		
3.2.1 Solubility	Not applicable	
3.2.2 Adsorption to containers	Not applicable	
3.2.3 Ratio of soil to solution	Not applicable	
3.2.4 Equilibration time determination	According to (a)" OECD-HPLC-method"[2]: Yes	
3.2.5 Stability test	Not applicable	
	4 RESULTS	
4.1 Preliminary Investigations	Not applicable	
4.2 Screening test: Adsorption	Not applicable	
4.3 Screening test: Desorption	Not applicable	
4.4 Calculations		
4.4.1 K_a , K_d	Not applicable	
4.4.2 Ka_{oc} , Kd_{oc}	A log K_{oc} value of 0.73 was determined for 1,2-benzisothiazolin-3-(2*H*)-one	**X**
4.5 Degradation product(s)	Not documented	

2 OECD (1999) OECD-Guidelines for the Testing of Chemicals. Proposal for a new guideline 121: Estimation of the adsorption coefficient (K_{oc}) on soil and on sewage sludge using High Performance Liquid Chromatography (HPLC), Draft Document (August 1999).

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.3/2 ADSORPTION / DESORPTION SCREENING TEST (02)

Annex Point IIA.7.7

	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	The adsorption coefficient (K_{oc}) of 1,2-benzisothiazolin-3-(2*H*)-one on soil and sewage sludge was estimated using HPLC. The retention time of at least 6 reference substances with known K_{oc} and the test substance was determined on a HPLC-DAD system and the capacity factor (k') was calculated. The log K_{oc} of the reference substances were plotted v k'. The slope and intercept were calculated by linear regression. The log K_{oc} of the test substance was calculated from this slope and intercept. This study was conducted according to OECD Guideline 121 and is described under point 3 with the following deviation: 1. No reference to duplicate determinations was made. However, this deviation is not considered to compromise the scientific validity of this study.
5.2 Results and discussion	The chromatogram of 1,2-benzisothiazolin-3-(2*H*)-one showed a peak around t_r = 5.6, just between urea (t_0) and acetanilide (the reference substance with the lowest logK_{oc} value). As 1,2-benzisothiazolin-3(2H)-one showed some retention it was possible to calculate a logK_{oc} value. However, the value was determined using extrapolation as t_r of 1,2-benzisothiazolin-3-(2*H*)-one was below the lowest calibration value found for all reference substances. The logK_{oc} value was determined to be 0.73. Please refer to Tables A7.1.3/2-1and A7.1.3/2-2 and Figure A7.1.3/2-1.
5.2.1 Adsorbed a.s. [%]	Not applicable
5.2.2 K_a	Not applicable
5.2.3 K_d	Not applicable
5.2.4 Ka_{oc}	A log K_{oc} value of 0.73 was determined for 1,2-benzisothiazolin-3-(2*H*)-one.
5.2.5 Ka/Kd	Not applicable
5.2.6 Degradation products	Not documented
5.3 Conclusion	From the retention time observed for 1,2-benzisothiazolin-3-(2*H*)-one the logK_{oc} was determined. This resulted in a logK_{oc} value of 0.73. The reproducibility and repeatability of the HPLC-DAD method was within the criteria mentioned in OECD Guideline 121.
5.3.1 Reliability	1
5.3.2 Deficiencies	Yes, One deviation was noted and is outlined under points 2.3 and 5.1. However, it does not compromise the scientific validity of this study.

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.1.3/2 ADSORPTION / DESORPTION SCREENING TEST (02)

Annex Point IIA.7.7

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	*March 2013.*
Materials and Methods	*Applicant's version is adopted with the following remarks:* *2.3. Applicant states that no duplicate determination was made. However, in study report and in Table 7.1.3.-1 and 7-1-3-2, there are two results, corresponding duplicate injections.* *3.3. There is no specification whetheter reference substances were added mixed or separately. In addition, test concentration (25.26 mg in 20 mL methanol) is higher than expected in the environment, and therefore, it cannot be considered a relevant concentration.*
Results and discussion	*RMS does not accept applicant version because of the following remarks:* ▪ *4.4.2. All log K_{oc} of reference points are above the test substance. According to the guidelines, logK_{oc} of at least one reference substance below the expected value of test substance should be used. If the results show that the logK_{ow} of the test substance is outside the calibration range the test should be repeated using different, more appropriate reference substances.* ▪ *According to OECD guideline 121" For ionisable substances, two tests should be performed with both ionised and non-ionised forms in appropriate buffer solutions but only in cases where at least 10 % of the test compound will be dissociated within pH 5.5 to 7.5". The applicant states that the whole test was performed at a pH of 5.5. This is indeed an environmental relevant parameter for soils, but the pKa of the test compound is of pKa 7.2. Thus, at the tested conditions, BIT molecules are mostly in their neutral form. The study of the adsorption of BIT using the same test but at neutral conditions is reported in Doc. III 7.1.3 (1), for which a LogK_{oc} value of 2.1 is obtained.*
Conclusion	*The study should be repeated using a different method, testing BIT with soil samples.*
Reliability	*3*
Acceptability	*Non-acceptable*
Remarks	*This study could only be used as additional information in the risk assessment.*

Table A7.1.3/2-1: Reference substance t_r, t_0, k', log k' and log K_{oc} values

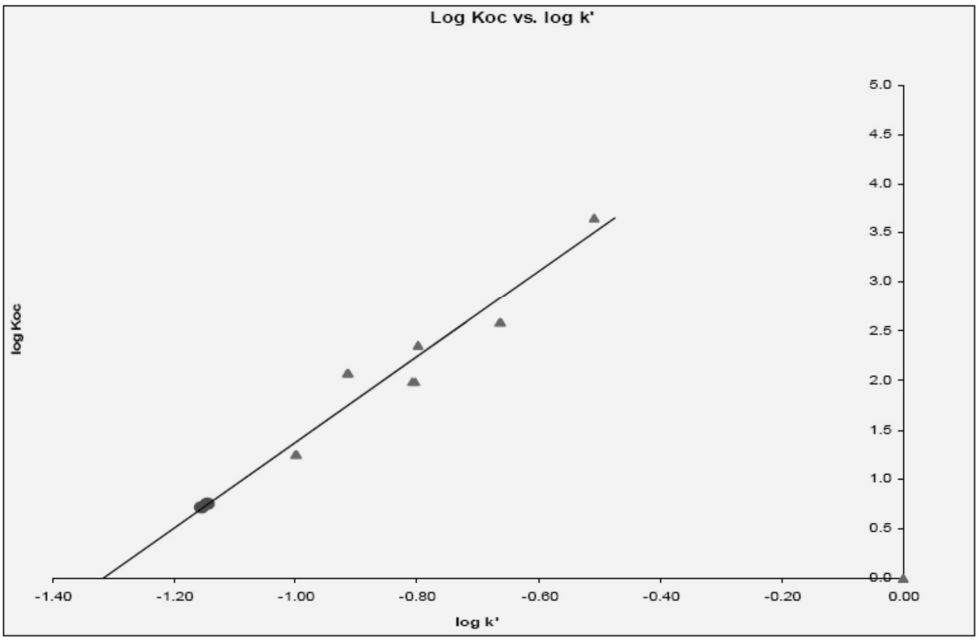
Reference Substance	Wavelength (nm)	t_r (min)	t_0 (min)	k'	logk'	log K_{oc} (1)*
Urea (= t_0)	200	5.209 5.205	5.209 5.205			
Acetanilide	245	5.730 5.727	5.209 5.205	0.100 0.100	-1.000 -0.999	1.25
Aniline	245	5.841 5.841	5.209 5.205	0.121 0.122	-0.916 -0.913	2.07
Monuron	245	6.019 6.023	5.209 5.205	0.156 0.157	-0.808 -0.804	1.99*
Carbendazim	245	6.035 6.032	5.209 5.205	0.159 0.159	-0.800 -0.799	2.35
Linuron	245	6.335 6.335	5.209 5.205	0.216 0.217	-0.665 -0.663	2.59
Pyrazophos	245	6.821 6.815	5.209 5.205	0.309 0.309	-0.509 -0.510	3.65

* values were obtained from OECD Guideline 121

Table A7.1.3/2-2: Results obtained for 1,2-benzisothiazolin-3-one (BIT)

Test Substance	Wavelength (nm)	t_r (min)	t_0 (min)	k'	logk'	logK_{oc}
BIT	318	5.582 5.571	5.209 5.205	0.072 0.070	-1.145 -1.153	0.742 0.708 mean 0.73

Figure A7.1.3/2-1: LogK_{oc} v logk' of the test substance and reference substance obtained with HPLC-DAD



Reference substance

Test substance

Test substance correlation coefficient (R) = 0.97
Reference substance correlation coefficient = 0.96

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	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X].**	
Detailed justification:	According to the 'Data requirements for biocidal product types, Version 4.3.2' (October, 2000), the requirement to carry out this study is based on the outcome of a water/sediment study. Water-sediment simulation tests are required if the biocide is directly emitted to water or if the solids water equilibrium partition coefficient (K_p) of the substance being investigated is > 2000. BIT, according to its recommended use, does not involve direct application to water. BIT is mainly used indoors and is not applied in considerable amounts. The K_p of BIT, derived from the equation $K_p = F_{oc} \times K_{oc}$, was calculated to be 1.3 and is thus below the threshold value set in the guidance (see above). Therefore, a water/sediment study and consequently a field study on accumulation in the sediment are not required to address this point.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2010*	
Evaluation of applicant's justification	*RMS accepts applicant's justification with the following remark:* *Reference to the outcome of the study 7.1.3 (2) is missing. In this study, a log Koc value of 2.1 was obtained. This will correspond to a Kp value of 12.6. Yet, this value is lower than 2000, and thus applicant's justification is accepted.*	
Conclusion	*Acceptable*	
Remarks		

Section A7 Subsection A7.2.1 Annex Point VII.4, XII.1.1	Ecotoxicological Profile Including Environmental Fate and Behaviour AEROBIC DEGRADATION IN SOIL - INITIAL STUDY	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	According to the 'Data requirements for biocidal product types, Version 4.3.2' (October, 2000), soil simulation tests are required if the solids- water equilibrium partition coefficient (K_p) of the substance being investigated is > 5000 or if the biocide is directly emitted to soil. According to the recommended use of BIT, as an in-can preservative, it does not involve direct application to soil. BIT is mainly used indoors and is not applied in considerable amounts. The Kp of BIT, derived from the equation Kp = Foc x Koc, was calculated to be 0.526 and is thus below the threshold value set in the guidance (see above). In addition, according to the "Reregistration Eligibility Decision (RED) for Benzisothiazoline-3-one" (page 30, United States Environmental Protection Agency (2005)), 1,2-benzisothiazolin-3-one breaks down quickly in aerobic soils, with a half-life of less than 24 hours in a sandy loam soil. Furthermore, in a risk assessment carried out with USES 4.0, the risk quotient for soil was found to be < 1, indicating no risk. It is therefore proposed that a study is not required to address this point.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2010*	
Evaluation of applicant's justification	*RMS accepts applicant's justification with the following remarks:* *Reference to the outcome of the study 7.1.3 (2) is missing. In this study, a $logK_{oc}$ value of 2.1 was obtained. This will correspond to a K_p value of 12.6. Yet, this value is lower than 2000, and thus applicant's justification is accepted.* *For the characterization of the exposure, total degradation rate for soil will not consider the DT_{50} value given in "Reregistration Eligibility Decision (RED) for Benzisothiazoline-3-one".* *Justification sentence " Furthermore, in a risk assessment carried out with USES 4.0, the risk quotient for soil was found to be < 1, indicating no risk " is not*	

Section A7 Subsection A7.2.1 Annex Point VII.4, XII.1.1	Ecotoxicological Profile Including Environmental Fate and Behaviour AEROBIC DEGRADATION IN SOIL - INITIAL STUDY
	applicable for all type of uses in PT 6 (see Doc. II-B). In addition, data analysis was carried out using EUSES 2.1.
Conclusion	*Acceptable*
Remarks	

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

Annex Point IIIA, VII.4, XII.1.1

Official use only

1 REFERENCE

1.1 Reference [redacted]

1.2 Data protection Yes

1.2.1 Data owner: Laboratorios Miret, S.A., LANXESS Deutschland GmbH, Lonza Ltd, Specialty Electronic Materials Switzerland GmbH (former The Dow Chemical Company), Thor GmbH, Troy Chemical Company BV

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

2 GUIDELINES AND QUALITY ASSURANCE

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

2.2 GLP Yes

2.3 Deviations No

3 MATERIALS AND METHODS

3.1 Test material Test substance details are summarised below

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

3.1.2 Labelled test material (Lot/Batch number; purity): 1,2-[ring-U-^{14}C]Benzisothiazol-3(2H)-one (thereafter referred to as [^{14}C]Benzisothiazolone); (CFQ43104; radiochemical purity: 96.6%)

3.1.3 Unlabelled test material (Lot/Batch number; purity, description): 1,2-Benzisothiazol-3(2H)-one; (MKBZ4459V; purity: 99.3%; white to yellow and faint beige to beige powder)

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

Annex Point IIIA, VII.4, XII.1.1

3.1.4	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)
3.1.5	Stability	Stability was determined before and after application. Test substance was stable during the application procedure.
3.1.6	Composition of Product	Not relevant as active substance was tested
3.2	**Test system**	Laboratory test
3.2.1	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.
3.2.2	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.
3.2.3	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.
3.2.4	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.
3.3	**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.
3.4	**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.

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3.5 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.

4 RESULTS

4.1 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.

4.2 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

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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.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 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.

5.2 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.

5.3 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.

5.3.1 Reliability: 1

5.3.2 Deficiencies: No

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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.

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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.

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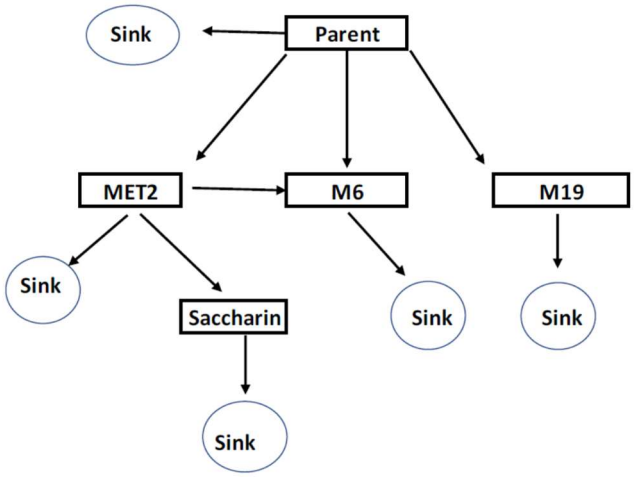
Figure 2. Simplified metabolic pathway used for metabolite kinetics



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

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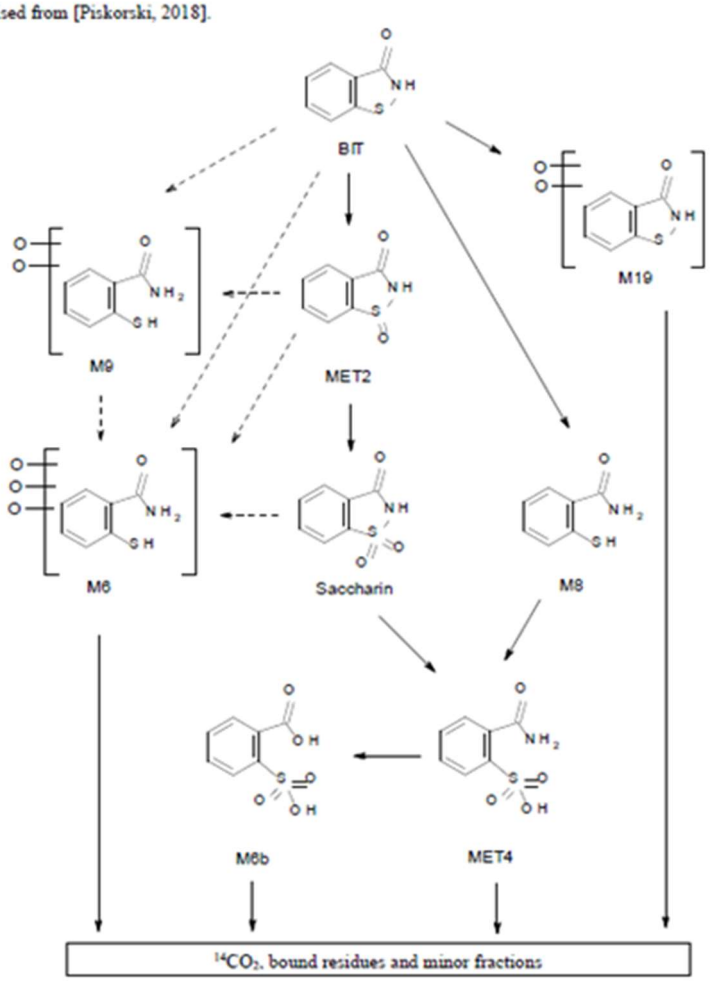
Figure 1. Proposed metabolic pathway (Piskorski, 2020b)

Revised from [Piskorski, 2018].



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:

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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.

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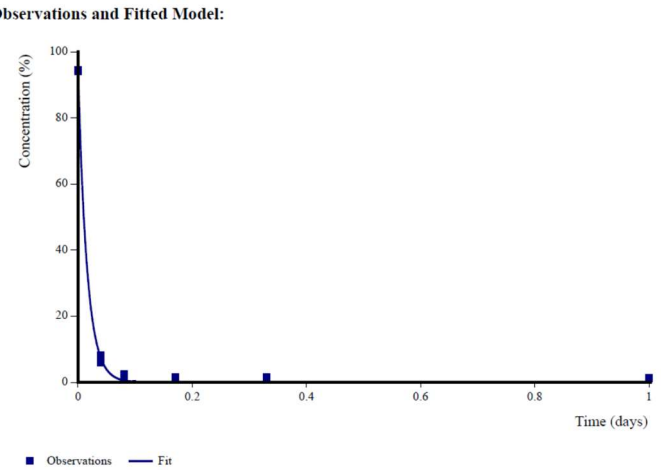
The next table shows parent results when all metabolites are included in Cake iteration process.

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.

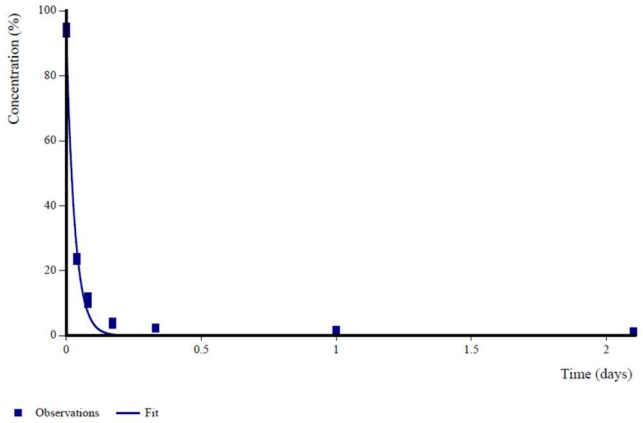
eCA notes that that due to the rapid disappearance of BIT, the number of data points before the DT50 occurs is limited in three of the soils, in fact only the initial value was measured as the following graphs show.

Soil I



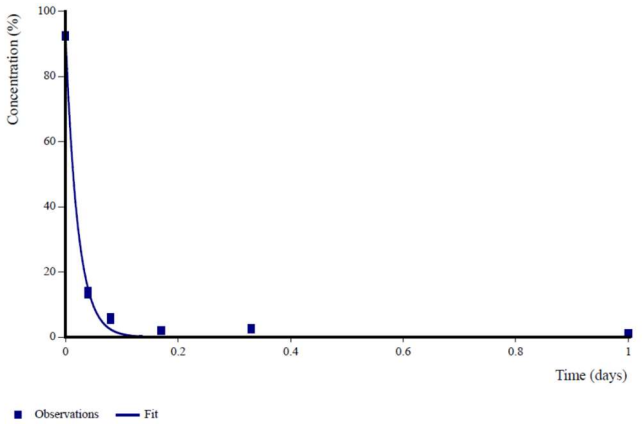
Soil II

Observations and Fitted Model:



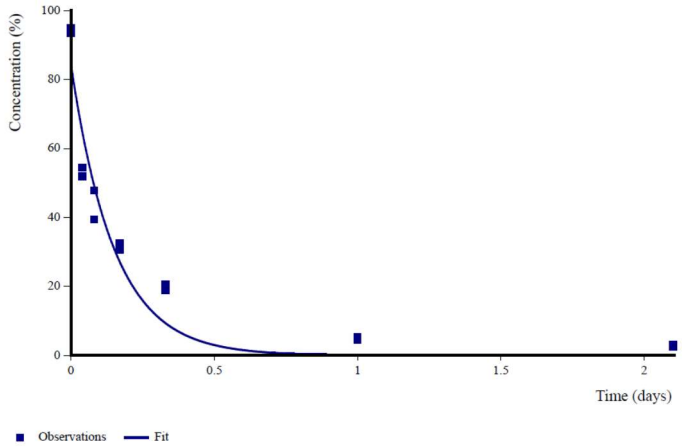
Soil III

Observations and Fitted Model:



Soil IV

Observations and Fitted Model:



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	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.

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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*
Acceptability	acceptable

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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	Leimersheim Germany	Mechtersheim Germany	Schmallenberg Germany	Schmallenberg Germany
Batch	**F2.4 0318**	**F5M 0318**	**01/18**	**01/18**
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**

Parameters	Soil I	Soil II	Soil III	Soil IV
	Speyer 2.4	**Speyer 5M**	**RefeSol 02-A**	**RefeSol 04-A**
During incubation (% OC)	**3.5**	**3.0**	**1.9**	**0.4**
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 LUFA, 67346 Speyer, Germany (Soil I and II; GLP) or by the Fraunhofer Institute, Germany (Soil III and IV; GLP)
** C/N ratio = % organic carbon / % nitrogen content
*** %OM = 1.724 x % organic carbon
**** **Determined under GLP by Agvise Laboratories, Northwood, ND 58267, USA**
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

1 REFERENCE

1.1 Reference [redacted]

1.2 Data protection Yes

Data owner: Laboratorios Miret, S.A., LANXESS Deutschland GmbH, Lonza Ltd, Specialty Electronic Materials Switzerland GmbH (former The Dow Chemical Company), Thor GmbH, Troy Chemical Company BV

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

2 GUIDELINES AND QUALITY ASSURANCE

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

2.2 GLP Yes

2.3 Deviations No

3 MATERIALS AND METHODS

3.1 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); (CFQ43104; radiochemical purity: 96.6%)
Unlabelled test material (Lot/Batch number; purity, description)	1,2-Benzisothiazol-3(2H)-one; (MKBZ4459V; purity: 99.3%; white to yellow and faint beige to beige powder)

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

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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) 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 the IES study # 20170175 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 %.
3.2 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.
3.3 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.
4 RESULTS	
4.1 Storage stability	Concentrated soil extracts, generated in the IES study # 20170175 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 IES study report # 20170175 or the study raw data. Sufficient stability during storage and presence of metabolite M6 could be confirmed.

4.2 Analytical results

The reference standard of 2-sulphamoylbenzoic acid (R12) was analysed by HPLC in water/MeCN (95/5) and in DMSO with three 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 IES study # 20170175. 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.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Concentrated soil extracts, generated in the IES study # 20170175 were used for further analytical work. Sufficient stability was verified by comparison of HPLC profiles obtained in study # 20170175 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.

5.2 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-

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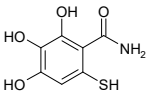
chromatography with the reference standards, including 2-sulphamoylbenzoic acid. The TLC analysis confirmed presence of an abundant metabolite, corresponding to the abundance of M6 that did not co-chromatograph with any of the available reference standards. In conclusion, following HPLC, TLC and LC-MS co-chromatography it could not be confirmed that metabolite M6 was 2-sulphamoylbenzoic acid.

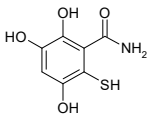
5.3 Conclusion

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.

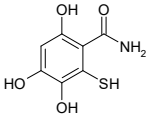
Within the original study (IES study # 20170175) the molecular weights and molecular formulae of M6 and M9 (probably transient metabolite of M6) were reported although the positions of oxidations could not be determined. However, based on the reported results, the likely structures of M6 are:



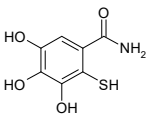
M6.1



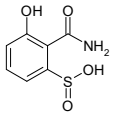
M6.2



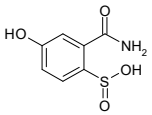
M6.3



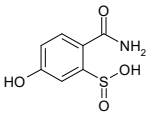
M6.4



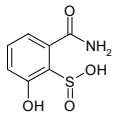
M6.5



M6.6

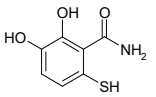


M6.7

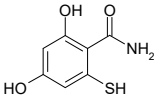


M6.8

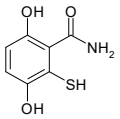
Similarly, based on the total information available of M6 likely structures, the likely structures of M9 are:



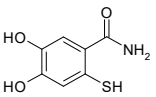
M9.1



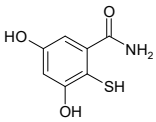
M9.2



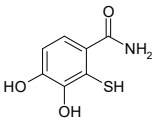
M9.3



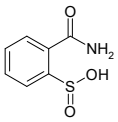
M9.4



M9.5



M9.6



M9.7

Reliability 1

Deficiencies No

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

Annex Point IIIA, VII.4, XII.1.1

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	**Official use only**
Other existing data [...]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure […]	**Other justification […]**	
Detailed justification:	According to the 'Data requirements for biocidal product types, Version 4.3.2' (October, 2000), soil simulation tests are required if the solids- water equilibrium partition coefficient (K_p) of the substance being investigated is > 5000 or if the biocide is directly emitted to soil. According to the recommended use of BIT, as an in-can preservative, it does not involve direct application to soil. BIT is mainly used indoors and is not applied in considerable amounts. The Kp of BIT, derived from the equation Kp = Foc x Koc, was calculated to be 0.526 and is thus below the threshold value set in the guidance (see above). In addition, according to the "Reregistration Eligibility Decision (RED) for Benzisothiazoline-3-one" (page 30, United States Environmental Protection Agency (2005)), 1,2-benzisothiazolin-3-one breaks down quickly in aerobic soils, with a half-life of less than 24 hours in a sandy loam soil. Furthermore, in a risk assessment carried out with USES 4.0, the risk quotient for soil was found to be < 1, indicating no risk. It is therefore proposed that a study is not required to address this point.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2010*	

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**
Evaluation of applicant's justification	*RMS accepts applicant's justification with the following remarks:* *Reference to the outcome of the study 7.1.3 (2) is missing. In this study, a logK_{oc} value of 2.1 was obtained. This will correspond to a K_p value of 12.6. Yet, this value is lower than 2000, and thus applicant's justification is accepted.* *For the characterization of the exposure, total degradation rate for soil will not consider the DT_{50} value given in "Reregistration Eligibility Decision (RED) for Benzisothiazoline-3-one".* *Justification sentence " Furthermore, in a risk assessment carried out with USES 4.0, the risk quotient for soil was found to be < 1, indicating no risk " is not applicable for all type of uses in PT 6 (see Doc. II-B). In addition, data analysis was carried out using EUSES 2.1.*
Conclusion	*Acceptable*
Remarks	

Section A7 **Subsection A7.2.2.2** **Annex Point VII.1.1, Annex VI**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **AEROBIC DEGRADATION IN SOIL - FURTHER STUDIES** **FIELD SOIL DISSIPATION AND ACCUMULATION**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	According to the 'Data requirements for biocidal product types, Version 4.3.2' (October, 2000), soil simulation tests are required if the solids- water equilibrium partition coefficient (K_p) of the substance being investigated is > 5000 or if the biocide is directly emitted to soil. Field soil accumulation tests are required if the $DT_{90field}$ is over one year and the $DT_{50field}$ is greater than 3 months. According to the recommended use of BIT, as an in-can preservative, it does not involve direct application to soil. BIT is mainly used indoors and is not applied in considerable amounts. The Kp of BIT, derived from the equation Kp = Foc x Koc, was calculated to be 0.526 and is thus below the threshold value set in the guidance (see above). In addition, according to the "Reregistration Eligibility Decision (RED) for Benzisothiazoline-3-one" (page 30, United States Environmental Protection Agency (2005)), 1,2-benzisothiazolin-3-one breaks down quickly in aerobic soils, with a DT_{50lab} of less than 24 hours in a sandy loam soil, which indicates that the $DT_{50field}$ would be below the threshold value set in the guidance (see above). Furthermore, in a risk assessment carried out with USES 4.0, the risk quotient for soil was found to be < 1, indicating no risk. It is therefore proposed that a study is not required to address this point.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2010*	

Section A7 **Subsection A7.2.2.2** **Annex Point VII.1.1, Annex VI**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **AEROBIC DEGRADATION IN SOIL - FURTHER STUDIES** **FIELD SOIL DISSIPATION AND ACCUMULATION**
Evaluation of applicant's justification	*RMS accepts applicant's justification with the following remarks:* *Reference to the outcome of the study 7.1.3 (2) is missing. In this study, a logK_{oc} value of 2.1 was obtained. This will correspond to a K_p value of 12.6. Yet, this value is lower than 2000, and thus applicant's justification is accepted.* *For the characterization of the exposure, total degradation rate for soil will not consider the DT_{50} value given in "Reregistration Eligibility Decision (RED) for Benzisothiazoline-3-one".* *Justification sentence " Furthermore, in a risk assessment carried out with USES 4.0, the risk quotient for soil was found to be < 1, indicating no risk " is not applicable for all type of uses in PT 6 (see Doc. II-B). In addition, data analysis was carried out using EUSES 2.1.*
Conclusion	*Acceptable*
Remarks	

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	**Official use only**
Other existing data [...]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure […]	**Other justification […]**	
Detailed justification:	According to the 'Data requirements for biocidal product types, Version 4.3.2' (October, 2000)', soil simulation tests are required if the solids- water equilibrium partition coefficient (K_p) of the substance being investigated is > 5000 or if the biocide is directly emitted to soil. According to the recommended use of BIT, as an in-can preservative, it does not involve direct application to soil. BIT is mainly used indoors and is not applied in considerable amounts. The Kp of BIT, derived from the equation Kp = Foc x Koc, was calculated to be 0.526 and is thus below the threshold value set in the guidance (see above). In addition, according to the "Reregistration Eligibility Decision (RED) for Benzisothiazoline-3-one" (page 30, United States Environmental Protection Agency (2005)), 1,2-benzisothiazolin-3-one breaks down quickly in aerobic soils, with a half-life of less than 24 hours in a sandy loam soil. Therefore, as BIT degrades so quickly it does not persist in a soil environment. Furthermore, in a risk assessment carried out with USES 4.0, the risk quotient for soil was found to be < 1, indicating no risk. It is therefore proposed that a study is not required to address this point.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2010*	

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 of applicant's justification	*RMS accepts applicant's justification with the following remarks:* *Reference to the outcome of the study 7.1.3 (2) is missing. In this study, a logK_{oc} value of 2.1 was obtained. This will correspond to a K_p value of 12.6. Yet, this value is lower than 2000, and thus applicant's justification is accepted.* *For the characterization of the exposure, total degradation rate for soil will not consider the DT_{50} value given in "Reregistration Eligibility Decision (RED) for Benzisothiazoline-3-one".* *Justification sentence " Furthermore, in a risk assessment carried out with USES 4.0, the risk quotient for soil was found to be < 1, indicating no risk " is not applicable for all type of uses in PT 6 (see Doc. II-B). In addition, data analysis was carried out using EUSES 2.1.*
Conclusion	*Acceptable*
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

1 REFERENCE

1.1 Reference	[redacted]
1.2 Data protection	Yes
Data owner	Laboratorios Miret, S.A., LANXESS Deutschland GmbH, Lonza Ltd, Specialty Electronic Materials Switzerland GmbH (former The Dow Chemical Company), Thor GmbH, Troy Chemical Company BV
Criteria for data protection	Data on existing a.s. submitted for first entry into the European list of approved biocidal active substance

2 GUIDELINES AND QUALITY ASSURANCE

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

3 MATERIALS AND METHODS

3.1 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); (CFQ43104; radiochemical purity: 96.6%)
Unlabelled test material (Lot/Batch number; purity, description)	1,2-Benzisothiazol-3(2H)-one; (MKBZ4459V; purity: 99.3%; 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.
3.2	**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).
3.3	**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.
3.4	**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.

4 RESULTS

4.1	**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.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**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 0.1 M

Section A7.2.2.3/01 Aerobic degradation in soil, further studies: Extent and nature of bound residues
Annex Point IIIA, XII.1.4

		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.
5.2	**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.
5.3	**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

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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 A7.2.2.4** **Annex Point XII.1.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **AEROBIC DEGRADATION IN SOIL - FURTHER STUDIES** **OTHER SOIL DEGRADATION STUDIES**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	According to the 'Data requirements for biocidal product types, Version 4.3.2' (October, 2000)', soil simulation tests are required if the solids- water equilibrium partition coefficient (K_p) of the substance being investigated is > 5000 or if the biocide is directly emitted to soil. According to the recommended use of BIT as an in-can preservative it does not involve direct application to soil. BIT is mainly used indoors and is not applied in considerable amounts. The Kp of BIT, derived from the equation Kp = Foc x Koc, was calculated to be 0.526 and is thus below the threshold value set in the guidance (see above). In addition, according to the "Reregistration Eligibility Decision (RED) for Benzisothiazoline-3-one" (page 30, United States Environmental Protection Agency (2005)), 1,2-benzisothiazolin-3-one breaks down quickly in aerobic soils, with a half-life of less than 24 hours in a sandy loam soil. Furthermore, in a risk assessment carried out with USES 4.0, the risk quotient for soil was found to be < 1, indicating no risk. It is therefore proposed that a study is not required to address this point.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2010*	

Section A7 **Subsection A7.2.2.4** **Annex Point XII.1.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **AEROBIC DEGRADATION IN SOIL - FURTHER STUDIES** **OTHER SOIL DEGRADATION STUDIES**
Evaluation of applicant's justification	*RMS accepts applicant's justification with the following remarks:* *Reference to the outcome of the study 7.1.3 (2) is missing. In this study, a logK_{oc} value of 2.1 was obtained. This will correspond to a K_p value of 12.6. Yet, this value is lower than 2000, and thus applicant's justification is accepted.* *For the characterization of the exposure, total degradation rate for soil will not consider the DT_{50} value given in "Reregistration Eligibility Decision (RED) for Benzisothiazoline-3-one".* *Justification sentence " Furthermore, in a risk assessment carried out with USES 4.0, the risk quotient for soil was found to be < 1, indicating no risk " is not applicable for all type of uses in PT 6 (see Doc. II-B). In addition, data analysis was carried out using EUSES 2.1.*
Conclusion	*Acceptable*
Remarks	

Section A7 **Subsection A7.2.3.1** **Annex Point IIIA XII 1.3**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ADSORPTION AND MOBILITY IN SOIL,** **ADSORPTION AND DESORPTION STUDIES**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [...]	**Other justification [ ]**	
Detailed justification:	According to the 'Data requirements for biocidal product types, Version 4.3.2' (October, 2000)', adsorption and desorption studies are required if a substance is used directly on, released to or disposed in/on soil in relevant amounts. These studies may also be required where the risk assessment results in a PEC/PNEC > 1 in soil or sediment, or the substance leaches to groundwater. BIT, according to its recommended use, does not involve direct application to soil. BIT is mainly used indoors and is not applied in considerable amounts. In a risk assessment carried out with USES 4.0, the risk quotient for soil was found to be < 1 and the predicted concentration of BIT in groundwater < 0.1µg/L. It can therefore be concluded that a full scale adsorption and desorption study is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*May 2011*	
Evaluation of applicant's justification	*Applicant's justification is accepted.*	
Conclusion	*Applicant's justification is acceptable.*	
Remarks		

Section A7 **Subsection A7.2.3.2** **Annex Point XII.1.3**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ADSORPTION AND MOBILITY IN SOIL – FURTHER STUDIES** **MOBILITY**	
	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:	According to the 'Data requirements for biocidal product types, Version 4.3.2' (October, 2000)', in most cases the mobility of a substance in soil can be estimated by means of running mathematical model calculations. Where it is indicated from data on adsorption and degradation in soil that relevant amounts of the substance may reach groundwater it may become necessary to carry out an outdoor confirmatory study. BIT, according to its recommended use does not involve direct application to soil. BIT is mainly used indoors and is not applied in considerable amounts. According to IIIA 7.1.3/1 "Estimation of the adsorption coefficient of 1,2-benzisothiazolin-3-one to sewage sludge using HPLC" De Vette, H.Q.M. and Jansen, J. (2002) and IIIA 7.1.3/2 "Estimation of the adsorption coefficient (K_{oc}) of BIT (1,2-benzisothiazolin-3-(2H)-one) on soil and sewage sludge using HPLC" Schouten, A. and Verhoef, A. (2005) the log K_{oc} values were 0.73 and 2.11 indicating a propensity not to strongly adsorb to soil (the average log K_{oc} value is 1.42, therefore the average K_{oc} value is 26.3). According to the McCall classification BIT is therefore highly mobile. However, in a risk assessment carried out with USES 4.0, the risk quotient for groundwater was found to be < 0.1 µg/l. It can therefore be concluded that BIT does not pose a risk to groundwater and hence further studies on the adsorption and mobility of BIT in soil are not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*September 2010*	

Section A7 **Subsection A7.2.3.2** **Annex Point XII.1.3**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ADSORPTION AND MOBILITY IN SOIL – FURTHER STUDIES** **MOBILITY**
Evaluation of applicant's justification	*Accept the applicant's justification*
Conclusion	*Acceptable*
Remarks	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.3.1

Annex Point: IIIA 12.3

PHOTOTRANSFORMATION IN AIR (ESTIMATION METHOD) (01)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Date of experimental work: March 27, 2007.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV Dow Benelux BV	
1.2.2	Companies with letter of access	Not applicable	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, Commission Directive 94/37/EC of July 22, 1994.	**X**
2.2	**GLP**	**Not applicable**	
2.3	**Deviations**	**No**	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Not applicable as 1,2-benzisothiazol-3-(2*H*)-one was simulated	
3.2	**Testing procedure**		
3.2.1	Test system	A computer modelling program AOP (Atmospheric Oxidation Program) was used to estimate the atmospheric half-life of 1,2-benzisothiazol-3-(2*H*)-one. The program estimated the rate constant for the atmospheric gas phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The reaction between ozone and olefinic/acetylenic compounds was also estimated. This was done by estimating the rate constant (k) of the active	**X**

Section A7 Subsection A7.3.1 Annex Point: IIIA 12.3	Ecotoxicological Profile Including Environmental Fate and Behaviour PHOTOTRANSFORMATION IN AIR (ESTIMATION METHOD) (01)	
	substance based on the chemical structure of 1,2-benzisothiazol-3-(2*H*)-one. The degradation of the active substance followed pseudo-first order kinetics. The rate constants were used by the program to calculate the atmospheric half-life for 1,2-benzisothiazol-3-(2*H*)-one based upon average concentrations of hydroxyl radicals and ozone.	
	4 RESULTS	
4.1 Rate constant (k)	AOP estimated the overall rate constant for the gas phase reaction between 1,2-benzisothiazol-3-(2*H*)-one and hydroxyl radicals (OH) to be 16.9594 x 10^{-12} cm^3/molecule-sec. No ozone reaction estimation was determined.	**X**
4.2 DT_{50}	The atmospheric half-life (DT_{50}) of 1,2-benzisothiazol-3-(2*H*)-one, as a result of gas-phase reactions with photochemically produced atmospheric hydroxyl radicals, was estimated to be 0.631 days (based on 12-hour days). No ozone reaction estimation was determined.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The rate constants and half-lives for reactions of 1,2-benzisothiazol-3-(2*H*)-one with OH radicals in the atmosphere were estimated using the Atmospheric oxidation program (AOP). Using the weighted global average OH radical concentration in the troposphere, the half-life of this process was calculated.	
5.2 Results and discussion		
5.2.1 Rate constant (k)	AOP estimated the overall rate constant for the gas phase reaction between 1,2-benzisothiazol-3-(2*H*)-one and hydroxyl radicals (OH) to be 16.9594 x 10^{-12} cm^3/molecule-sec.	
5.2.2 DT_{50}	The atmospheric half-life (DT_{50}) of 1,2-benzisothiazol-3-(2*H*)-one, as a result of gas-phase reactions with photochemically produced atmospheric hydroxyl radicals, was estimated to be 0.631 days (based on 12-hour days).	
5.3 Conclusion	According to the Atkinson method of calculation, the main route of degradation of 1,2-benzisothiazol-3-(2*H*)-one, in air is *via* the reaction with hydroxyl radicals with a DT_{50} of 0.631 days (based on 12-hour days).	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.3.1	
Annex Point: IIIA 12.3	**PHOTOTRANSFORMATION IN AIR (ESTIMATION METHOD) (01)**

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*May 2013.*
Materials and Methods	*Applicant's version is accepted with the following comments:* *2.1. The guideline refers to Plant Protection Products Directive. This Directive states: "An estimation of the photochemical oxidative degradation (indirect phototransformation) of the active substance, must be submitted.", but it does not provide further guidelines on the method of this estimation.* *Nevertheless, RMS accepts the Atmospheric Oxidation Program, taking into account the OECD Environmental Monograph nº 67. As stated is this document, the overall good agreement of experimental and calculated data justifies the application of this program for the estimation of rate constants.* *3.2.1. Test report only considers photodegradation of BIT due to reactions with hydroxyl radicals, not considering other reactive species such as NO_3 and O_3 radicals.* *In addition, the test report does not provide the average concentrations of tropospheric hydroxyl and ozone radicals considered in the AOP program to estimate the rate constants.*
Results and discussion	*Applicant's version is accepted but with the following comments:* *Results do not provide any estimation of degradation products formed during photochemical reactions.*
Conclusion	*According to the Atkinson method of calculation, the main route of degradation of 1,2-benzisothiazol-3(2H)-one, in air is via the reaction with hydroxyl radicals with a DT_{50} of 0.946 days (based on 24-hour days).*
Reliability	*1*
Acceptability	***Acceptable***
Remarks	*The half-life of BIT for the reaction with OH-radicals taking into account the concentration of OH-radicals in atmosphere of 5×10^5 molecules $\cdot$ cm^{-3} as recommended in the TGD (2003) is equivalent to 22.705 hours (24-h day). This value will be used in the risk assessment.*

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

DISTRIBUTION IN THE ENVIRONMENT

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Date of experimental work: July 13, 2007	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV Dow Benelux BV	
1.2.2	Companies with letter of access	Not applicable	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not applicable	
2.2	**GLP**	Not applicable	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Not applicable	
3.2	**Testing procedure**		
3.2.1	Test system	The Mackay Level I fugacity model was used to simulate the equilibrium distribution of a fixed quantity of conserved (i.e. non-reacting) 1,2-benzisothiazol-3-(2*H*)-one in a closed environment at equilibrium. No degrading reactions, advective processes or intermedia transport processes (e.g. wet deposition or sedimentation) were considered.	

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** **DISTRIBUTION IN THE ENVIRONMENT**	
		Three types of chemicals are considered in this model – Type 1: chemicals that partition into all media, Type 2: involatile chemicals and Type 3: chemicals with zero or near-zero solubility. 1,2-benzisothiazol-3-(2*H*)-one was assessed under Type 1. Physical-chemical properties and partition coefficient data were input values used by the model to derive environmental properties such as volume, density and organic matter. These parameters were then used to quantify 1,2-benzisothiazol-3-(2*H*)-one behaviour in an evaluative environment. Please refer to Table A7.3.2-1 and A7.3.2-2 for input parameters and environmental properties generated by the model. Distribution was simulated for the following homogenous environmental media (or compartments): air, water, soil, sediment, suspended sediment, fish and aerosols.	
		4 RESULTS	
4.1	**Fagacity**	2.62×10^{-5} μPa	
4.2	**Distribution**	1,2-benzisothiazol-3-(2*H*)-one was predicted to partition predominantly to water (97.77%) and to a much lesser extent to sediment (0.05%) and to soil (2.2%). Insignificant amounts are anticipated to be distributed to the air (0.0004%) and to the suspended sediment (0.0015%).	**X**
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.4	**Materials and methods**	The Mackay Level I fugacity model was used to simulate the equilibrium distribution of 1,2-benzisothiazol-3-(2*H*)-one in a closed environment at equilibrium. The physical-chemical properties, partition coefficient data and user-defined volumes and densities were used to quantify the behaviour of 1,2-benzisothiazol-3-(2*H*)-one for the following homogenous environmental compartments: air, water, soil, sediment, suspended sediment, fish and aerosols.	
5.5	**Results and discussion**		
5.2.3	Fugacity	2.62×10^{-5} μPa	
5.2.4	Distribution	1,2-benzisothiazol-3-(2*H*)-one was predicted to partition predominantly to water (97.77%) and to a much lesser extent to sediment (0.05%) and to soil (2.2%). Insignificant amounts are	

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** **DISTRIBUTION IN THE ENVIRONMENT**	
		anticipated to be distributed to the air (0.0004%) and to the suspended sediment (0.0015%). Please refer to Table A7.3.2-3 and Figure A7.3.2-1.	
5.6	**Conclusion**	1,2-benzisothiazol-3-(2*H*)-one has a fugacity value of 2.62 x 10^{-5} µPa and is found to partition predominantly to water.	**X**
5.3.3	Reliability	1	
5.3.4	Deficiencies	None	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*January 2010*
Materials and Methods	*Applicant's version is accepted*
Results and discussion	*Applicant's version is accepted*
Conclusion	*1,2-benzisothiazol-3-(2H)-one has a fugacity value of 2.62 x 10^{-5} µPa and is found to partition predominantly to water.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A7.3.2-1: Physical and partition coefficient input data for Level I Fugacity Model

Input Parameter	Value
Molecular mass (g/mol)	151.19
Data temperature (°C)	25
Water solubility (g/m^3)	1118
Vapour pressure (Pa)	1.5 x 10^{-4}
Log K_{ow}	1.4
Melting point (°C)	160
Amount of chemical (kg)	40,000

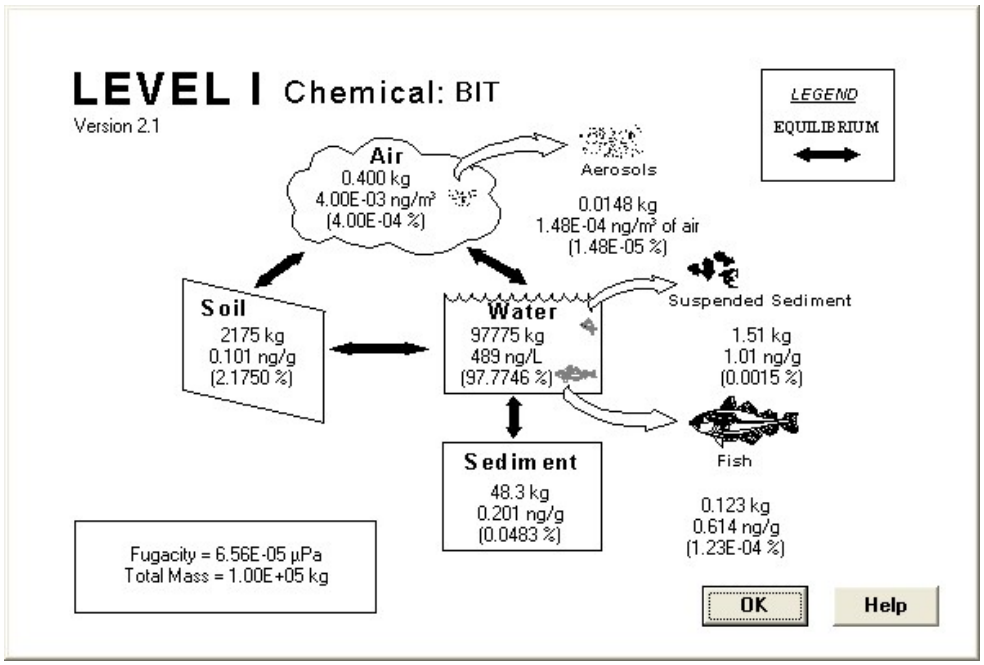
Table A7.3.2-2: Environmental properties for 1,2-benzisothiazol-3-(2*H*)-one as generated by the Level I Fugacity model

Environmental properties	Air	Water	Soil	Sediment	Suspended sediment	Fish	Aerosol
Volume, V (m^3)	1.00 x 10^{14}	2.00 x 10^{11}	9.00 x 10^9	1.00 x 10^8	1.00 x 10^6	2.00 x 10^5	2000
Density (kg/m^3)	1.185	1000	2400	2400	1500	1000	2000
Organic carbon (g/g)	-	-	0.02	0.04	0.2	-	-

Table A7.3.2-3: Phase properties and composition of 1,2-benzisothiazol-3(2*H*)-one in the relevant environmmental compartments

Model output	Air	Water	Soil	Sediment	Suspended sediment	Fish	Aerosol
Z (mol/m^{-3}.Pa)	4.03 x 10^{-4}	4.93 x 10^{-4}	2.44 x 10^4	4.87 x 10^4	1.52 x 10^5	6.19 x 10^4	7.46 x 10^5
VZ (mol/Pa)	4.03 x 10^{10}	9.86 x 10^{15}	2.19 x 10^{14}	4.87 x 10^{12}	1.52 x 10^{11}	1.24 x 10^{10}	1.49 x 10^9
Concentration (mol/m^3)	1.06 x 10^{-14}	1.29 x 10^{-6}	6.39 x 10^{-7}	1.28 x 10^{-6}	4.00 x 10^{-6}	1.62 x 10^{-6}	1.96 x 10^{-5}
Amount (%)	4 x 10^{-4}	97.7746	2.1750	0.0483	0.0015	1.23 x 10^{-4}	1.48 x 10^{-5}

Figure A7.3.2-1: Distribution of 1,2-benzisothiazol-3(2*H*)-one in the environment as predicted by the Mackay Level 1 Fugacity Model



Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.1/1

Annex Point IIA VII.7.1

ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Oncorhynchus mykiss*

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: June 25, 2002 – June 29, 2002.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test was carried out in accordance with OECD Guideline No. 203, "Fish, acute toxicity test"; E.C. method, "C.1, Acute Toxicity for Fish" and US EPA Ecological Effects Test Guidelines OPPTS 850.1075, "Fish Acute Toxicity Test, Freshwater and Marine", EPA 712-C-96-118.	
2.2	**GLP**	Yes, certified by the Inspectorate for Health Protection, Commodities and Veterinary Public Health of the Ministry of Health, Welfare and Sport from The Netherlands	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazoline-3-one (BIT)	
3.1.1	Lot/Batch number	BT 17301	
3.1.2	Specification	Please refer to Doc. III-A, 2/1	
3.1.3	Purity	97.42%	
3.1.4	Composition of Product	Not applicable	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.1/1 ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Oncorhynchus mykiss*

Annex Point IIA VII.7.1

3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	HPLC with UV detection.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
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	The dilution water used was Laboratory tap water. Details are given in Table A7.4.1.1/1-1.	
3.4.2	Test organisms	Fresh-water fish species *Oncorhynchus mykiss,* details are given in Table A7.4.1.1/1-2.	**X**
3.4.3	Test system	Details are given in Table A7.4.1.1/1-3.	
3.4.4	Test conditions	Details are given in Table A7.4.1.1/1-4.	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality, behavioural responses and clinical symptoms.	
3.4.7	Sampling	Observations for mortality, behavioural responses and clinical symptoms were made at 3, 6, 24, 48, 72 and 96 hours of exposure. Temperature, pH and dissolved oxygen content of the test media were measured daily before and after each renewal of the media. Total hardness was analysed daily.	
3.4.8	Monitoring of TS concentration	Yes; 200 mL samples from a test substance solution (100 mg of BIT dissolved in 10 L of water: 10 mg/L BIT), control (without BIT) and vehicle control were taken at 0 h and 24 h intervals.	
3.4.9	Statistics	An estimate of the 96 h LC_{50} of BIT value and its confidence interval was calculated using the Probit analysis method (Finney, 1971).	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.1/1 ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Oncorhynchus mykiss*

Annex Point IIA VII.7.1

		4 RESULTS	
4.1	**Limit Test**	Not performed, however a range-finding test was performed.	
4.1.1	Concentration	0, 1.00, 5.00 and 10.00 mg BIT/L	
4.1.2	Number/ percentage of animals showing adverse effects	The per cent mortalities observed were 10, 90 and 100% at the test concentrations of 1.00, 5.00 and 10.00 mg BIT/L, respectively. No mortalities were observed in the control and vehicle control groups.	
4.1.3	Nature of adverse effects	Mortality. No other effects were documented.	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0.80, 1.30, 2.10, 3.40 and 5.51 mg BIT/L.	**X**
4.2.2	Actual concentrations of test substance	The test concentrations selected were not detectable by the analytical method, so a known detectable concentration of 10 mg/L was prepared to perform the active ingredient content and stability of the test solution. The nominal concentration was therefore 10 mg/L (purity 71.08 %) and the actual concentration was found to be 7.108 mg/L. After dosing, the measured concentration was 6.82 mg BIT/L, 96% of the actual concentration. After 24 hours, the measured concentration was 6.57 mg/L, 92% of the actual concentration.	**X**
4.2.3	Effect data (Mortality)	Data are provided in Table A7.4.1.1/1-5 and Table A7.4.1.1/1-6.	
4.2.4	Concentration / response curve	The regression equation established was $Y=3.704+4.024\chi$, where Y is Probit mortality and χ is log concentration (mg/L) of BIT	
4.2.5	Other effects	Loss of equilibrium, sluggishness, swimming at surface, dark pigmentation, lying on bottom and rapid respiration were observed at 5.51 mg/L test concentration; Loss of equilibrium, sluggishness, swimming at surface, light pigmentation and lying on bottom at 3.40 mg/L; Loss of equilibrium, sluggishness, dark pigmentation and lying on bottom at 2.10 mg/L; Loss of equilibrium, sluggishness and dark pigmentation at 1.30 mg/L; No clinical symptoms were observed at the test concentration of 0.8 mg/L.	
4.3	**Results of controls**		

Section A7 Subsection A7.4.1.1/1 Annex Point IIA VII.7.1		Ecotoxicological Profile Including Environmental Fate and Behaviour ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Oncorhynchus mykiss*	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were observed in the control and vehicle control groups.	
4.3.2	Nature of adverse effects	Not applicable	
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**	The toxicity of 1,2-benzisothiazoline-3-one (BIT) to the fresh-water fish *Oncorhynchus mykiss* was determined in a 96-hour semi-static acute toxicity test. The concentrations tested were 0, 0.80, 1.30, 2.10, 3.40 and 5.51 mg/L. The test was conducted according to OECD guideline 203 and EU guideline C.1 with no deviations and is described under point 3.	
5.2	**Results and discussion**	The concentration of BIT was found to be more than 80% of the nominal concentration selected, during the 96% h exposure period. Since the actual concentrations were within 80% of the nominal concentrations, the test results are expressed as nominal concentrations. Mortality at 0, 3, 6, 24, 48, 72 and 96 hours is given in Table A7.4.1.1/1-5. The per cent mortality observed at 96 h were 20, 50, 80 and 100 at the test concentrations of 1.30, 2.10, 3.40 and 5.51 mg BIT/L. Loss of equilibrium, sluggishness, swimming at surface, dark or light pigmentation, lying on bottom and rapid respiration were the clinical symptoms observed at concentrations above 0.80 mg/L. At 0.80 mg/L and the control concentrations tested, there were no adverse effects observed with respect to survival or condition. The test met the validity criteria established in the recommended Guideline. There was no mortality in the control and the dissolved oxygen concentration was above 60% saturation. The average percentage of the substance present during the test was >80% of initial concentration.	
	LC_0	Not calculated	

Section A7 **Subsection A7.4.1.1/1** **Annex Point IIA VII.7.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Oncorhynchus mykiss***	
	LC_{50}	2.10 mg/L (with a 95% fiducial limits of 1.59-2.77 mg/L)
	LC_{100}	Not calculated
5.3 Conclusion		Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.
5.3.1	Other Conclusions	No
5.3.2	Reliability	1
5.3.3	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 comments:* *3.4.2. The size of test organisms employed in this test exceed the recommended in OECD Guideline No. 203.This document recommends a total length of test fish of 5 cm (±1 cm), while individuals used for this study were among 7.17 and 7.63 cm. The length and weight of the fish are related to the sensitivity of animals towards the toxicant. Larger fish may be less sensitive. However the resulting values of the endpoints are similar to other tests with the same species thus the test results can be considered as reliable.* *4.2.1. According to OECD Guideline No. 203, concentrations of test substance must follow a geometric series.* *4.2.2. In section "materials and methods", the purity of BIT is reported to be 97.42%. (on dry basis). However, the purity of BIT reported in the analysis of test concentration is 71.08%. (concentration of BIT in water during the exposure period).* *In addition, concentration of BIT after 24 h is measured with higher concentration than main test, assuming that degradation rate of BIT is similar in test concentrations.*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.1/1

Annex Point IIA VII.7.1

ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Oncorhynchus mykiss*

Results and discussion	*Applicant's version is accepted despite methodological deficiencies* *The effects values (LC_{50}, LC_{100}, LC_0) derived in the study report are for the wet material, in order to obtain the corresponding values for BIT without water, the purity of 71.08% must be taken into account resulting in the following effect values for pure BIT:* *Table A7.4.1.1/1-6: Effect data based on nominal concentrations*

	96 h [mg/L]	***95 % c.l.***
LC_0	*0.57*	-
LC_{50}	*1.49*	*1.13-1.97*
LC_{100}	*3.92*	-

Conclusion	*Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Table A7.4.1.1/1-1: Dilution water

Criteria	Details
Source	Laboratory tap water
Alkalinity	Not documented
Hardness	204.0 mg/L ($CaCO_3$)
pH	7.69-7.72
Oxygen content (%)	97.7-110.0
Conductance	Not documented
Holding water different from dilution water	No

Table A7.4.1.1/1-2: Test organisms

Criteria	Details
Species/strain	*Oncorhynchus mykiss*
Source	Deputy director of Fisheries, Indo-Norwegian Trout Farming Project, India
Wild caught	No
Age/size	Age not documented Size: 7.17-7.63 cm
Kind of food	"Trout starter feed"
Amount of food	Not documented
Feeding frequency	Daily
Pretreatment	Fish were allowed to acclimatise for 12 days. Feeding was withheld 48 hours before exposure to BIT
Feeding of animals during test	No

Table A7.4.1.1/1-3: Test system

Criteria	Details
Test type	Semi-static
Renewal of test solution	Daily replacement of the exposure media
Volume of test vessels	120 L
Volume/animal	10 L/fish
Number of animals/vessel	10 fish/vessel
Number of vessels/ concentration	1 vessel/concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.1/1-4: Test conditions

Criteria	Details
Test temperature	13.0 – 14.3 ºC

Dissolved oxygen	87.8 to 114.6% as air saturation
pH	7.68-8.00
Adjustment of pH	Not documented
Aeration of dilution water	Not documented
Intensity of irradiation	Not documented
Photoperiod	16 hours light

Table A7.4.1.1/1-5: Acute toxicity to *Oncorhynchus mykiss* in a semi-static study with 1,2-benzisothiazol-3-(2*H*)-one

Nominal concentration (mg/L) of 1,2-benzisothiazol-3-(2*H*)-one (BIT)	Mortality data						% Mortality
	3 h	6 h	24 h	48 h	72 h	96 h	96 h
0 (control)	0/10	0/10	0/10	0/10	0/10	0/10	0
47.4 mg acetone/L (vehicle control)	0/10	0/10	0/10	0/10	0/10	0/10	0
0.80	0/10	0/10	0/10	0/10	0/10	0/10	0
1.30	0/10	0/10	0/10	0/10	1/10	1/10	20
2.10	0/10	0/10	0/10	1/10	2/10	2/10	50
3.40	0/10	0/10	1/10	1/10	3/10	3/10	80
5.51	0/10	0/10	2/10	2/10	5/10	1/10	100

Table A7.4.1.1/1-6: Effect data based on nominal concentrations

	48 h [mg/L]	95 % c.l.	96 h [mg/L]	95 % c.l.
LC_0	-	-	0.80	-
LC_{50}	-	-	2.10	1.59-2.77
LC_{100}	-	-	5.51	-

Table A7.4.1.1/1-7: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled
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
Criteria for poorly soluble test substances	Not applicable

Section A7
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Ecotoxicological Profile Including Environmental Fate and Behaviour

ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Brachydanio rerio*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: September 24, 2001 – October 20, 2001.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access	Dow Benelux BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test was carried out in accordance with OECD Guideline no. 203 "Fish, acute toxicity test" and EU Guideline C.1.	
2.2	**GLP**	Yes (certified by the Inspectorate for Health Protection and Veterinary Public Health, Ministry of Health, Welfare and Sport, The Netherlands).	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	BT 12000	
3.1.2	Specification	Please refer to Doc. III-A, 2/2	
3.1.3	Purity	98%	
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 A7.4.1.1/2 ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Brachydanio rerio*

Annex Point IIA VII.7.1

3.1.6	Method of analysis	HPLC with UV detection.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
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	The dilution water used was DSWL-E prepared from ground water. Details are given in Table A7.4.1.1/2-1.	
3.4.2	Test organisms	Fresh-water fish species *Brachydanio rerio,* details are given in Table A7.4.1.1/2-2.	**X**
3.4.3	Test system	Details are given in Table A7.4.1.1/2-3.	
3.4.4	Test conditions	Details are given in Table A7.4.1.1/2-4.	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality and condition (visually observable morphological or behavioural effects)	
3.4.7	Sampling	The temperature in the control medium was measured at the beginning of the test, at each replacement time (in the newly prepared control vessel and in the corresponding "spent" control vessel) and at the end of the test. The pH and the oxygen concentration of the exposure media were measured at the beginning and at the end of the test, as well as at each replacement time (in the spent media just before replacement and in the newly prepared media just after dosing). Observations for mortality and condition were made at 3, 24, 48, 72 and 96 hours.	
3.4.8	Monitoring of TS concentration	Yes; Samples from the exposure media of 0.56, 3.2 and 10 mg/L and the control were taken at 0 h (newly prepared media) and 24 h (spent media after 24 h), except the sample of 10 mg/L which was taken at 3 h because all animals were dead at that exposure time.	

Section A7 Subsection A7.4.1.1/2 Annex Point IIA VII.7.1		Ecotoxicological Profile Including Environmental Fate and Behaviour ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Brachydanio rerio*	
3.4.9	Statistics	The LC_{50} values and their confidence interval were calculated by means of a parametric model.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentration	Not applicable	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L	
4.2.2	Actual concentrations of test substance	After dosing the measured concentrations were between 102% and 139% of the nominal concentration. After 24 hours, just before replacement, the measured concentrations were between 104% and 114% of the nominal concentration.	
4.2.3	Effect data (Mortality)	Data are provided in Table A7.4.1.1/2-5 and Table A7.4.1.1/2-6.	
4.2.4	Concentration / response curve	The slope of the concentration-effect curve was 0.21 (95% confidence interval: 0.10-0.32).	
4.2.5	Other effects	None	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were observed in the control group	
4.3.2	Nature of adverse effects	Not applicable	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.1/2

Annex Point IIA VII.7.1

ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Brachydanio rerio*

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**	The toxicity of 1,2-benzisothiazol-3-(2*H*)-one (BIT) to the fresh-water fish *Brachydanio rerio* was determined in a 96-hour semi-static acute toxicity test. The concentrations tested were 0, 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L. The test was conducted according to OECD guideline 203 and EU guideline C.1 with no deviations and is described under point 3.
5.2	**Results and discussion**	After dosing, the measured concentrations were between 102% and 139% of the nominal concentration. After 24 hours, (just before replacement), the measured concentrations were between 104% and 114% of the nominal concentration. Since the actual concentrations were within 20% of the nominal concentrations, the test results are expressed as nominal concentrations. Mortality at 0, 3, 24, 48, 72 and 96 hours is given in Table A7.4.1.1/2-5. At 10 mg/L, all animals were dead. At 3.2 mg/L, partial mortality occurred. At 1.8 mg/L and the lower concentrations tested, there were no adverse effects observed with respect to survival or condition. The test met the validity criteria established in the recommended Guideline. There was no mortality in the control and the dissolved oxygen concentration was above 5.1 mg/L. The average percentage of the substance present during the test was > 80% of initial concentration. The 96 h NOEC value for survival and condition was 1.8 m/L. The LC_{50} value at 96 h was 4.9 mg/L with 95% confidence intervals of 3.9-6.1 mg/L. The LC_{100} value at 96 h was 10 mg/L.
3.2.1	LC_0	Not documented
3.2.2	LC_{50}	4.9 mg/L (with a 95% confidence interval of 3.9-6.1 mg/L)
3.2.3	LC_{100}	10 mg/L
5.3	**Conclusion**	Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.
5.3.4	Other Conclusions	No

Section A7 Subsection A7.4.1.1/2 Annex Point IIA VII.7.1	Ecotoxicological Profile Including Environmental Fate and Behaviour ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Brachydanio rerio*	
5.3.5 Reliability	1	
5.3.6 Deficiencies	No	

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*February 2010*
Materials and Methods	*Applicant's version is accepted*
Results and discussion	*Applicant's version is accepted*
Conclusion	*Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A7.4.1.1/2-1: Dilution water

Criteria	Details
Source	Ground water from a locality near Linschoten in The Netherlands
Alkalinity	Not documented
Hardness	210 mg/L ($CaCO_3$)
pH	8.0-8.5
Oxygen content	Not documented

Conductance	Not documented
Holding water different from dilution water	No

Table A7.4.1.1/2-2: Test organisms

Criteria	Details
Species/strain	*Brachydanio rerio*
Source	The commercial hatchery Atlanta, Hellevoetsluis, The Netherlands.
Wild caught	No
Age/size	Age not documented Size 2.2 ± 0.22 cm
Kind of food	Not documented
Amount of food	Not documented
Feeding frequency	Not documented
Pretreatment	Not documented
Feeding of animals during test	No

Table A7.4.1.1/2-3: Test system

Criteria	Details
Test type	Semi-static
Renewal of test solution	Daily replacement of the exposure media
Volume of test vessels	2 L
Volume/animal	0.15 L/fish
Number of animals/vessel	10 fish/vessel
Number of vessels/ concentration	1 vessel/concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.1/2-4: Test conditions

Criteria	Details
Test temperature	24.3 – 25.4 ºC
Dissolved oxygen	Lowest measured oxygen concentration = 8.0 mg O_2/L
pH	7.9 – 8.2
Adjustment of pH	Not documented
Aeration of dilution water	Yes, the exposure media were slightly aerated and replaced daily.
Intensity of irradiation	Not documented
Photoperiod	16 hours

Table A7.4.1.1/2-5: Mortality data of *Brachydanio rerio* in a semi-static acute toxicity study with 1,2-benzisothiazol-3-(2*H*)-one

Nominal concentration (mg/L) of 1,2-benzisothiazol-3-(2*H*)-one (BIT)	Mortality data					
	0 h	3 h	24 h	48 h	72 h	96 h
0	0/10	0/10	0/10	0/10	0/10	0/10
0.56	0/10	0/10	0/10	0/10	0/10	0/10
1.0	0/10	0/10	0/10	0/10	0/10	0/10
1.8	0/10	0/10	0/10	0/10	0/10	0/10
3.2	0/10	2/10	2/10	2/10	2/10	2/10
5.6	0/10	2/10	6/10	6/10	6/10	6/10
10	0/10	10/10	-	-	-	-

Table A7.4.1.1/2-6: Effect data based on nominal concentrations

	48 h [mg/L]	95 % c.l.	96 h [mg/L]	95 % c.l.
LC_0	1.8	-	1.8	-
LC_{50}	4.9	3.9-6.1	4.9	3.9-6.1
LC_{100}	10	-	10	-

Table A7.4.1.1/2-7: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled
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
Criteria for poorly soluble test substances	Not applicable

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.1/3

Annex Point IIA VII.7.1

ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Oncorhynchus mykiss*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: July 7, 1997 – October 6, 1997	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access	Rohm and Haas	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted in accordance with U.S. EPA Pesticide Assessment Guidelines, Subdivision E, Section 72-1. The method used is equivalent to the prescribed method OECD 203 "Fish, Acute Toxicity Test".	
2.2	**GLP**	Yes, with the exception of water and food characterisations, however this in not considered to compromise the scientific validity of the study.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]® (1,2-benzisothiazol-3-(2*H*)-one)	
3.1.1	Lot/Batch number	060793	
3.1.2	Specification	Please refer to Doc. III-A, 2/2	
3.1.3	Purity	99.29%	
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 A7.4.1.1/3

Annex Point IIA VII.7.1

ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Oncorhynchus mykiss*

3.1.6	Method of analysis	UV absorbance	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	No	
3.4	**Testing procedure**		
3.4.1	Dilution water	Details are given in Table A7.4.1.1/3-1.	
3.4.2	Test organisms	*Oncorhynchus mykiss,* details are given in Table A7.4.1.1/3-2.	**X**
3.4.3	Test system	Details are given in Table A7.4.1.1/3-3.	
3.4.4	Test conditions	Details are given in Table A7.4.1.1/3-4.	**X**
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality and abnormal behavioural responses.	
3.4.7	Sampling	Temperature, dissolved oxygen and pH were measured at 0, 24, 48, 72 and 96 hours in each vessel. In addition, hourly measurement of temperature was made in one vessel for the duration of the test. Observations for mortality and stress were made at 1, 24, 48, 72 and 96 hours.	
3.4.8	Monitoring of TS concentration	Yes Analytical measurements of the test substance were made on samples of test solutions collected at 0 and 96 hours or whenever 100 % mortality was observed in any replicate test concentration.	
3.4.9	Statistics	A computer program developed by C.E. Stephan and ASTM was used to compute point and interval (i.e, confidence interval) estimate of EC_{50}.	**X**
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentration	Not applicable	

Section A7 **Subsection A7.4.1.1/3** **Annex Point IIA VII.7.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Oncorhynchus mykiss***	
4.1.2 Number/ percentage of animals showing adverse effects	Not applicable	
4.1.3 Nature of adverse effects	Not applicable	
4.2 Results test substance		
4.2.1 Initial concentrations of test substance	Data are provided in Table A7.4.1.1/3-5	
4.2.2 Actual concentrations of test substance	Data are provided in Table A7.4.1.1/3-5	
4.2.3 Effect data (Mortality)	Please refer to Tables A7.4.1.1/3-6 and A7.4.1.1/3-7.	
4.2.4 Concentration / response curve	Not documented	
4.2.5 Other effects	Some fish were observed to be quiescent, surfacing, gyrating, swimming upside down and/or ceasing to swim or have labored respiration.	
4.3 Results of controls		
4.3.1 Number/ percentage of animals showing adverse effects	Please refer to Table A7.4.1.1/3-6.	
4.3.2 Nature of adverse effects	No adverse effects were observed.	
4.4 Test with reference substance	Not performed	
4.4.1 Concentrations	Not applicable	
4.4.2 Results	Not applicable	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.1/3 ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Oncorhynchus mykiss*

Annex Point IIA VII.7.1

		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The toxicity of [redacted]® (1,2-benzisothiazol-3-(2*H*)-one) to *Oncorhynchus mykiss* was determined in a 96-hour static acute toxicity test. The nominal concentrations tested were 0, 0.31, 0.65, 1.25, 2.5, 5.0 and 10.0 mg/L. The test was conducted according to US EPA Pesticide Assessment Guidelines Subdivision E 72-1, which is equivalent to OECD test guideline 203: "Fish, Acute Toxicity Test" and is described under point 3.
5.2	**Results and discussion**	Mortality at 24, 48, 72 and 96 hours is given in Table A7.4.1.1/3-6. Complete mortality was observed in the 5.0 and 10.0 mg/L concentrations following 24 hours of exposure. Complete mortality was observed in the 2.5 mg/L concentration following 48 hours of exposure. No mortality was observed at the 0.31 mg/L concentration. Some fish were observed to be quiescent, surfacing, gyrating, swimming upside down and/or ceasing to swim or have laboured respiration in the 0.31, 0.65, 1.25, 2.5 and 10.0 mg/L concentrations. The test met the validity criteria established in the recommended Guideline. There was no mortality in the control and the dissolved oxygen concentration was above 60%. The average percentage of the substance present during the test was > 80% of initial concentration. The LC_{50} value at 96 h was calculated to be 0.74 mg/L based on mortality with 95% confidence intervals of 0.62 – 0.87 mg/L. The acute NOEC was determined to be 0.31 mg/L.
	LC_0	Not documented
	LC_{50}	0.74 mg/L (with a 95% confidence interval of 0.62 – 0.87 mg/L)
	LC_{100}	Not documented
5.3	**Conclusion**	Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A7
Subsection A7.4.1.1/3
Annex Point IIA VII.7.1

Ecotoxicological Profile Including Environmental Fate and Behaviour

ACUTE TOXICITY OF BIT TO FISH-FRESH WATER, *Oncorhynchus mykiss*

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2012.*
Materials and Methods	*Applicant's version is accepted with the following comments:* *3.4.2. The size of test organisms employed in this test is different than the recommended in OECD Guideline No. 203.This document recommends a total length of test fish of 5cm (±1 cm), while in this study, smaller individuals were used, with an average size of 2.9 cm.* *3.4.4. Temperature during the test was among 11.5 and 13.2ºC. This temperature is slightly lower than recommended in OECD Guideline No. 203 (13-17ºC).*
Results and discussion	*Applicant's version is accepted.*
Conclusion	*Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*This study was performed with a static test, in which solutions remain unchanged throughout the duration of the test. Due to the photodegradation rate of BIT, RMS would recommend choosing a semi-static test or a flow-through test for this substance. However, according to the monitoring of test substance concentration, there are no significant changes in concentrations of BIT in test solutions during the test.* *Key study.*

Table A7.4.1.1/3-1: Dilution water

Criteria	Details
Source	Laboratory fresh water (mixture of deionised water and reagent grade chemicals)
Alkalinity	60 mg/L ($CaCO_3$)
Hardness	84 mg/L ($CaCO_3$)
pH	7.3

Oxygen content	9.7 mg/L
Conductance	290 µmhos/cm
Holding water different from dilution	No

Table A7.4.1.1/3-2: Test organisms

Criteria	Details
Species/strain	*Rainbow Trout*, *Oncorhynchus mykiss*
Source	Mt. Lassen Trout Farm, California, USA
Wild caught	No; Farmed
Age/size	5 weeks / 29.0 ± 1.2 mm
Kind of food	Purina® Trout Chow®, Fish Feed #3 and Prime Flake Red
Amount of food	Not documented
Feeding frequency	Not documented
Pretreatment	Acclimation period = 14 days. Fish were fed up to 48 hrs prior to the experiment
Feeding of animals during test	No

Table A7.4.1.1/3-3: Test system

Criteria	Details
Test type	Static
Renewal of test solution	No
Volume of test vessels	19.5 L glass jars
Loading	Not in excess of 0.5 g fish/ L
Volume/animal	1.5 L / fish
Number of animals/vessel	10 fish per vessel
Number of vessels/ concentration	2 vessel/concentration
Test performed in closed vessels due to significant volatility of TS	Not documented

Table A7.4.1.1/3-4: Test conditions

Criteria	Details
Test temperature	11.5 – 13.2°C
Dissolved oxygen	6.6 – 9.9 mg/L
pH	6.8 – 7.4
Adjustment of pH	Not documented
Aeration of dilution water	Not documented
Intensity of irradiation	Not documented
Photoperiod	16 hours

Table A7.4.1.1/3-5: Initial and actual concentrations of BIT

Nominal concentrations of BIT (mg/L)	Actual Concentration mg/L (hours)					Mean concentration (mg/L)	Percent Nominal Recovered
	0	24	48	72	96		
0 (Dilution water control)	0.00	-	-	-	0.34	0.17	-
0.31	0.34	-	-	-	0.49	0.42	135.5
0.65	0.59	-	-	-	0.68	0.64	98.5
1.25	1.15	-	1.31	-	1.31	1.26	100.8
2.50	2.22	-	2.22	-	-	2.22	88.8
5.00	4.45	4.60	-	-	-	4.53	90.6
10.00	9.03	8.93	-	-	-	8.98	89.8

Table A7.4.1.1/3-6: Mortality data

Nominal concentrations of BIT (mg/L)	Mortality							
	Number				Percentage (cumulative)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0	0/20	0/20	0/20	0/20	0	0	0	0
0.31	0/20	0/20	0/20	0/20	0	0	0	0
0.65	1/20	4/20	7/20	7/20	5	20	35	35
1.25	5/20	18/20	19/20	19/20	25	90	95	95
2.50	17/20	20/20	-	-	85	100	-	-
5.00	20/20	-	-	-	100	-	-	-
10.00	20/20	-	-	-	100	-	-	-

Table A7.4.1.1/3-7: Effect data based on nominal concentrations

	48 h (mg/L)	95 % c.l.	96 h (mg/L)	95 % c.l.
LC_{50}	0.84	0.72 – 0.99	0.74	0.62 – 0.87
NOEC	0.31	-	0.31	-

Table A7.4.1.1/3-8: Validity criteria for acute fish test according to OECD Guideline 203

	Fulfilled
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

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.2/1 ACUTE TOXICITY TO INVERTEBRATES

Annex Point IIA VII.7.2 *Daphnia magna*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: May 28, 2002 – May 30, 2002.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test was carried out in accordance with OECD Guideline No. 202, "*Daphnia* sp. Acute Immobilisation Test"; E.C. method, "C.2, Acute Toxicity for *Daphnia*"; and US EPA OPPTS 850.1010 "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids", EPA 712-C-96-114.	
2.2	**GLP**	Yes, certified by the Inspectorate for Health Protection, Commodities and Veterinary Public Health of the Ministry of Health, Welfare and Sport from The Netherlands	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazoline-3-one (BIT)	
3.1.1	Lot/Batch number	BT 17301	
3.1.2	Specification	Please refer to Doc. III-A, 2/1	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**		
Subsection A7.4.1.2/1	**ACUTE TOXICITY TO INVERTEBRATES**		
Annex Point IIA VII.7.2	*Daphnia magna*		
3.1.3	Purity	97.42%	**X**
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	HPLC with UV detection.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	Yes, Potassium dichromate	
3.3.1	Method of analysis for reference substance	Not documented	
3.4	**Testing procedure**		
3.4.1	Dilution water	*Daphnia* culture water. Details are given in Table A7.4.1.2/1-1.	**X**
3.4.2	Test organisms	*Daphnia magna,* details are given in Table A7.4.1.2/1-2.	**X**
3.4.3	Test system	Details are given in Table A7.4.1.2/1-3.	
3.4.4	Test conditions	Details are given in Table A7.4.1.2/1-4.	**X**
3.4.5	Duration of the test	48 hours	

Section A7 **Subsection A7.4.1.2/1** **Annex Point IIA VII.7.2**		**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ACUTE TOXICITY TO INVERTEBRATES** *Daphnia magna*	
3.4.6	Test parameter	Behavioural symptoms including immobility	
3.4.7	Sampling	Observations for immobilisation and other behavioural symptoms were made at 0, 12, 24, 36 and 48 hours of exposure. Temperature, dissolved oxygen and pH were measured at 0, 12, 24, 36 and 48 hours of exposure. Total hardness of the test media was measured prior to treatment.	
3.4.8	Monitoring of TS concentration	Yes; Two 200 mL replicates of a test substance solution (10 mg of BIT dissolved in 1000 mL of water: 10 mg/L BIT), control (without BIT) and vehicle control were taken at 0 h and 12 h intervals.	
3.4.9	Statistics	From the immobility data, the 24 and 48 h EC_{50} (for acute immobilisation) with 95% fiducial limits were calculated using Probit analysis method (Finney, 1971) using an in-house validated software.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentration	Not documented	
4.1.2	Number/ percentage of animals showing adverse effects	Not documented	
4.1.3	Nature of adverse effects	Not documented	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0.20, 0.35, 0.63, 1.11 and 1.96 mg BIT/L Data are provided in Table A7.4.1.2/1-5	
4.2.2	Actual concentratio	The test concentrations selected were not detectable by the analytical method, so a known detectable concentration of 10 mg/L was prepared	

Section A7 Subsection A7.4.1.2/1 Annex Point IIA VII.7.2		Ecotoxicological Profile Including Environmental Fate and Behaviour ACUTE TOXICITY TO INVERTEBRATES *Daphnia magna*	
	ns of test substance	to demonstrate the active ingredient content and stability of the test solution. The nominal concentration was therefore 10 mg/L (purity 71.08 %) and the actual concentration was found to be 7.108 mg/L. After dosing, the mean measured concentration was 5.96 mg BIT/L, 84% of the actual concentration. After 12 hours, the mean measured concentration was 5.63 mg/L, 79% of the actual concentration. Data are provided in Table A7.4.1.2/1-5	
4.2.3	Effect data (Immobilisation)	Data are provided in Table A7.4.1.2/1-6 and Table A7.4.1.2/1-7.	
4.2.4	Concentration / response curve	The regression equations established were: 24 h: Y=5.213+2.899 χ 48 h: Y=5.732+3.357 χ where Y is Probit mortality and χ is log concentration (mg/L) of BIT	
4.2.5	Other effects	At 12 hours, observations of lethargy, animals caught on the container and swimming on surface were recorded in the treated groups. At 24 hours, lethargy, flared carapace and swimming on surface were recorded in some animals from the treated groups. At 36 hours, lethargy and flared carapace were recorded in some animals from the treated groups. At 48 hours, some animals were observed to have a flared carapace or to be lethargic, swimming on the bottom of the test vessel, swimming on surface and caught on the container.	
4.3	**Results of controls**	No immobility or other behavioural symptoms were observed in the control and vehicle control groups.	
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	0 (control without potassium dichromate), 0.07, 0.12, 0.20, 0.34 and 0.58 mg potassium dichromate /L	
4.4.2	Results	Data are provided in Table 7.4.1.2/1-6	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.4.1.2/1	**ACUTE TOXICITY TO INVERTEBRATES**
Annex Point IIA VII.7.2	*Daphnia magna*

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The toxicity of 1,2-benzisothiazol-3-(2*H*)-one (BIT) to the fresh-water crustacean *Daphnia magna* was determined in a 48-hour semi-static acute toxicity test. The nominal concentrations tested were 0, 0.20, 0.35, 0.63, 1.11 and 1.96 mg BIT/L. The test was conducted according to OECD guideline 202, EU guideline C.2, and US EPA OPPTS 850.1010, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids", with no deviations and is described under point 3.	
5.2	**Results and discussion**	Active substance analysis of the test media showed that the concentrations of BIT remained within the guidelines limits of 80 % of the nominal concentrations throughout the test period (48 h). Since the actual concentrations were within 80% of the nominal concentrations, the test results are expressed as nominal concentrations. Immobilisation data at 24 and 48 hours are given in Table A7.4.1.2/1-6. The per cent immobilisation observed at 24 h was 0, 0, 35, 65, and 85 % at the test concentrations of 0.20, 0.35, 0.63, 1.11 and 1.96 mg BIT/L, respectively. 0, 20, 55, 80, and 100 % immobility was observed in the 0.20, 0.35, 0.63, 1.11 and 1.96 mg/L nominal concentrations, respectively, following 48 hours of exposure. During the test, observations of lethargy, swimming on the bottom of the test vessel, flared carapace, swimming on surface and animals caught on the container were recorded in some animals from the treated groups. No immobility or other behavioural symptoms were observed in the control and vehicle control groups. Results of the validity and reliability of test system was confirmed by conducting a positive control study using potassium dichromate as the reference substance. The test met the validity criteria established in the recommended Guideline. There was no immobility in the control and the dissolved oxygen level was above 60% of the initial oxygen. The average percentage of the substance present during the test was > 80% of initial concentration. The 24 h EC_{50} value was determined to be 0.84 mg/L with fiducial limits 0.62 and 1.14 mg/L. The 48 h EC_{50} value was determined to be 0.61 mg/L with fiducial limits 0.48 and 0.77 mg/L. The maximum concentration of BIT causing no immobilisation and the lowest	

Section A7 Subsection A7.4.1.2/1 Annex Point IIA VII.7.2	Ecotoxicological Profile Including Environmental Fate and Behaviour ACUTE TOXICITY TO INVERTEBRATES *Daphnia magna*	
	concentration tested causing 100 % immobilisation within the 48 h test period were 0.20 and 1.96 mg/L	
5.2.1 EC_0	0.20 mg/L	
5.2.2 EC_{50}	0.61 mg/L (with 95 % fiducial limits 0.48 and 0.77 mg/L)	
5.2.3 EC_{100}	1.96 mg/L	
5.3 Conclusion	Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*April 2015*
Materials and Methods	*Applicant's version is accepted with the following comments:* *3.1.3.: Purity of BIT is reported to be 97.42%. However, the purity of BIT reported in the analysis of test concentration is 71.08%. RMS does not understand why the given value of 71.08% of BIT in water during the exposure period is different of the purity of 97.42% on dry basis because the stock solution was prepared by dissolving the test substance in water.*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.2/1 ACUTE TOXICITY TO INVERTEBRATES

Annex Point IIA VII.7.2 *Daphnia magna*

Results and discussion

Applicant's version is accepted but with the following comments:

4.2.2. The actual concentration measured after 12 h is 5.63 mg BIT/L, which is 79% of the actual concentration. According to guidelines, the results can be based on nominal values if there is evidence that the concentration of the test substance has been satisfactorily maintained within ± 20 per cent of the nominal or measured initial concentration, but OECD guidelines state that the concentration of the substance tested should be measured as a minimum, at the highest and lowest test concentration, at beginning and end of the test and the actual concentration was only measured for 10 mg/L of BIT. The reason for this was that selected test concentrations were not detectable by the analytical method.

Table A7.4.1.2/1-5: Nominal and actual concentrations of test substance

Nominal concentration (mg/L) of 1,2-benzisothiazol-3(2H)-one (BIT)	***Actual concentration (mg/L) of BIT***	***Mean Analytical Concentration mg/L (hours)***		***Mean detected concentration (%)***		***Mean Concentration (mg/L)***	***Percent Nominal Recovered***
		0	***12***	***0***	***12***		
0 (Control)	-	-	-	-	-	-	-
0 (Vehice control)	-	-	-	-	-	-	-
10.00	*7.108*	*5.96*	*5.63*	*83.77*	*79.13*	*5.79*	*81.45%*

5.2.2. The following recalculated result for EC_{50} at 48 h is the one which should taken into account for the risk assessment, because the purity of 71.08% must be considered resulting in the following effect value for pure BIT.

EC_{50} (48 h)= 0.43 mg BIT/L.

Conclusion *Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.*

Reliability *3*

Acceptability *Non-acceptable.*

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.4.1.2/1	**ACUTE TOXICITY TO INVERTEBRATES**
Annex Point IIA VII.7.2	*Daphnia magna*

Remarks	*The purity of BIT reported in the analysis of test concentration is 71.08%. If this is true, then Daphnias were exposed to lower concentrations. However there are several purity values given in the lab report. The RMS does not have sufficient confidence in the purity values.* *The table showing the mean measured concentrations of the active substance showed that concentrations did not remain stable; however this was only shown for the highest concentration in a separate analysis because the selected test concentrations were not detectable by the analytical method. This adds uncertainty to the derived endpoints.* *For the reasons explained above, RMS does not accept this test.*

Table A7.4.1.2/1-1: Dilution water

Criteria	Details
Source	Daphnia culture water
Alkalinity	Not documented
Hardness	210 mg/L ($CaCO_3$)
pH	7.8±0
Ca / Mg ratio	Not documented
Na / K ratio	Not documented
Oxygen content	8.15±0.21
Conductance	Not documented
Holding water different from dilution water	No

Table A7.4.1.2/1-2: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	Aquatic section, Department of Ecotoxicology, Jai Research Foundation, India
Age	≤ 24 hours
Breeding method	Not documented
Kind of food	Unicellular algae, Chlorella vulgaris
Amount of food	Not documented
Feeding frequency	Not documented
Pretreatment	First instar Daphnids were separated from the adults in the culture of Daphnia being acclimatised for the study and were immediately transferred to labelled glass beakers using a micropipette.
Feeding of animals during test	No

Table A7.4.1.2/1-3: Test system

Criteria	Details
Renewal of test solution	Test solutions were changed at each 12 h interval during the exposure period.
Volume of test vessels	600 mL
Volume/animal	100 mL/Daphnid
Number of animals/vessel	5 Daphnids/vessel
Number of vessels/ concentration	4 vessels/concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2/1-4: Test conditions

Criteria	Details
Test temperature	18.4-18.5 ºC
Dissolved oxygen	7.32-7.67 mg/L
pH	7.54-7.63
Adjustment of pH	Not documented
Aeration of dilution water	Not documented
Quality/Intensity of irradiation	Not documented
Photoperiod	16 hours

Table A7.4.1.2/1-5: Nominal and actual concentrations of test substance

Nominal concentration (mg/L) of 1,2-benzisothiazol-3-(2*H*)-one (BIT)	Actual concentration (mg/L) of BIT	Mean Analytical Concentration mg/L (hours)		Mean Concentration (mg/L)	Percent Nominal Recovered
		0	12		
0 (Control)	-	-	-	-	-
0 (Vehice control)	-	-	-	-	-
10.00	7.108	5.96	5.63	5.79	81.53%

Table A7.4.1.2/1-6: Immobilisation data of *Daphnia magna* in a semi-static acute toxicity study with 1,2-benzisothiazol-3-(2*H*)-one

Nominal concentration (mg/L) of 1,2-benzisothiazol-3-(2*H*)-one	Immobilisation		Oxygen [mg/L] 48 h	pH 48 h	Temperature [°C] 48 h
	24 h	48 h			
0	0% (0/20)	0% (0/20)	7.37-7.33	7.55-7.57	18.5
0.0002 mL/mL (vehicle control)	0% (0/20)	0% (0/20)	7.47-7.40	7.55-7.57	18.5
0.20	0% (0/20)	0% (0/20)	7.60-7.55	7.57-7.59	18.5
0.35	0% (0/20)	20% (4/20)	7.57-7.53	7.58-7.60	18.5
0.63	35% (7/20)	55% (11/20)	7.58-7.51	7.60-7.62	18.5
1.11	65% (13/20)	80% (16/20)	7.64-7.59	7.61-7.63	18.5
1.96	85% (17/20)	100% (20/20)	7.65-7.63	7.62-7.63	18.5

Table A7.4.1.2/1-7: Effect data: EC_{50} and NOEC values

	EC50 (mg/L)	95 % fiducial limits	NOEC (mg/L)
24 h	0.84	0.62-1.14	-
48 h	0.61	0.48-0.77	-

Table A7.4.1.2/1-8: Immobilisation data of *Daphnia magna* in a semi-static acute toxicity study with reference substance (Potassium Dichromate)

Nominal concentration (mg/L) of Potasium Dichromate	Immobilisation	
	24 h	48 h
Control	0% (0/20)	0% (0/20)
0.07	0% (0/20)	0% (0/20)
0.12	0% (0/20)	40% (8/20)
0.20	0% (0/20)	65% (13/20)
0.34	35% (7/20)	85% (17/20)
0.58	60% (12/20)	100% (20/20)

Table A7.4.1.2/1-9: Validity criteria for acute daphnia immobilisation test

	fulfilled
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	Not applicable

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Annex Point IIA VII.7.2

Ecotoxicological Profile Including Environmental Fate and Behaviour

ACUTE TOXICITY TO INVERTEBRATES

Daphnia magna

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: September 26, 2001 – December 13, 2001.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access	Dow Benelux BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test method was based on OECD Guideline no. 202 "*Daphnia* sp., Acute Immobilisation Test" and EU Guideline C.2.	
2.2	**GLP**	Yes (certified by the Inspectorate for Health Protection and Veterinary Public Health, Ministry of Health, Welfare and Sport, The Netherlands).	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	BT 12000	
3.1.2	Specification	Please refer to Doc. III-A, 2/2	
3.1.3	Purity	98%	
3.1.4	Composition of Product	Not applicable	

Section A7 Subsection A7.4.1.2/2 Annex Point IIA VII.7.2		Ecotoxicological Profile Including Environmental Fate and Behaviour ACUTE TOXICITY TO INVERTEBRATES *Daphnia magna*	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	HPLC with UV detection.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
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	The dilution water used was DSWL-E prepared from ground water. Details are given in Table A7.4.1.2/2-1.	
3.4.2	Test organisms	*Daphnia magna,* details are given in Table A7.4.1.2/2-2.	
3.4.3	Test system	Details are given in Table A7.4.1.2/2-3.	
3.4.4	Test conditions	Details are given in Table A7.4.1.2/2-4.	**X**
3.4.5	Duration of the test	48 hours	
3.4.6	Test parameter	Immobility and condition (visually observable morphological or behavioural effects)	
3.4.7	Sampling	The temperature in one of the control vessels was measured at the beginning and at the end of the test. The pH and oxygen concentration were measured at the beginning and the end of the test in all the concentration groups. Observations for immobility and condition were made at 24 and 48 hours.	
3.4.8	Monitoring of TS concentration	Yes; Samples from the exposure media of 0.56, 3.2 and 10 mg/L and the control were taken at 0 h (newly prepared media) and 48 h (spent media).	
3.4.9	Statistics	EC_{50} values and their confidence interval were calculated by means of a parametric model.	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.2/2 ACUTE TOXICITY TO INVERTEBRATES

Annex Point IIA VII.7.2

Daphnia magna

	4 RESULTS	
4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
4.1.3	Nature of adverse effects	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L
4.2.2	Actual concentrations of test substance	Please refer to Table A7.4.1.2/2-5. After dosing the measured concentrations were between 103% and 114% of the nominal concentration. At the end of the test the same media were sampled again and appeared to be between 102% and 105% of the nominal concentration. The average percentage present during the test was 105%.
4.2.3	Effect data (Immobilisation)	Data are provided in Table A7.4.1.2/2-6 and Table A7.4.1.2/2-7.
4.2.4	Concentration / response curve	The slope of the concentration-effect curve was 5.7 (95% confidence interval: 4.3-8.6).
4.2.5	Other effects	Some animals at the concentrations of 1.0, 1.8, 3.2, 5.6 and 10 mg/L were mobile according to the definition given in the guidelines but their movements were slower. Some animals at the concentrations of 3.2 and 5.6 mg/L were mobile but they swam with irregular movements.
4.3	**Results of controls**	No adverse effects were observed in the control group
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not applicable

Section A7 **Subsection A7.4.1.2/2** **Annex Point IIA VII.7.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ACUTE TOXICITY TO INVERTEBRATES** *Daphnia magna*	
4.4.2 Results	Not applicable	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The toxicity of 1,2-benzisothiazol-3-(2*H*)-one (BIT) to the fresh-water crustacean *Daphnia magna* was determined in a 48-hour static acute toxicity test. The concentrations tested were 0, 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L. The test was conducted according to OECD guideline 202 and EU guideline C.2 with no deviations and is described under point 3.	
5.2 Results and discussion	After dosing the measured concentrations were between 103% and 114% of the nominal concentrations. At the end of the test the same media were sampled again and appeared to be between 102% and 105% of the nominal concentrations. The average percentage present during the test was 105%. Since the actual concentrations were within 20% of the nominal concentrations, the test results are expressed as nominal concentrations. Immobilisation data at 0, 24 and 48 hours are given in Table A7.4.1.2/2-6. At 5.6 and 10 mg/L all animals were immobile. At 0.56 and 1.8 mg/L there were no adverse effects observed with respect to mobility or condition, respectively. The test met the validity criteria established in the recommended Guideline. There was no immobility in the control and the dissolved oxygen concentration was above 5.4 mg/L. The average percentage of the substance present during the test was > 80% of initial concentration. The 48 h NOEC values were 1.8 and 0.56 mg/L for mobility and condition, respectively. The EC_{50} value at 48 h was 4.0 mg/L with 95% confidence intervals of 3.5-4.6 mg/L. The EC100 value at 48 h was 5.6 mg/L.	
5.2.1 EC_0	1.8 mg/L	
5.2.2 EC_{50}	4.0 mg/L (with a 95% confidence interval of 3.5-4.6 mg/L)	
5.2.3 EC_{100}	5.6 mg/L	
5.3 Conclusion	Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.	
5.3.1 Reliability	1	

Section A7 **Subsection A7.4.1.2/2** **Annex Point IIA VII.7.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ACUTE TOXICITY TO INVERTEBRATES** *Daphnia magna*
5.3.2 Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*February 2010*
Materials and Methods	*Applicant's version is accepted with the following comments:* ▪ *The test was performed using a static system without replacement of test solution.* ▪ *4.4. No test with reference substance was performed. Although not obligatory, it is recommended.*
Results and discussion	*Applicant's version is accepted*
Conclusion	*Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

Table A7.4.1.2/2-1: Dilution water

Criteria	Details
Source	Ground water from a locality near Linschoten (the Netherlands)
Alkalinity	Not documented
Hardness	213 mg/L ($CaCO_3$)
pH	8.0-8.5
Ca / Mg ratio	1 : 0.54
Na / K ratio	1 : 0.15
Oxygen content	Not documented
Conductance	Not documented
Holding water different from dilution water	No

Table A7.4.1.2/2-2: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	Laboratory culture
Age	One day
Breeding method	Every week the cultures are started with 150 Daphnids in 4 L of dilution water. The medium is completely replaced at least once a week. At the same time all young born are removed.
Kind of food	Algal cells (*Chlorella*)
Amount of food	4 x 109 algal cells and 0.13 g of yeast per 4 L.
Feeding frequency	Daily
Pretreatment	No
Feeding of animals during test	No

Table A7.4.1.2/2-3: Test system

Criteria	Details
Renewal of test solution	No
Volume of test vessels	150 mL
Volume/animal	20 mL/Daphnid
Number of animals/vessel	5 Daphnids/vessel
Number of vessels/ concentration	4 vessels/concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2/2-4: Test conditions

Criteria	Details
Test temperature	20.0 - 20.1 ºC
Dissolved oxygen	9.3 - 9.8 mg/L
pH	8.1-8.3
Adjustment of pH	Not documented
Aeration of dilution water	No
Quality/Intensity of irradiation	Not documented
Photoperiod	16 h light/8 h dark

Table A7.4.1.2/2-5: Nominal and actual concentrations of test substance

Nominal concentration (mg/L) of 1,2-benzisothiazol-3-(2*H*)-one (BIT)	Measured concentrations			
	t = 0 h		t = 48 h	
	mg/L	% deviation from nominal	mg/L	% deviation from nominal
0	< 0.1	-	< 0.1	-
0.56	0.64	14.3	0.59	5.4
3.2	3.31	3.4	3.25	1.6

10	10.48	4.8	10.15	1.5

Table A7.4.1.2/2-6: Immobilisation data for *Daphnia magna* in a static acute toxicity study with 1,2-benzisothiazol-3-(2*H*)-one

Nominal concentration (mg/L) of 1,2-benzisothiazol-3-(2*H*)-one (BIT)	Number of immobile Daphnids			Oxygen [mg/L] 48 h	pH 48 h	Temperature [°C] 48 h
	0 h	24 h	48 h			
0	0/20	0/20	0/20	9.4	8.1	20
0.56	0/20	0/20	0/20	9.4	8.1	-
1.0	0/20	0/20	0/20	9.4-9.5	8.1	-
1.8	0/20	0/20	0/20	9.3	8.1	-
3.2	0/20	0/20	2/20	9.3	8.1	-
5.6	0/20	11/20	20/20	9.3	8.1	-
10	0/20	16/20	20/20	9.3	8.1	-

Table A7.4.1.2/2-7: Effect data based on nominal concentrations

	EC_{50}	95 % c.l.	NOEC	EC_{100}
24 h [mg/L]	6.3	5.4-7.2	-	-
48 h [mg/L]	4.0	3.5-4.6	1.8 (mobility) 0.56 (condition)	5.6

Table A7.4.1.2/2-8: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202

	fulfilled
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	Not applicable

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.2/3 ACUTE TOXICITY TO INVERTEBRATES

Annex Point IIA VII.7.2

Daphnia magna

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: July 15, 1997 – October 6, 1997.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access	Dow Benelux BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test method was based on US EPA Pesticide Assessment Guidelines Subdivision E 72-2, which is equivalent to OECD test Guideline 202, "*Daphnia* sp., Acute Immobilisation Test".	
2.2	**GLP**	Yes, with the exception of water and food characterisations, however this in not considered to compromise the scientific validity of the study.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]® (1,2-benzisothiazol-3-(2*H*)-one)	
3.1.1	Lot/Batch number	060793	
3.1.2	Specification	Please refer to Doc. III-A, 2/2	
3.1.3	Purity	99.29%	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	

Section A7 Subsection A7.4.1.2/3 Annex Point IIA VII.7.2		Ecotoxicological Profile Including Environmental Fate and Behaviour ACUTE TOXICITY TO INVERTEBRATES *Daphnia magna*	
3.1.6	Method of analysis	UV absorbance	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
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	Details are given in Table A7.4.1.2/3-1.	**X**
3.4.2	Test organisms	*Daphnia magna,* details are given in Table A7.4.1.2/3-2.	**X**
3.4.3	Test system	Details are given in Table A7.4.1.2/3-3.	**X**
3.4.4	Test conditions	Details are given in Table A7.4.1.2/3-4.	
3.4.5	Duration of the test	48 hours	
3.4.6	Test parameter	Immobility, stress and mortality	
3.4.7	Sampling	Temperature, dissolved oxygen and pH were measured at 0 and 24 h in a surrogate vessel. In addition, hourly measurement of temperature was made in one vessel for the duration of the test. Observations for mortality, immobilisation and stress were made at 24 and 48 hours.	
3.4.8	Monitoring of TS concentration	Yes; Analytical measurements of test substance were made on samples of test solutions collected at time 0 and 48 hours or whenever 100% mortality was observed in any replicate test concentration.	
3.4.9	Statistics	A computer program developed by C.E. Stephan and ASTM was used to compute point and interval (i.e, confidence interval) estimate of EC_{50}.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentration	Not applicable	

Section A7 Subsection A7.4.1.2/3 Annex Point IIA VII.7.2		Ecotoxicological Profile Including Environmental Fate and Behaviour ACUTE TOXICITY TO INVERTEBRATES *Daphnia magna*	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Data are provided in Table A7.4.1.2/3-5	
4.2.2	Actual concentrations of test substance	Data are provided in Table A7.4.1.2/3-5	
4.2.3	Effect data (Immobilisation)	Data are provided in Table A7.4.1.2/3-6 and Table A7.4.1.2/3-7.	
4.2.4	Concentration / response curve	Not documented	
4.2.5	Other effects	During the test, some organisms were observed quiescent in the 1.25 and 2.5 mg/L nominal concentrations.	
4.3	**Results of controls**	No adverse effects were observed in the control group.	
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**	The toxicity of [redacted]® (1,2-benzisothiazol-3-(2*H*)-one) to the fresh-water crustacean *Daphnia magna* was determined in a 48-hour static acute toxicity test. The nominal concentrations tested were 0, 0.31, 0.65, 1.25, 2.5, 5.0 and 10.0 mg/L. The test was conducted according to US EPA Pesticide Assessment Guidelines Subdivision E 72-2, which is equivalent to OECD test Guideline 202, "*Daphnia* sp., Acute Immobilisation Test" with no deviations and is described under point 3.	

Section A7 **Subsection A7.4.1.2/3** **Annex Point IIA VII.7.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ACUTE TOXICITY TO INVERTEBRATES** *Daphnia magna*
5.2 Results and discussion	Mean analytical measurements of test substance concentrations in test solution ranged from 88.8 to 116.1% of the nominal concentrations. Mortality and immobilisation data at 24 and 48 hours are given in Table A7.4.1.2/3-6. During the test some organisms were observed quiescent in the 1.25 and 2.5 mg/L nominal concentrations. 10, 40, 100 and 100% mortality was observed in the 1.25, 2.5, 5.0 and 10.0 mg/L nominal concentrations, respectively, following 48 hours of exposure. The test met the validity criteria established in the recommended Guideline. There was no immobility in the control and the dissolved oxygen level was above 60% of the initial oxygen. The average percentage of the substance present during the test was > 80% of initial concentration. The nominal (analytical) 48 h EC_{50} value was determined to be 2.44 (2.24) mg/L. The No Observed Effect Concentration (NOEC) was determined to be 0.65 (0.59) mg/L.
5.2.4 EC_0	Not determined
5.2.5 EC_{50}	2.44 mg/L with a 95% confidence interval of 2.07-2.95 mg/L (nominal concentration)
5.2.6 EC_{100}	Not determined
5.3 Conclusion	Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.
5.3.3 Reliability	1
5.3.4 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 comments:* *3.3. No positive control with reference substance was performed.* *3.4.3. For this test, two groups of 10 animals each was used. According to OECD Guideline No 202, it is preferable to use four groups of five animals each.*
Results and discussion	*Applicant's version is accepted*

Section A7
Subsection A7.4.1.2/3
Annex Point IIA VII.7.2

Ecotoxicological Profile Including Environmental Fate and Behaviour

ACUTE TOXICITY TO INVERTEBRATES

Daphnia magna

Conclusion	*The 48-hour EC_{50} of 2.24 mg a.s./L based on measured concentrations is taken for the risk assessment.* *Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	*Key Study.*

Table A7.4.1.2/3-1: Dilution water

Criteria	Details
Source	Laboratory fresh water (mixture of deionised water and reagent grade chemicals)
Alkalinity	60 mg/L ($CaCO_3$)
Hardness	88 mg/L ($CaCO_3$)
pH	8.0
Ca / Mg ratio	Not documented
Na / K ratio	Not documented
Oxygen content	Not documented
Conductance	300 µmhos/cm
Holding water different from dilution water	No

Table A7.4.1.2/3-2: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	Laboratory aquaculture (original source was Aquatic Research Organisms, New Hampshire, USA).
Age	< 24 hours
Breeding method	All animals received a regular supply of laboratory fresh water and were maintained at test conditions prior to testing. Water used during the production of neonates was also used as diluent water for the duration of the test.
Kind of food	Freshwater algae, *Selenastrum capricornutum*
Amount of food	Not documented
Feeding frequency	Not documented
Pretreatment	At least 24 hours prior to the start of the test, gravid adult Daphnids were transferred to fresh diluent water in brood vessels. Adult Daphnids were not fed during the production of neonates. First instar neonates produced during the 24-hour period immediately prior to test initiation were used for testing.
Feeding of animals during test	No

Table A7.4.1.2/3-3: Test system

Criteria	Details
Renewal of test solution	The exposure media were not replaced
Volume of test vessels	400 mL
Volume/animal	25 mL/Daphnid
Number of animals/vessel	10 Daphnids/vessel
Number of vessels/ concentration	2 vessels/concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2/3-4: Test conditions

Criteria	Details
Test temperature	20.3- 20.8 ºC
Dissolved oxygen	7.7-8.4 mg/L
pH	7.7-8.0
Adjustment of pH	Not documented
Aeration of dilution water	Not documented
Quality/Intensity of irradiation	540-1080 lux
Photoperiod	16 hours

Table A7.4.1.2/3-5: Nominal and actual concentrations of test substance

Nominal concentration (mg/L) of 1,2-benzisothiazol-3-(2*H*)-one (BIT)	Analytical Concentration mg/L (hours)			Mean Concentration (mg/L)	Percent Nominal Recovered
	0	24	48		
Control	0.00	-	0.00	0.00	-
0.31	0.43	-	0.28	0.36	116.1
0.65	0.65	-	0.53	0.59	90.8
1.25	1.18	-	1.03	1.11	88.8
2.50	2.28	-	2.34	2.31	92.4
5.00	4.51	-	4.80	4.66	93.2
10.00	9.00	9.82	-	9.41	94.1

Table A7.4.1.2/3-6: Percent mortalities/immobilisation data of *Daphnia magna* in a static acute toxicity study with 1,2-benzisothiazol-3-(2*H*)-one

Nominal concentration (mg/L) of 1,2-benzisothiazol-3-(2*H*)-one	Percent mortality/immobilisation		Oxygen [mg/L] 48 h	pH 48 h	Temperature [°C] 48 h
	24 h	48 h			
0	0% (0/20)	0% (0/20)	8.4	7.7	20.7
0.31	0% (0/10)	0% (0/10)	8.4	7.7	20.6
0.65	0% (0/20)	0% (0/20)	8.4	7.7	20.5
1.25	5% (1/20)	10% (2/20)	8.4	7.7	20.5
2.5	30% (6/20)	40% (8/20)	8.4	7.7	20.5
5.0	35% (7/20)	100% (20/20)	8.4	7.7	20.4
10.0	100% (20/20)	100% (20/20)	8.4	7.7	20.3

Table A7.4.1.2/3-7: Effect data: EC_{50} and NOEC values

	EC50 (mg/L) Nominal (Analytical)	95 % confidence limits Nominal (Analytical)	NOEC (mg/l) Nominal (Analytical)
24 h	4.29 (3.98)	3.40-5.54 (3.14-5.17)	1.25 (1.11)
48 h	2.44 (2.24)	2.07-2.95 (1.84-2.73)	0.65 (0.59)

Table A7.4.1.2/3-8: Validity criteria for acute daphnia immobilisation test

	fulfilled
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	Not applicable

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.3/1 (01) GROWTH INHIBITION TEST ON ALGAE

Annex Point IIA VII.7.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]. (2002), Alga (*Selenastrum capricornutum*), growth inhibition test with BIT, JAI Research foundation, Department of Ecotoxicology, Valvada-396 108, Dist. Valsad, Gujarat, India, unpublished report No.: 3801 Dates of experimental work: May 23, 2002 – May 26, 2002.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test was carried out in accordance with OECD Guideline No. 201, "Alga, Growth Inhibition Test"; and E.C. method C.3, "Algal Inhibition Test".	
2.2	**GLP**	Yes, certified by the Inspectorate for Health Protection, Commodities and Veterinary Public Health of the Ministry of Health, Welfare and Sport from The Netherlands	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazoline-3-one (BIT)	
3.1.1	Lot/Batch number	BT17301	
3.1.2	Specification	Please refer to Doc. III-A, 2/1	
3.1.3	Purity	97.42%	**X**
3.1.4	Composition of Product	Not applicable	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.3/1 (01) GROWTH INHIBITION TEST ON ALGAE

Annex Point IIA VII.7.3

		1 REFERENCE	Official use only
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	HPLC with UV detection.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Culture medium	Liquid nutrient medium prepared according to OECD Guideline 201. Details are given in Table A7.4.1.3/1-1.	
3.4.2	Test organisms	*Selenastrum capricornutum*, details are given in Table A7.4.1.3/1-2	
3.4.3	Test system	Details are given in Table A7.4.1.3/1-3	
3.4.4	Test conditions	Details are given in Table A7.4.1.3/1-4	
3.4.5	Duration of the test	72 h	
3.4.6	Test parameter	Growth Inhibition, Reduction in Grow Rate	
3.4.7	Sampling	A volume of 10 mL of the test culture was collected from each replicate flask at 24, 48 and 72 h and the cell concentration of each sample was determined using a haemocytometer and microscope.	
3.4.8	Monitoring of TS concentration	Yes; 50 mL samples from a test substance solution (10 mg of BIT dissolved in 1 mL of acetone, and then made up to 100 mL adding alga culture medium: 100 µg/mL BIT), control (without BIT) and vehicle control were taken at 0 h and 72 h intervals.	
3.4.9	Statistics	The EC_{50} (0-72 h) with its associated 95% fiducial limits of BIT was calculated using the Probit analysis method (Finney, 1991).	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.3/1 (01) GROWTH INHIBITION TEST ON ALGAE

Annex Point IIA VII.7.3

		1 REFERENCE	Official use only
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0.002, 0.004, 0.008, 0.016, 0.032, 0.064 and 0.128 µg BIT/mL.	
4.2.2	Actual concentrations of test substance	The test concentrations selected were not detectable by the analytical method, so a known detectable concentration of 10 µg/mL was prepared to perform the active ingredient content and stability of the test solution. The nominal concentration was therefore 10 µg/mL (purity 71.08 %) and the actual concentration was found to be 7.108 µg/mL. After dosing the measured concentration was 6.604 µg BIT/mL, 93% of the actual concentration. After 72 hours, the measured concentration was 5.989 µg BIT/mL, 84% of the actual concentration.	**X**
4.2.3	Growth curves	The regression equations established were: Growth Inhibition: $Y=8.573+1.862\ \chi$ Reduction in Growth Rate: $Y=6.782+1.241\ \chi$ Please see Figures A7.4.1.3/1-1 and A7.4.1.3/1-2	
4.2.4	Concentration / response curve	A probit regression line was drawn for growth inhibition and growth rates by plotting the probit inhibition values (Y) against the corresponding log concentration (χ). Please see Figures A7.4.1.3/1-3 and A7.4.1.3/1-4	
4.2.5	Cell concentration data	Data are provided in table A7.4.1.3/1-5	
4.2.6	Effect data (cell multiplication inhibition)	Please refer to Table A7.4.1.3/1-6	
4.2.7	Other observed effects	None	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.3/1 (01) GROWTH INHIBITION TEST ON ALGAE

Annex Point IIA VII.7.3

		1 REFERENCE	Official use only
4.3	**Results of controls**	Normal cell growth was observed in the control group over 72 h (22.5 times in control and 22.3 times in vehicle control). For the vehicle control (acetone), E_bC_{50} and E_rC_{50} were 0.0	
4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The growth inhibition of 1,2-benzisothiazol-3-(2*H*)-one (BIT) to the fresh-water alga *Selenastrum capricornutum* was determined in a 72-hour test comparing data obtained from each treatment group with that of the vehicle (acetone) control group. The concentrations tested were 0, 0.002, 0.004, 0.008, 0.016, 0.032, 0.064 and 0.128 µg BIT/mL. The test was conducted according to OECD Guideline 201 and EU Guideline C.3 with no deviations and is described under point 3.	
5.2	**Results and discussion**	Active substance analysis showed that the concentrations of BIT were within the guideline limits (6.604 µg BIT/mL at 0 h and 5.989 µg BIT/mL at 72 h). The pH (7.4-8.0), temperature (22-24ºC) and illumination (7400-7410 lux) recorded during the study period were within the guideline limits. In the treated and control group, cell growth increased with time and reached a peak by 72 h. Normal cell growth was observed in the control group. At 72 h concentrations of 0.002, 0.004, 0.008, 0.016, 0.032, 0.064 and 0.128 µg BIT/mL recorded 4.88, 16.87, 37.15, 62.31, 82.43, 94.42, 99.90 per cent growth inhibition (E_bC) and 1.43, 5.30, 13.36, 26.77, 43.77, 66.07, 73.51 per cent growth rate reduction (E_rC), respectively. The test met the validity criteria established in the recommended Guideline. Cell concentration in control cultures increased at least by a factor of 16 within 3 days. The average percentage of the substance present during the test was > 80% of initial concentration.	
5.2.1	NOE_rC	0.002 mg/L	
5.2.2	E_rC_{50}	0.037 mg/L	
5.2.3	E_bC_{50}	0.012 mg/L	
5.3	**Conclusion**	Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.3/1 (01) GROWTH INHIBITION TEST ON ALGAE

Annex Point IIA VII.7.3

		1 REFERENCE	Official use only
		the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Abril 2015.*
Materials and Methods	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.3/1 (01) GROWTH INHIBITION TEST ON ALGAE

Annex Point IIA VII.7.3

	1 REFERENCE	Official use only
Results and discussion	*The test fulfills the Validity criteria in OECD 201, but not completely:* • *It fulfills exponential growth by more than a factor of 16.* • *Mean coefficient of variation section by section is 0.47 and 0.48 for the control and vehicle control respectively. None of them meet the 35%* criteria. • *Coefficient of variation of average specific growth rates for 72h = 0.021 meeting the 8% criteria.* • *Initial cell density is 12050 cells/ml. Guideline recommends a cell density equal to 10000 cells/ml.* *The biological section of the study can be considered good. Between control and vehicle control, each containing 6 replicates, there are not important differences in cell density values. The study is done under GLP.* *The study does not fulfill the second criterion by a 13%. Nevertheless when the study was done, the second criterion did not apply. Besides, there are other cases where a study not fulfilling the second criterion has been accepted. This is the case of MIT.* *Finally, despite there is no analytical verification at the concentrations tested, the study provides data that shows that concentrations of the test substance are maintained within 20% of nominal concentrations making it possible to calculate endpoints based on nominal concentrations. Proper chemical analysis probably would have lead to even lower test concentrations.*	
Conclusion	*The test is valid, but the effects values were recalculated based on the content of 71.08% purity of BIT and nominal concentrations resulting in an ErC50 = 0.011 mg BIT/l and an ErC10 = 0.0029 mg BIT/l at 48h*	
Reliability	*2*	
Acceptability	*Acceptable*	

Section A7

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Annex Point IIA VII.7.3

Ecotoxicological Profile Including Environmental Fate and Behaviour

GROWTH INHIBITION TEST ON ALGAE

1 REFERENCE — **Official use 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

Growth rate	eCA		
	ErC50	ErC10	NOEC
0-24	0.011 (0.0047-0.017)	0.0029 (0.0022-0.0041)	0.0028
0-48	0.017 (0.015-0.019)	0.0032 (0.0023-0.0040)	0.0028
0-72	0.026 (0.021-0.031)	0.0044 (0.0035-0.0052)	0.0028

Table A7.4.1.3/1-1: Composition of algal nutrient medium

Nutrient	Final concentration in culture medium (mg/L)
NH_4Cl	15.0
$MgCl_2.6H_2O$	12.0
$CaCl_2.2H_2O$	18.0
$MgSO_4.7H_2O$	15.0
KH_2PO_4	1.6
$FeCl_3.6H_2O$	0.08
$Na_2EDTA.2H_2O$	0.1
H_3BO_3	0.185
$MnCl_2.4H_2O$	0.415
$ZnCl_2$,	0.003
$CoCl_2.6H_20$	0.0015
$CuCl_2.2H_2O$	0.00001
$Na_2MoO_4.2H_2O$	0.007
NaHCO3	50.0

Table A7.4.1.3/1-2: Test organisms

Criteria	Details
Species	*Selenastrum capricornutum*
Strain	CCAP 278/4
Source	Culture Collection of Algae and Protozoa, CEH Windermere, UK.
Laboratory culture	Yes
Method of cultivation	Static condition
Pretreatment	Not documented
Initial cell concentration	12050 cells/mL

Table A7.4.1.3/1-3: Test system

Criteria	Details
Volume of culture flasks	250 mL
Culturing apparatus	Haemocytometer
Light quality	Not documented
Procedure for suspending algae	Agitating continuously at 100 rpm using an orbital shaker
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.3/1-4: Test conditions

Criteria	Details
Test temperature	22-24ºC
pH	7.4-8.0
Aeration of dilution water	No
Light intensity	7405 lux
Photoperiod	Not documented

Table A7.4.1.3/1-5: Cell concentration data

Test-Substance Concentration (nominal) [µg/mL]	Cell concentrations (mean values) [cells/mL]							
	measured				Percent of control			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
Control	12050	59167	111250	271250	100	100.00	100.00	100.00
Vehicle control	12050	60000	117083	269167	100	101.41	105.24	99.23
0.002	12050	59167	110000	257500	100	100.00	98.88	94.93
0.004	12050	52500	97500	228333	100	88.73	87.64	84.18
0.008	12050	41667	76667	177500	100	70.42	68.91	65.44
0.016	12050	24167	53333	117500	100	40.85	47.94	43.32
0.032	12050	16667	28333	69167	100	28.17	25.47	25.50
0.064	12050	10000	18333	35000	100	16.90	16.48	12.90
0.128	12050	5833	10833	27500	100	9.86	9.74	10.14
Temperature [°C]	22-24	22-24	23-24	22-24				
pH	7.7-7.8	-	-	7.6-7.7				

Table A7.4.1.3/1-6: Growth inhibition and EC_{50} values with BIT

Nominal Concentration [µg/mL]		Per cent Growth Inhibition (E_bC_{50})	Per cent Reduction in Growth Rate (E_rC_{50})
Vehicle control		0.0	0.0
0.002		4.88	1.43
0.004		16.87	5.30
0.008		37.15	13.36
0.016		62.31	26.77
0.032		82.43	43.77
0.064		94.42	66.07
0.128		99.90	73.51
EC_{50} (µg/mL)		0.012	0.037
95% fiducial limits (µg/mL)	Lower	0.007	0.017
	Upper	0.020	0.081

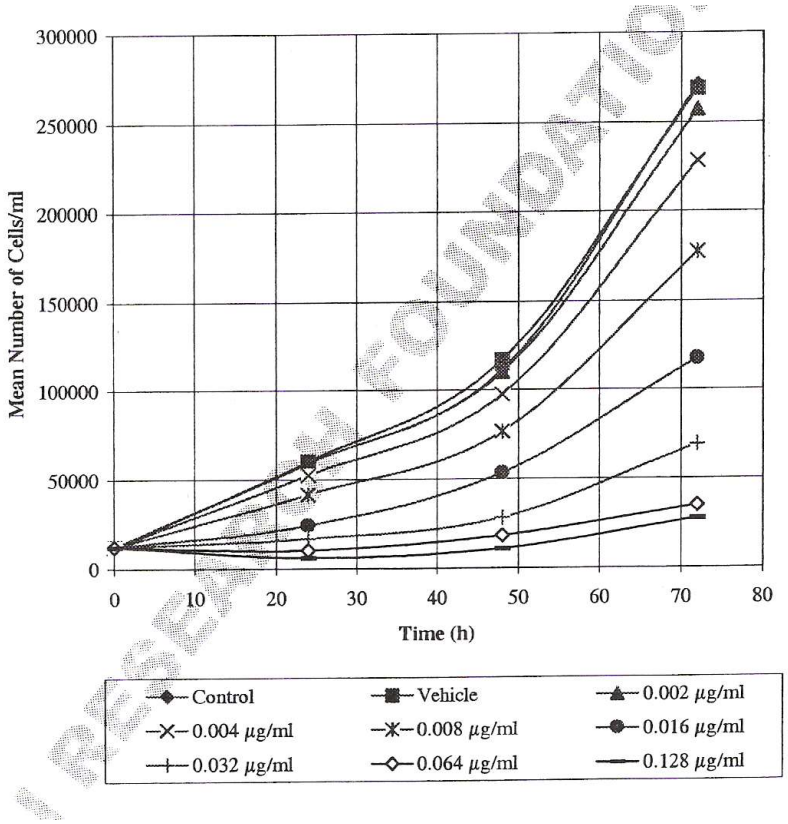

Figure A7.4.1.3/1-1: Growth curves for algal growth inhibition test with BIT

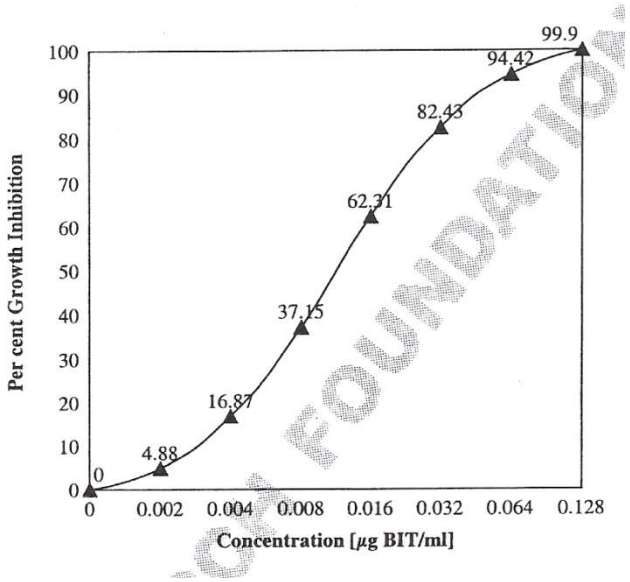

Figure A7.4.1.3/1-2: Growth inhibition curve for *Selenastrum capricornutum* exposed to BIT

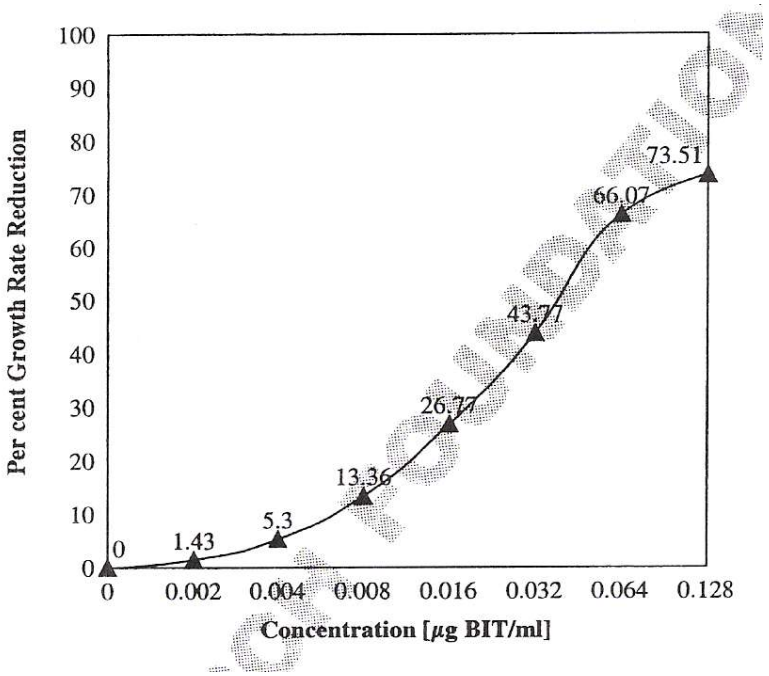

Figure A7.4.1.3/1-3: Growth rate reduction curve for *Selenastrum capricornutum* exposed to BIT

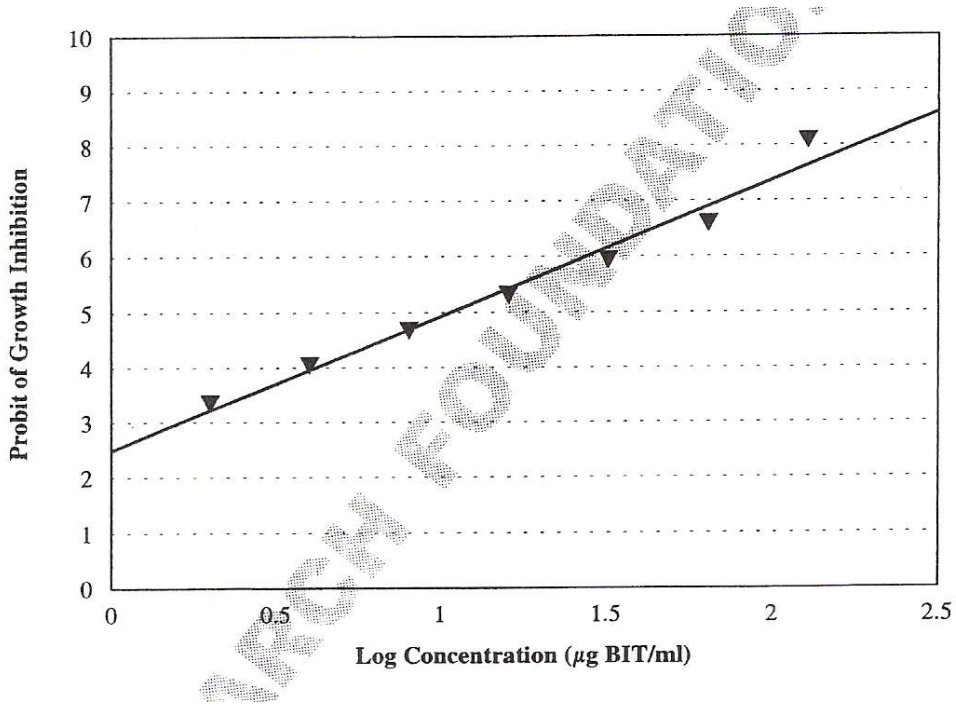

Figure A7.4.1.3/1-4: Probit regression line (growth inhibition) for algal growth inhibition test with BIT

Table A7.4.1.3/1-7: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	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

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Subsection A7.4.1.3/2
Annex Point IIA VII.7.3

Ecotoxicological Profile Including Environmental Fate and Behaviour

GROWTH INHIBITION TEST ON ALGAE

Selenastrum capricornutum

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: November 13, 2001 – November 19, 2001.	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access	Dow Benelux BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test was carried out in accordance with OECD Guideline No. 201, "Alga, Growth Inhibition Test"; and EC method C3, "Algal Inhibition Test".	
2.2	**GLP**	**Yes (certified by the Inspectorate for Health Protection and Veterinary Public Health, Ministry of Health, Welfare and Sport, The Netherlands).**	
2.3	**Deviations**	**No**	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	BT 12000	
3.1.2	Specification	Please refer to Doc. III-A, 2/2	
3.1.3	Purity	98%	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.3/2 GROWTH INHIBITION TEST ON ALGAE

Annex Point IIA VII.7.3

Selenastrum capricornutum

		1 REFERENCE	Official use only
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	HPLC with UV detection.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	$K_2Cr_2O_7$ and/or 3,5-dichlorophenol	
3.3.1	Method of analysis for reference substance	Not documented	
3.4	**Testing procedure**		
3.4.1	Culture medium	The medium was prepared from concentrated stock solutions in ultra pure water. The composition of the nutrient medium is shown in Table A7.4.1.3/2-1. The medium was sterilised by micropore filtration and contained 150 mg/L $NaHCO_3$ (not 50 mg/L as specified in the OECD Guideline, in order to improve the buffer capacity of the medium). The medium contained Fe-citrate, because the growth of the algae would be erratic in the absence of complexed iron.	
3.4.2	Test organisms	*Selenastrum capricornutum*, details are given in Table A7.4.1.3/2-2.	**X**
3.4.3	Test system	Details are given in Table A7.4.1.3/2-3.	
3.4.4	Test conditions	Details are given in Table A7.4.1.3/2-4.	**X**
3.4.5	Duration of the test	72 hours	
3.4.6	Test parameter	Growth inhibition	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.3/2 GROWTH INHIBITION TEST ON ALGAE

Annex Point IIA VII.7.3

Selenastrum capricornutum

		1 REFERENCE	Official use only
3.4.7	Sampling	Algal densities (cells/mL) and algal biovolume (µm³/mL) were determined after 23, 48 and 71 hours. Measured values were corrected for the background values in the appropriate blanks.	
3.4.8	Monitoring of TS concentration	At 0 and 72 hours.	
3.4.9	Statistics	The EC values with respect to the growth rate during exponential growth (E_rC values) were calculated by means of a parametric model assuming a constant error per measurement. This calculation method is based on the assumptions given in the recommended Guideline and has been validated in international ring tests. EC values with respect to the area under the growth curve (E_bC values) were calculated by linear interpolation of a plot of the percentage reduction in growth (I_A) against the log concentration of the test substance. The NOEC was also determined. An effect was considered significant, when the average inhibition of the growth rate was higher than 5%. Statistical significance was determined with a one tailed t-test (a = 5%). In addition model calculations were carried out using the DEBtox software package. Model parameters for population growth and their asymptotic standard deviation and correlation coefficients were estimated. The NEC (no-effect concentration) was calculated from the Profile Ln Likelihood function.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0 (control), 0.03, 0.10, 0.33, 1.0 and 3.3 mg/L	
4.2.2	Actual concentrations of test substance	Chemical analyses were carried out for the concentrations of 0, 0.03, 0.33 and 3.3. Measured concentrations were: At the start of the test: < 0.03, < 0.03, 0.32 and 3.2, respectively. At the end of the test: < 0.03, < 0.03, 0.19 and 3.2, respectively.	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.3/2 GROWTH INHIBITION TEST ON ALGAE

Annex Point IIA VII.7.3

Selenastrum capricornutum

		1 REFERENCE	Official use only
4.2.3	Growth curves	Please refer to Figure A7.4.1.3/2-1.	
4.2.4	Concentration / response curve	Please refer to Figure A7.4.1.3/2-2.	
4.2.5	Cell concentration data	Data are provided in Table A7.4.1.3/2-5.	
4.2.6	Effect data (cell multiplication inhibition)	The 72-hour E_rC_{50} and E_bC_{50} values were estimated to be 1.13 mg/L and 0.25 mg/L, respectively, when corrected for measured test substance concentrations. The NOEC and the NEC were determined to be 0.02 and < 0.02 mg/L, respectively, when corrected for measured test substance concentrations.	
4.2.7	Other observed effects	Microscopic inspection of the morphology of algal cells in the pre-culture at the start of the test revealed normal cells. At the end of the test no abnormal cells were observed in the cultures containing nominal BIT concentrations up to 1.0 mg/L. At the highest test substance concentration no algal cells were observed.	
4.3	**Results of controls**	The mean cell density of the controls at 71 hours was 31.3×10^4 cells/mL and 30.0×10^4 cells/mL.	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not documented	
4.4.2	Results	The results of these reference tests demonstrate a constant quality of the test system during an extended period of at least 15 years. The resulting EC_{50} values are comparable to the mean values found in international ring tests.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The toxicity of 1,2-benzisothiazol-3-(2*H*)-one (BIT) to the fresh water green alga *Selenastrum capricornutum* was determined in a 72-hour growth inhibition test. The concentrations tested were 0, 0.03, 0.10, 0.33, 1.0 and 3.3 mg/L. The test was conducted according to OECD guideline 201 and EU guideline C3 with no deviations and is described under point 3.	
5.2	**Results and discussion**	The measured concentration of the lowest nominal test concentration (0.03 mg/L) was not valid because it was near the detection limit. The	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.3/2 GROWTH INHIBITION TEST ON ALGAE

Annex Point IIA VII.7.3

Selenastrum capricornutum

		1 REFERENCE	Official use only
		measured concentration of the test concentration 0.33 mg/L was measured to be 97% of the nominal concentration at the start of the test and 58% of the nominal concentration at the end of the study. The measured concentration of 3.3 mg/l appeared to be 97% of the nominal concentration. Mean cell densities at 0, 23, 48 and 71 hours are given in Table A7.4.1.3/2-5. The individual growth rates and percent values for the levels of inhibition are given in Table A7.4.1.3/2-6. The test met the validity criteria established in the recommended Guideline. The control growth rate (0.070 h^{-1}) was higher than the minimum cell multiplication factor of 16 during a three day test given in the Guideline (0.038 h^{-1}) and the concentrations of the test substance were > 80% of the initial concentrations throughout the duration of the study. The statistical endpoints included in the model calculations demonstrated a significant effect on the growth rate. The difference between the E_rC_{50} and the E_bC_{50} values, therefore, has no toxicological relevance. The E_rC_{50} value of 1.13 mg/L (measured) is considered to provide the best expression of the toxic effect of BIT on algal growth.	
5.2.4	NOE_rC	0.02 mg/L	
5.2.5	E_rC_{50}	1.13 mg/L (with a 95% confidence interval of 0.78-1.73 mg/L)	
5.2.6	E_bC_{50}	0.25 mg/L (with a 95% confidence interval of 0.25-0.85 mg/L)	
5.3	**Conclusion**	Based upon the criteria established in Council Directive 67/548/EEC and all relevant aquatic endpoints, BIT is classified as dangerous to the environment and very toxic to aquatic organisms and should therefore be assigned the symbol N and the R phrase R50.	
5.3.3	Reliability	1	
5.3.4	Deficiencies	**No**	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.3/2

Annex Point IIA VII.7.3

GROWTH INHIBITION TEST ON ALGAE

Selenastrum capricornutum

	1 REFERENCE	**Official use only**
Date	*March 2015.*	
Materials and Methods	*Applicant's version is accepted*	
Results and discussion	*This test fulfills the validity criteria in OECD 201:* • *It fulfills exponential growth by more than a factor of 16.* • *Mean coefficient of variation section by section at 72h = 0.12. It meets the 35% criteria.* • *Coefficient of variation of average specific growth rates for 72h = 0.016 meeting the 7% criteria.* *Initial cell density is 3000 cells/ml. Guideline recommends a cell density range of 5000- 10000 cells/ml. The value is a 40% lower than the lowest range.*	
Conclusion	*The study fulfils all criteria but has a lower cell density than the recommended. It has been decided to consider the study valid for the risk assessment.* *Since there is not measured data for all concentrations RMS recalculated endpoints using nominal concentrations. The lowest endpoint is at 24. ErC50 = 0.48 mg BIT/l and an ErC10 = 0.16 mg BIT/l.*	
Reliability	*2*	
Acceptability	*Acceptable*	
Remarks	***<u>Calculation of endpoints:</u>*** *Initially the Applicant calculated the endpoints based on nominal concentrations because measured concentrations at 72 h were below the limit of quantification (LOQ).* *However after working group discussions in November 2015 and an adhoc working group in 2016 endpoints were recalculated taking into account the mode of action of isotiazolinones. Its biocidal effect is described as a two-step process involving rapid inhibition of growth and metabolism leading to a loss in viability of the cells. These effects occur within minutes at the enzymatic level and can result in loss of viability within hours of exposure.* *This rapid mode of action of the isothiazolinone is apparent in certain algal studies. The growth curves indicate strong effect within the first 24h of exposure and a recovery of growth which is dependent on dosing concentrations. Based on this observation and information on the mode of action of the biocide, it has been suggested to estimate effects on algae after 24 hours of exposure based on initial measured concentrations in place of the geometric mean measured concentration*	

over the test duration (0-72 hours). This approach was accepted in the review of other isothiazolinones in the BPD/BPR review for DCOIT and MIT. If the most sensitive time period is not 24 hours, the kinetics of BIT have to be considered.

This approach was used bu eCA to recalculate endpoints. The Statistical Package R has been used to asses which model (i.e.: log-logistic, weibul, likewise) best fits to the data. eCA has estimated the endpoints fitting the curve taking into account negative growths and later calculating the 50% of the upper asymptote of the curve. NOEC values are estimated using Dunnett's test. In this case, endpoints have been calculated using nominal concentrations since there is not data for all initial measured concentrations. The lowest endpoint is at 24. The LOQ of the analytical method was 0.01 mg BIT/L.

For each of the studies it has been assessed when the strongest effect occurs and the endpoint has been estimated accordingly.

It has been also assessed that none of the assumptions of non-linear regression have been violated (correct regression model, variance homogeneity and normal distributions of measurements errors)

At the same time and as part of the adhoc, the different applicants re-evaluated their studies according to this uniform approach. 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 using SAS version 9.2 (SAS Institute, Cary, North Carolina). Since the variability of the growth of the controls alga at 24 hours leads to uncertainty in both the NOEC and the ErC10 derivation, the ErC10 is suggested as the chronic endpoint, as its determination is independent from the study design and is consistently derived for all the algal studies.

Concentrations

Nominal [mg/L BIT]	Actual [mg/L BIT] 0h	Actual [mg/L BIT]72h
0.00	**0**	**0**
0.03	**0.015**	**0.015**
0.10	**-**	**-**
0.33	**0.32**	**0.19**
1.00	**-**	**-**
3.30	**3.2**	**3.2**

Endpoints

The results from the above explained analysis are.

Growth rate	Applicant		eCA with initial measured concentrations		
	ErC50 (95%)	ErC10 (95%)	ErC50	ErC10	NOEC
0-24	0.447 (0.309 -0.639)	0.22 (0.164 - 0.295)	0.48 (0.39 – 0.57)	0.16 (0.088 – 0.24)	0,1

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Subsection A7.4.1.3/2

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GROWTH INHIBITION TEST ON ALGAE

Selenastrum capricornutum

1 REFERENCE | **Official use only**

0-48	0.543 (0.44 - 0.677)	0.306 (0.251 -0.372)	0.64 (0.59 – 0.68)	0.3 (0.25 – 0.35)	0,03
0-72	0.607 (0.492 -0.753)	0.28 (0.231 - 0.338)	0.67 (0.61 – 0.74)	0.25 (0.2 – 0.3)	0,0,3

The endpoints at 24h calculated by eCA are used for the risk assessment.

Table A7.4.1.3/2-1: Composition of algal nutrient medium

Nutrient	Final concentration in culture medium (mg/L)
NH_4Cl	15
$MgCl_2.6H_2O$	12
$CaCl_2.2H_2O$	18
$MgSO_4.7H_2O$	15
KH_2PO_4	1.6
Fe-citrate.$3H_2O$	0.08
$Na_2EDTA.2H_2O$	0.1
H_3BO_3	0.185
$MnCl_2.4H_2O$	0.415
$ZnSO_4.7H_2O$	0.0063
$CoCl_2.6H_20$	0.0015
$CuSO_4.5H_2O$	0.000015
$Na_2MoO_4.2H_2O$	0.007
$NaHCO_3$	150

Table A7.4.1.3/2-2: Test organisms

Criteria	Details
Species	*Selenastrum capricornutum*
Strain	CCAP 278/4
Source	CCAP, The Freshwater Biological Association, UK
Laboratory culture	Yes
Method of cultivation	A pre-culture growing in exponential phase was prepared according to the recommendations of OECD Guideline 201, under the following conditions: Temperature: 23 ± 2°C Illumination: 60 – 120 µmol.s^{-1}.m^{-2} Shaking: 100 rpm
Pretreatment	Not documented
Initial cell concentration	A suspension containing 3 x 10^5 cells/mL was prepared by dilution of a pre-culture containing 2.9 x 10^6 cells/mL

Table A7.4.1.3/2-3: Test system

Criteria	Details
Volume of culture flasks	200 mL
Culturing apparatus	Coulter electronic particle counter
Light quality	Fluorescent lamps
Procedure for suspending algae	Agitation at 100 rpm on an orbital shaker
Number of vessels/ concentration	3 replicates per test concentration, 6 control flasks containing algae only and a single background concentration series containing test substance without algae
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.3/2-4: Test conditions

Criteria	Details
Test temperature	21.1 - 23.6 °C (mean 22.7 °C)
pH	0 hours: 8.0 72 hours: 8.1 – 8.2
Aeration of dilution water	Not documented
Light intensity	65 – 74 µmol.s^{-1}.m^{-2}
Photoperiod	Continuous illumination

Table A7.4.1.3/2-5: Cell concentration data of Selenastrum capricornutum at different concentrations of 1,2-benzisothiazol-3-(2*H*)-one (BIT)

Nominal concentration (mg/L) of 1,2-benzisothiazol-3-(2*H*)-one (BIT)	Mean cell density measurements (cells/mL x 10^4) corrected for background			
	0 hours	23 hours	48 hours	71 hours
0	0.3	1.2	6.1	31.3
0	0.3	1.1	6.0	30.0
0.03	0.3	1.1	5.5	25.1
0.10	0.3	1.0	5.0	21.4
0.33	0.3	0.7	4.0	13.9
1.0	0.3	0.3	0.4	0.8
3.3	0.3	0.3	0.2	0.2

Table A7.4.1.3/2-6: Individual growth rates and % inhibition of *Selenastrum capricornutum* at different concentrations of 1,2-benzisothiazol-3-(2*H*)-one (BIT)

Nominal concentration of 1,2-benzisothiazol-3-(2*H*)-one (BIT) (mg/L)	Growth rate	% Inhibition
0	1.61	-3.0
0	1.56	0.2
0	1.54	1.5
0	1.57	-0.5
0	1.56	0.2
0	1.54	1.5
0.03	1.51	3.6
0.03	1.48	5.1
0.03	1.49	4.3
0.1	1.45	7.5
0.1	1.44	7.6
0.1	1.44	8.0
0.33	1.28	17.9
0.33	1.32	15.7
0.33	1.28	17.9
1.0	0.43	72.4
1.0	0.30	80.8
1.0	0.28	82.0
3.3	0.17	89.3
3.3	-0.10	106.2
3.3	-0.06	104.0

Table A7.4.1.3/2-7: Measured EC_{50} values by biomass integral (EbC50 value) and growth rate (E_rC_{50} value) for 1,2-benzisothiazol-3-(2*H*)-one (BIT)

Period of exposure (hours)	E_bC_{50} value (mg/L)	E_rC_{50} value (mg/L)
0 to 72	0.25	1.13

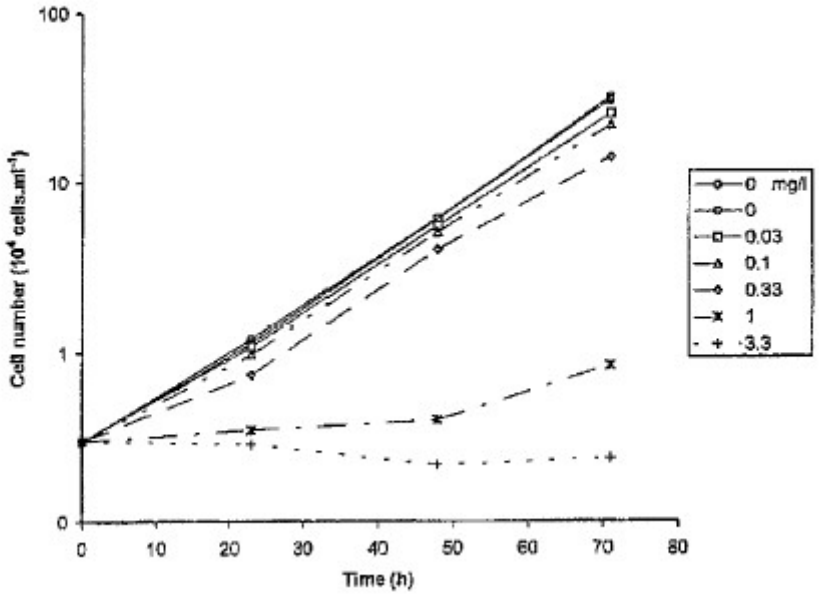

Figure A7.4.1.3/2-1: Growth curves of Selenastrum capricornutum at different concentrations of 1,2-benzisothiazol-3-(2*H*)-one (BIT)

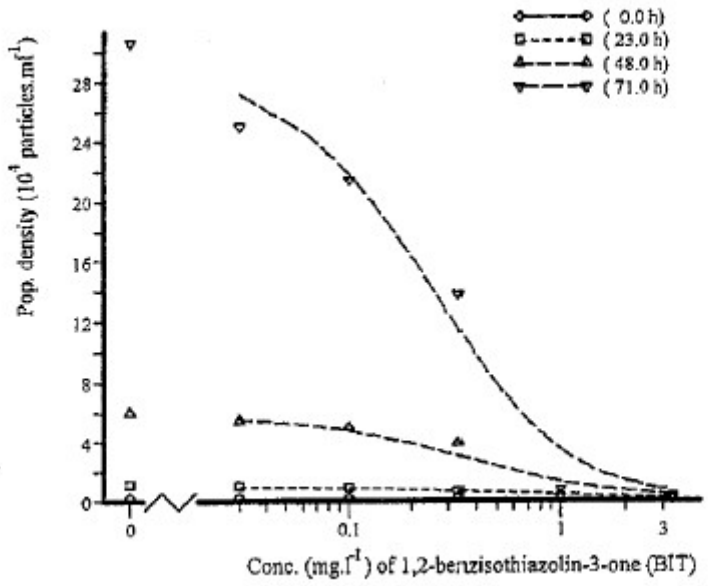

Figure A7.4.1.3/2-2: 1,2-benzisothiazol-3-(2*H*)-one (BIT) concentration-effect curves for Selenastrum capricornutum after different incubation periods

Table A7.4.1.3/2-7: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	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	Not applicable

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.4/1 INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Annex Point IIA VII.7.4

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Date of experimental work: October 12, 2002.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Benelux BV	
1.2.2	Companies with letter of access	Troy Chemical Company BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test was carried out in accordance with OECD Guideline No. 209, "Activated Sludge, Respiration Inhibition Test"; and E.C. method, "C.11, Activated Sludge, Respiration Inhibition Test".	
2.2	**GLP**	Yes, certified by the Inspectorate for Health Protection, Commodities and Veterinary Public Health of the Ministry of Health, Welfare and Sport from The Netherlands	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazoline-3-one (BIT)	
3.1.1	Lot/Batch number	BT 17301	
3.1.2	Specification	Please refer to Doc. III-A, 2/1	
3.1.3	Purity	97.42%	
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 A7.4.1.4/1 INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Annex Point IIA VII.7.4

		1 REFERENCE	Official use only
3.1.6	Method of analysis	Not documented	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	Yes; 3,5-dichlorophenol	
3.3.1	Method of analysis for reference substance	Not documented	
3.4	**Testing procedure**		
3.4.1	Culture medium	Synthetic sewage feed, prepared according to OECD guideline 209. Details are given in table A7.4.1.4/1-1	
3.4.2	Inoculum / test organism	Activated sludge from domestic sewage treatment plant. Details are given in Table A7.4.1.4/1-2	
3.4.3	Test system	Details are given in Table A7.4.1.4/1-3	
3.4.4	Test conditions	Details are given in table A7.4.1.4/1-4	**X**
3.4.5	Duration of the test	3 hours	
3.4.6	Test parameter	Respiration inhibition	
3.4.7	Analytical parameter	Dissolved oxygen measurement	
3.4.8	Sampling	One minute intervals over a period of ten minutes.	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Three controls: two test solutions with inoculum but without BIT, another with BIT (32 mg/L) and without inoculum (as abiotic control)	
3.4.11	Statistics	The EC_{50} of both the reference substance and the test substance were calculated using percent respiration inhibition data at different test concentrations following Probit analysis method (Finney, 1971).	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.4/1 INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Annex Point IIA VII.7.4

			Official use only
		1 REFERENCE	
		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**		
4.2.1	Initial concentration of test substance	2.0, 4.0, 8.0, 16.0 and 32 mg BIT/L	
4.2.2	Actual concentrations of test substance	Not documented	
4.2.3	Growth curves	Not documented	
4.2.4	Cell concentration data	Not documented	
4.2.5	Concentration/ response curve	Please refer to Figure A7.4.1.4/1-1 The regression equations established were: Test solution treated with 3,5-Dichlorophenol: $Y=2.334+2.55\ \chi$ Test solution treated with BIT: $Y=4.038+1.613\ \chi$ where Y is % inhibition and χ is log concentration (mg/L)	
4.2.6	Effect data	Data are presented in Table A7.4.1.4/1-5. The EC_{50} was determined as 3.946 mg/L with the 95% fiducial limits 3.202 to 4.863. The mean respiration rate observed at the concentrations 2.0, 4.0, 8.0, 16.0 and 32.0 mg BIT/L were 12.75, 7.93, 4.10, 2.12, and 1.51 mg O_2/L/h, respectively.	
4.2.7	Other observed effects	No	
4.3	**Results of controls**	The mean respiration rate observed in controls with inoculum but without BIT were 16.81 and 15.84 mg O_2/L/h. The mean respiration rate in the abiotic control was 0.24 mg O_2/L/h.	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.4/1 INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Annex Point IIA VII.7.4

		1 REFERENCE	Official use only
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	5.0, 9.0, 16.2 and 29.16 mg/L of 3,5-dichlorophenol	
4.4.2	Results	The EC_{50} was determined as 11.032 mg/L with the 95% fiducial limits 9.800 to 12.419	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The respiration inhibition of 1,2-benzisothiazol-3-(2*H*)-one (BIT) on the activated sludge from a domestic sewage treatment plant was determined measuring the dissolved oxygen after 3 hours of exposure. The nominal concentrations tested were 2.0, 4.0, 8.0, 16.0 and 32.0 mg BIT/L. The test was conducted according to OECD guideline 209 and EU guideline C.11 with no deviations and is described under point 3.	
5.2	**Results and discussion**	The mean respiration rate observed at the concentrations 2.0, 4.0, 8.0, 16.0 and 32.0 mg BIT/L were 12.75, 7.93, 4.10, 2.12, and 1.51 mg O_2/L/h, respectively. The per cent inhibition of respiration calculated at the concentrations of 2.0, 4.0, 8.0, 16.0 and 32.0 mg BIT/L were 21.90, 51.42, 74.89, 87.01 and 90.75 %, respectively. The mean respiration rate observed in controls with inoculum but without BIT were 16.81 and 15.84 mg O_2/L/h, respectively. The mean respiration rate in the abiotic control was 0.24 mg O_2/L/h. The EC_{50} value for BIT was determined to be 3.946 mg/L with fiducial limits 3.202 and 4.863 mg/L. Results of the validity and reliability of test system were confirmed by conducting an assessment using 3,5-dichlorophenol as the reference substance. The EC_{50} value of 3,5-dichlorophenol was determined to be 11.032 mg/L, which was within the acceptable range (5 to 30 mg/L) as per the guidelines.	
5.2.1	EC_{20}	Not documented	
5.2.2	EC_{50}	3.946 mg/L	
5.2.3	EC_{80}	Not documented	
5.3	**Conclusion**	The test met the validity criteria established in the recommended Guideline. The respiration rates of the two controls are within 15%	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.4/1 INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

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		1 REFERENCE	Official use only
		of each other, and the EC_{50} of the reference substance is in the accepted range. The respiration inhibition and EC_{50} data obtained reveal that the compound is relatively more toxic to heterogeneous bacterial populations present in the collected activated sludge than the reference substance.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*April 2010*
Materials and Methods	*Applicant's version is accepted with the following comments:* *3.4.2. The initial cell concentration in this test (3 g suspended solid per liter) is lower than recommended in OECD guidelines (4 g suspended solid per liter).*
Results and discussion	*Applicant's version is accepted.*
Conclusion	*The test met the validity criteria established in the recommended Guideline. The respiration rates of the two controls are within 15% of each other, and the EC_{50} of the reference substance is in the accepted range.* *The respiration inhibition and EC_{50} data obtained reveal that the compound is relatively more toxic to heterogeneous bacterial populations present in the collected activated sludge than the reference substance.* *Additionally eCA calculated EC10 using linear regression and the Michaelis Menten model which results in a EC10 = 0.55 mg a.s. /l BIT.*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	*Key Study.*

Table A7.4.1.4/1-1: Composition of synthetic sewage feed

Nutrient	Amounts to be dissolved in 1000 mL of distilled water (g)
Peptone	16.0
Meat extract	11.0
Urea	3.0
NaCl	0.7
$CaCl_2.2H_2O$	0.4
$MgSO_4.7H_2O$	0.2
K_2HPO_4	2.8

Table A7.4.1.4/1-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Not applicable
Strain	Not applicable
Source	Sewage treatment plant treating domestic sewage
Sampling site	Rashtriya Chemicals & Fertilizers Ltd., India
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Washed three times with tap water and centrifuged at 2000 rpm for 10 minutes
Pretreatment	Drying
Initial cell concentration	3 g suspended solid per liter

Table A7.4.1.4/1-3: Test system

Criteria	Details
Culturing apparatus	Glass beakers as test vessels and BOD bottles for measuring the respiration rate
Number of culture flasks/concentration	1 culture flask/concentration
Aeration device	Not documented
Measuring equipment	pH meter (range 7.62 - 7.83), dissolved oxygen meter
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.4/1-4: Test conditions

Criteria	Details
Test temperature	20 ± 2 º C
pH	7.62-7.83
Aeration of dilution water	Yes

Suspended solids concentration	3 g suspended solid/L

Table A7.4.1.4/1-5: Results of the activated sludge respiration inhibition test with 1,2-benzisothiazol-3-(2*H*)-one and the reference substance 3,5-dichlorophenol

Nominal concentration of test substance (mg/L)	pH	Respiration rate (mg O_2/L/h)	% Inhibition
1,2-benzisothiazol-3-(2*H*)-one			
0 (Control 1)	7.62	16.81	-
0 (Control 2)	7.65	15.84	-
2.0	7.76	12.75	21.90
4.0	7.80	7.93	51.42
8.0	7.83	4.10	74.89
16.0	7.80	2.12	87.01
32.0	7.77	1.51	90.75
3,5-dichlorophenol			
5.0	7.65	13.70	16.08
9.0	7.70	9.36	42.66
16.2	7.71	4.48	72.56
29.6	7.83	2.53	84.50
0 (Abiotic control)	7.73	0.24	-

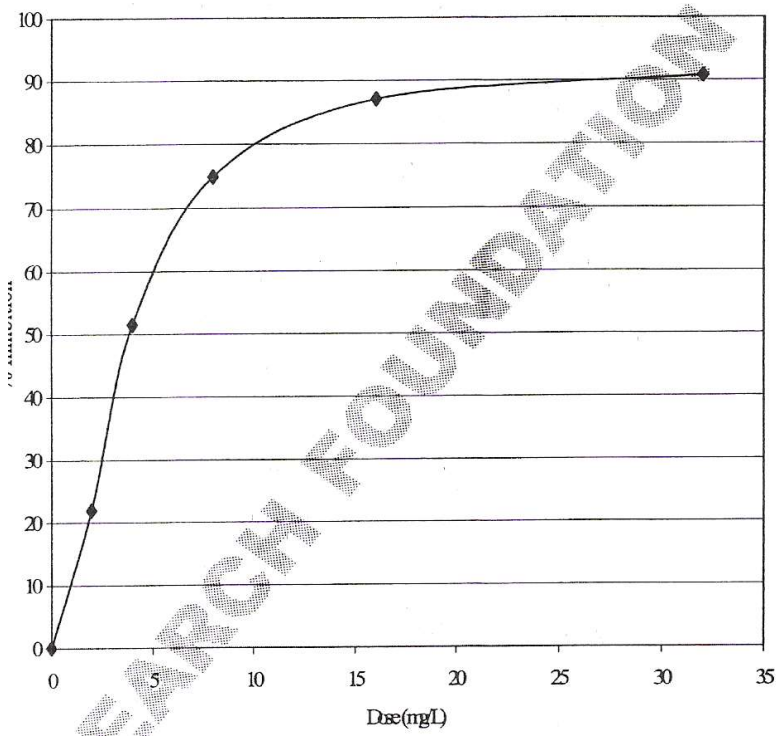

Figure A7.4.1.4/1-1: 1,2-benzisothiazol-3-(2*H*)-one concentration-effect curve for activated sludge

Table A7.4.1.4/1-6: Validity criteria for activated sludge respiration inhibition test according to OECD Guideline 209

	fulfilled
Respiration rates of the two controls are within 15% of each other	Yes
The EC_{50} of the reference substance is in the accepted range (5 to 30 mg/L for 3,5-dichlorophenol)	Yes

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Ecotoxicological Profile Including Environmental Fate and Behaviour

INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Activated sludge

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: September 19, 2001	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access	Dow Benelux BV	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test was carried out in accordance with OECD Guideline No. 209, "Activated Sludge, Respiration Inhibition Test"	
2.2	**GLP**	Yes (certified by the Inspectorate for Health Protection and Veterinary Public Health, Ministry of Health, Welfare and Sport, The Netherlands).	
2.3	**Deviations**	Yes, the study deviated from the prescribed guideline in the following way: The variation in the controls of the test was not within the limits of the guideline. However, this variation did not occur in four other tests carried out in parallel using the same sludge. It was therefore considered unlikely that the activated sludge was not suitable and the values of one of the parallel tests were used for the EC_{50} calculation. However, this deviation is not considered to effect the scientific validity of the study.	**X**
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-benzisothiazolin-3-one (BIT)	
3.1.1	Lot/Batch number	BT 12000	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.4/2 INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Annex Point IIA VII.7.4

Activated sludge

		1 REFERENCE	Official use only
3.1.2	Specification	Please refer to Doc. III-A, 2/2	
3.1.3	Purity	98%	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	Not documented	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	Yes, 3,5-dichlorophenol	
3.3.2	Method of analysis for reference substance	Not documented	
3.4	**Testing procedure**		
3.4.1	Culture medium	Synthetic sewage feed, prepared according to OECD Guideline 209. Details are given in Table A7.4.1.4/2-1.	
3.4.2	Inoculum / test organism	Details are given in Table A7.4.1.4/2-2.	
3.4.3	Test system	Details are given in Table A7.4.1.4/2-3.	
3.4.4	Test conditions	Details are given in Table A7.4.1.4/2-4.	
3.4.5	Duration of the test	3 hours	
3.4.6	Test parameter	Respiration inhibition	
3.4.7	Analytical parameter	Oxygen concentration	
3.4.8	Sampling	The decrease in the oxygen concentration in the mixtures was measured every 1.5 minute during a period of about 10-11 minutes.	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.4/2 INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Annex Point IIA VII.7.4

Activated sludge

		1 REFERENCE	Official use only
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Control mixtures of 16 mL synthetic sewage feed and 284 mL dilution water were prepared in 1-litre glass beakers.	
3.4.11	Statistics	The respiration rate (in mg O_2 per g dry weight active sludge per hour) was calculated by linear regression of the oxygen concentration readings in the linear part of the oxygen depletion curve.	
		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**		
4.2.1	Initial concentration of test substance	1.0, 3.2, 10.1, 32 and 100 mg/L	
4.2.2	Actual concentrations of test substance	Not documented	
4.2.3	Growth curves	Not documented	
4.2.4	Cell concentration data	Dry weight was found at a mixed liquor suspended solids level of 4.2 g/L.	
4.2.5	Concentration/ response curve	Please refer to Figure A7.4.1.4/2-1.	
4.2.6	Effect data	Data are presented in Table A7.4.1.4/2-5. The EC_{50} value was calculated to be 10.6 mg/L with a 95% confidence interval of 7.3-15.4 mg/L. The EC_{20} value was calculated to be 5.3 mg/L with a 95% confidence interval of 4.2-6.8 mg/L. The EC_{80} value was calculated to be 21.1 mg/L with a 95% confidence interval of 14.7-30.2 mg/L.	

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Subsection A7.4.1.4/2 INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Annex Point IIA VII.7.4

Activated sludge

		1 REFERENCE	Official use only
4.2.7	Other observed effects	No	
4.3	**Results of controls**	The variation in the controls of the test was not within the limits of the guideline. However, this variation did not occur in four other tests carried out in parallel using the same sludge. It was therefore considered unlikely that the activated sludge was not suitable and the values of one of the parallel tests were used for the EC_{50} calculation. The respiration rates of two controls of the parallel test were found to be 14.3 and 16.3 mg O_2/ g.h, respectively. The difference between the two values amounted to 13.4% being within the validity criterion (15%) established in the guideline.	
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	5, 12 and 30 mg/L of 3,5-dichlorophenol	
4.4.2	Results	EC_{50} value = 7.8 mg/L	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The effect of 1,2-benzisothiazol-3-(2*H*)-one (BIT) on the respiration rate of a sample of activated sludge during an incubation period of three hours, was determined in a respiration inhibition test. The concentrations tested were 0, 1.0, 3.2, 10.1, 32 and 100 mg/L. The test was conducted according to OECD guideline 209 with no deviations and is described under point 3.	
5.2	**Results and discussion**	The variation in the controls of the test was not within the limits of the guideline. However, this variation did not occur in four other tests carried out in parallel using the same sludge. It was therefore considered unlikely that the activated sludge was not suitable and the values of one of the parallel tests were used for the EC_{50} calculation. The respiration rates of two controls of the parallel test were found to be 14.3 and 16.3 mg O_2/g/h, respectively. The difference between the two values amounted to 13.4% being within the validity criterion (15%) established in the guideline. Results of the validity and reliability of test system were confirmed by conducting an assessment using 3,5-dichlorophenol as the	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.1.4/2 INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Annex Point IIA VII.7.4

Activated sludge

		1 REFERENCE	Official use only
		reference substance. The EC_{50} of 3,5-dichlorophenol was 7.8 mg/L which also is in the accepted range (5-30 mg/L). Results of the activated sludge respiration inhibition test with 1,2-benzisothiazol-3-(2*H*)-one and the reference substance are given in Table A7.4.1.4/2-5.	
5.2.1	EC_{20}	5.3 mg/L (with a 95% confidence interval of 4.2-6.8 mg/L)	
5.2.2	EC_{50}	10.6 mg/L (with a 95% confidence interval of 7.3-15.4 mg/L)	
5.2.3	EC_{80}	21.1 mg/L (with a 95% confidence interval of 14.7-30.2 mg/L)	
5.3	**Conclusion**	The EC_{50} value was calculated to be 10.6 mg/L with a 95% confidence interval of 7.3-15.4 mg/L. The EC_{20} value was calculated to be 5.3 mg/L with a 95% confidence interval of 4.2-6.8 mg/L. The EC_{80} value was calculated to be 21.1 mg/L with a 95% confidence interval of 14.7-30.2 mg/L.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	Yes The variation in the controls of the test was not within the limits of the guideline. However, this variation did not occur in four other tests carried out in parallel using the same sludge. It was therefore considered unlikely that the activated sludge was not suitable and the values of one of the parallel tests were used for the EC_{50} calculation.	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2010

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Subsection A7.4.1.4/2
Annex Point IIA VII.7.4

Ecotoxicological Profile Including Environmental Fate and Behaviour

INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)

Activated sludge

	1 REFERENCE	**Official use only**
Materials and Methods	Applicant's version is accepted with the following comments: 2.3. Deviations. The variation in the controls of the test was not within the limits of the guideline. However, this variation did not occur in four other tests carried out in parallel using the same sludge. It was therefore considered unlikely that the activated sludge was not suitable and the values of one of the parallel tests were used for the EC_{50} calculation.	
Results and discussion	Applicant's version is accepted, despite the variation in the controls of the test.	
Conclusion	The EC_{50} value was calculated to be 10.6 mg/L with a 95% confidence interval of 7.3-15.4 mg/L. The EC_{20} value was calculated to be 5.3 mg/L with a 95% confidence interval of 4.2-6.8 mg/L. The EC_{80} value was calculated to be 21.1 mg/L with a 95% confidence interval of 14.7-30.2 mg/L. Additionally eCA calculated EC10 using linear regression and the Michaelis Menten model which results in a EC10 = 2.45 mg a.s. /l BIT.	
Reliability	2	
Acceptability	Acceptable	
Remarks		

Table A7.4.1.4/2-1: Composition of synthetic sewage feed

Nutrient	Amounts to be dissolved in two litres of ultrapure water (g)
Peptone	32
Meat extract	22
Urea	6
NaCl	1.4
$CaCl_2.2H_2O$	0.8
$MgSO_4.7H_2O$	0.4
K_2HPO_4	5.6

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

Criteria	Details
Nature	Activated sludge
Species	Not applicable
Strain	Not applicable
Source	Oxidation ditch which is used to treat domestic sewage.
Sampling site	Oxidation ditch situated at the district of Hazerswoude Dorp, the Netherlands.
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Approximately 10 litres of activated sludge were centrifuged and the supernatant discarded. The activated sludge was washed three times by centrifugation (10 min at 2000 rpm) and re-suspended twice in tap water and twice in dilution water.
Pretreatment	Fifty ml of synthetic sewage feed was added daily per litre of sludge suspension.
Initial cell concentration	Dry weight was found at a mixed liquor suspended solids level of 4.2 g/L.

Table A7.4.1.4/2-3: Test system

Criteria	Details
Culturing apparatus	1 litre glass beakers for incubation period. 300 mL conical flasks for oxygen measurements.
Number of culture flasks/concentration	1 culture flask/concentration
Aeration device	Not documented
Measuring equipment	Oxygen electrode
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.4/2-4: Test conditions

Criteria	Details
Test temperature	Approximately 20 ºC
pH	7.2-7.7
Aeration of dilution water	Not documented
Suspended solids concentration	4.2 g/L

Table A7.4.1.4/2-5: Results of the activated sludge respiration inhibition test with 1,2-benzisothiazol-3-(2*H*)-one and the reference substance 3,5-dichlorophenol

Nominal concentration of test substance (mg/L)	pH	Respiration rate (mg O_2/g.h)	% Inhibition
1,2-benzisothiazol-3-(2*H*)-one			
0 (Initial control)	7.7	13.31 14.32	-
0 (Final control)	7.6	17.61 16.32	-
1.0	7.7	16.0	-4.2%
3.2	7.7	15.4	-0.9%
10	7.6	7.8	49.3%
32	7.5	2.3	84.9%
100	7.2	0.5	96.4%
3,5-dichlorophenol			
0 (Initial control)	7.6	15.5	-
0 (Final control)	7.7	17.1	-
5	7.7	11.4	30.2%
12	7.6	4.8	70.7%
30	7.6	2.4	85.4%

[1] As the control values had a high variation, the control values of a parallel test with the same sludge were used for the end point calculations.
[2] Values of the parallel control test (approximately the same average).

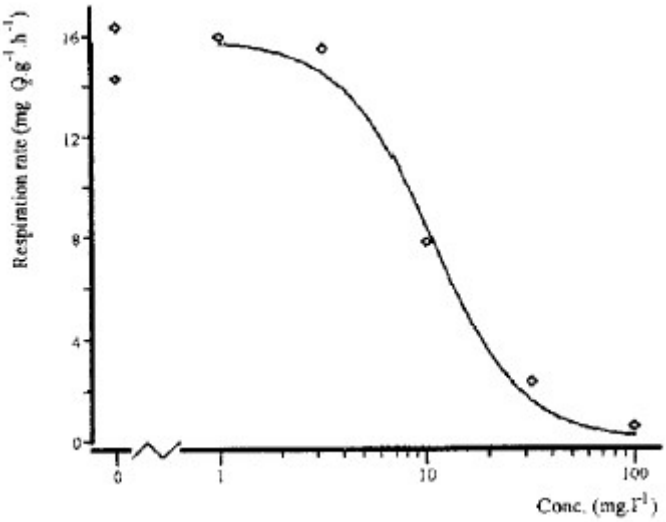


Figure A7.4.1.4/2-1: 1,2-benzisothiazol-3-(2*H*)-one concentration-effect curve for activated sludge

Table A7.4.1.4/2-6: Validity criteria for activated sludge respiration inhibition test according to OECD Guideline 209

	fulfilled
Respiration rates of the two controls are within 15% of each other	Yes
The EC_{50} of the reference substance is in the accepted range (5 to 30 mg/L for 3,5-dichlorophenol)	Yes

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	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X].**	
Detailed justification:	It is proposed that this point is not relevant to BIT as significant exposure of the aquatic environment is unlikely to occur and based on the physico-chemical properties of BIT, there is little or no risk of bioconcentration in aquatic species. According to the Technical Guidance Document (TGD) on Risk Assessment (ECB Part II, 2003), substances with a log K_{ow} < 3 are unlikely to bioaccumulate and the risk of secondary poisoning is minimal. BIT has an estimated log K_{ow} of 1.4, indicating little or no risk of secondary poisoning in the aquatic food chain. A risk assessment has been carried out to investigate the potential for secondary poisoning in birds and mammals as a result of the consumption of aquatic species exposed to BIT (Doc. II-C, 2.4). The results were found to be well below the trigger value of 1, indicating negligible risk of secondary poisoning in the aquatic food chain. According to the TGD, if measured BCF values are not available, the BCF can be predicted from the relationship between the K_{ow} and BCF. The linear equation provided only allows for the calculation of a BCF for substances with a log K_{ow} of > 2. As the log K_{ow} of BIT is 1.4, this equation cannot be applied. Consequently the BCF value for BIT is taken to be 3.09 L/kg as calculated for fish using the USES modelling system. The TGD states that substances with a log K_{ow} of < 4.5 are likely to have a bioconcentration factor (BCF) of < 2000. The BCF value for BIT for fish is significantly lower than this and would indicate that bioconcentration in fish tissues is highly unlikely. The product is not intended for release to watercourses and is recommended not to be used on or near water. It can therefore be concluded that in the unlikely event that exposure of the aquatic environment to BIT should occur, there is little or no risk of bioconcentration in aquatic species.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	

Section A7 **Subsection A7.4.2** **Annex Point IIA, VII.7.5**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ESTIMATION OF BIOCONCENTRATION**
Date	*August 2010*
Evaluation of applicant's justification	*Applicant's justification accepted*
Conclusion	*Applicant's justification accepted*
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	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X].**	
Detailed justification:	According to the "Data requirements for biocidal product types, Version 4.3.2 (October 2000)", this test is usually not required, as it does not add information as needed in the risk assessment and the current test guidelines are not sufficient. Therefore, no studies are presented to address this point.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*August 2010*	
Evaluation of applicant's justification	*Applicant's justification is accepted.*	
Conclusion	*Applicant's justification is acceptable.*	
Remarks		

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.3.2/1

Annex Point IIIA XIII 2.2

EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: September 22, 2006 – November 2, 2006.	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Rohm and Hass	
1.2.2	Companies with letter of access	Troy Chemical Company B.V.	
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.**	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test was carried out in accordance with OECD Guideline No. 210, "Fish, Early Life-Stage Toxicity Test", US EPA Ecological Effects Test Guidelines OPPTS 850.1400, "Fish Early Life-Stage Toxicity Test", ASTM Standard E1241-98, "Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish"; and US EPA Standard Evaluation Procedure, "Fish Early Life-Stage Test".	
2.2	**GLP**	Yes, self-certified	
2.3	**Deviations**	**No**	
		3 METHOD	
3.1	**Test material**	1,2-Benzisothiazolin-3-one	
3.1.1	Lot/Batch number	2005-051	
3.1.2	Specification	As given under point 3.1.3	
3.1.3	Purity	89.8%	

Section A7 Subsection A7.4.3.2/1 Annex Point IIIA XIII 2.2		Ecotoxicological Profile Including Environmental Fate and Behaviour EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	HPLC with UV detection.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	No	
3.2.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	Details are given in Table A7.4.3.2-1.	
3.4.2	Test organisms	*Pimephales promelas*, (Fathead Minnow), details are given in Table A7.4.3.2-2.	
3.4.3	Handling of embryos and larvae (OECD 210/212)	The embryos were removed from spawning substrates and examined to select viable specimens < 24 h old. To initiate the test, embryos were distributed among incubation cups (constructed from glass cylinders with 425 µm nylon screen attached to the bottom), until each contained 20 embryos. One cup was placed in each treatment and control test chamber. The cups were suspended in the water column of each test chamber and attached to a rocker arm, the reciprocating motion of which, facilitated circulation of test water around the embryos. After a 5-day embryo hatching period, the larvae were released to their respective test chambers. Newly-hatched larvae were fed live brine shrimp nauplii (*Artemia* sp.) three times per day during the first seven days. Thereafter, they were fed three times per day on weekdays and twice per day on weekends.	
3.4.4	Test system	Details are given in Table A7.4.3.2-3.	
3.4.5	Test conditions	Details are given in Table A7.4.3.2-4.	**X**
3.4.6	Duration of the test	33 days	

Section A7 **Subsection A7.4.3.2/1** **Annex Point IIIA XIII 2.2**		**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH**
3.4.7	Test parameter(s)	Time to hatch, hatching success, and post-hatch growth and survival were evaluated.
3.4.8	Examination / Sampling	During the first day of exposure, embryos were observed twice for mortality and eggs with fungus. Thereafter, until hatching was complete, observations of embryo mortality and the removal of dead embryos were performed once daily. From day 5, during the 28-day post-hatch exposure period, the larvae were observed daily to evaluate mortality, clinical signs of toxicity and abnormal behaviour. Total length, and wet and dry weights were measured at the conclusion of the 28-day post-hatch exposure period.
3.4.9	Monitoring of TS concentration	Yes; Water samples were collected from one test chamber of each treatment and control group, two days prior to the start of the test (after conditioning the diluter for four days). Samples were collected from alternating replicate test chambers in each treatment and control group on days 0, 7, 14, 21, 28 and 33 to determine concentrations of the test substance in the test chambers.
3.4.10	Statistics	Data from the negative and solvent control groups for each parameter were compared using a t-test. Hatching success and survival, considered to be discrete-variable data, were analyzed using the Chi-square and Fisher's Exact tests to identify treatment groups that showed a statistically significant difference from the pooled control. Growth data, considered as continuous variables, were evaluated for normality using the Shapiro-Wilk test, and for homogeneity of variance using Levene's test. Those treatments that were significantly different from the control means were identified using Dunnett's t-test. All statistical tests were performed using SAS software.
		4 RESULTS
4.1	**Range finding test**	Performed
4.1.1	Concentrations	Test concentrations for the main study were based on a range-finding test. Concentrations from this study were not documented in the report.

Section A7 Subsection A7.4.3.2/1 Annex Point IIIA XIII 2.2		Ecotoxicological Profile Including Environmental Fate and Behaviour EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH	
4.1.2	Number/ percentage of animals showing adverse effects	Not documented	
4.1.3	Nature of adverse effects	Not documented	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Please refer to Table A7.4.3.2-5	
4.2.2	Actual concentrations of test substance	Please refer to Table A7.4.3.2-5	**X**
4.2.3	Effect data	Please refer to Table A7.4.3.2-6.	
4.2.4	Concentration / response curve	Not documented	
4.2.5	Other effects	There were a few sporadic observations of organisms that appeared weak or small in size, but these did not occur in a concentration-responsive pattern. Between days 2 and 4 of the post-hatch period, several fish in the 2.4 mg/L treatment group were observed to be surfacing, but from day 5 to test termination, surviving fish in the group generally appeared normal. Up to day 20, post-hatch, several fish in the 4.8 mg/L group were observed to be weak, surfacing, swimming erratically, or with morphological abnormalities such as crooked spines. Most of these apparently weakened fish died prior to test termination, and at 28 days post-match, all surviving fish appeared normal.	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	Please refer to Table A7.4.3.2-6.	
4.3.2	Nature of adverse effects	Please refer to Table A7.4.3.2-6.	
4.4	**Test with reference substance**	Not performed	

Section A7 Subsection A7.4.3.2/1 Annex Point IIIA XIII 2.2	Ecotoxicological Profile Including Environmental Fate and Behaviour EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH		
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.3.2/1

Annex Point IIIA XIII 2.2

EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH

	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The toxicity of 1,2-benzisothiazol-3-(2*H*)-one to the early life stage development and growth of *Pimephales promelas* was determined under flow-trough test conditions for 33 days. The test was conducted according to OECD test guideline 210: "Fish, Early-life Stage Toxicity Test", US EPA OPPTS 850.1400, "Fish Early Life-Stage Toxicity Test", ASTM Standard E1241-98, "Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish"; and US EPA Standard Evaluation Procedure, "Fish Early Life-Stage Test", with no deviations and is described under point 3.

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.3.2/1 EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH

Annex Point IIIA XIII 2.2

5.2	**Results and discussion**	The mean measured test concentrations were 0.28, 0.59, 1.2, 2.4 and 4.8 mg/L, which represented 90, 94, 92 and 96% of nominal concentrations, respectively. The results of the study were based on the mean measured concentrations. All water quality parameters were within acceptable limits during the test. Temperatures in the test chambers ranged from 24.1 to 25.7ºC during the test, and the continuous temperature measurements ranged from approximately 24 to 25.5ºC. Dissolved oxygen concentrations remained ≥ 6.9 mg/L (≥ 84% of saturation) throughout the test. Hatching success in the negative and solvent control groups were 96 and 95%, respectively. The difference in hatching success between the control groups was not statistically significant. Hatching successes in the 0.28, 0.59, 1.2, 2.4 and 4.8 mg/L treatment groups were 100, 98, 99, 94 and 99%, respectively. There were no statistically significant differences between the hatching success of the treated embryos and the controls. Consequently, the NOEC for hatching success was 4.8 mg/L (the higher concentration tested). Larval survival in the negative and solvent control groups were 95 and 89 %, respectively. The difference in larval survival between the control groups was not statistically significant. Larval survival in the 0.28, 0.59, 1.2, 2.4 and 4.8 mg/L treatment groups was 88, 90, 84, 55 and 37%, respectively. There were statistically significant decreases in survival in the 1.2, 2.4 and 4.8 mg/L groups in comparison to the controls. Consequently, the LOEC for larval survival was 1.2 mg/L and the NOEC was 0.59 mg/L. During the post-hatch period, several fish in the treated groups were observed to be surfacing, weak, swimming erratically, or with morphological abnormalities. Differences in mean total length and dry weight between the control groups were not statistically significant. Significant differences were observed in the mean wet weight of fish in the negative and solvent control groups. Therefore, treatment group mean wet weights were compared to the solvent control. Groups that showed significant effects on larval survival were excluded from analysis of growth. There was a statistically significant decrease in mean total length and mean wet weight of fish in the 0.59 mg/L treatment group. Consequently, the LOEC for growth was 0.59 mg/L and the NOEC was 0.28 mg/L. Data are present in Tables A7.4.3.2-6 and A7.4.3.2-7.
5.2.1	NOEC	0.28 mg/L
5.2.2	LOEC	0.59 mg/L

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.4.3.2/1	**EFFECTS ON REPRODUCTION AND GROWTH RATE OF FISH**
Annex Point IIIA XIII 2.2	

5.3	**Conclusion**	Based on mean measured concentrations, the most sensitive NOEC for 1,2-benzisothiazolin-3-(2*H*)-one was determined to be 0.28 mg/L for a reduction of growth.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	**No**	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2012.*
Materials and Methods	*Applicant's version is accepted with the following comment:* *3.4.5 Test conditions, the water temperature differ more than ±1.5 ºC*
Results and discussion	*Applicant's version is adopted*
Conclusion	*Applicant's version is adopted*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*Key Study.*

Table A7.4.3.2-1: Dilution water

Criteria	Details
Source	Well water
Alkalinity	181-182 mg as $CaCO_3$
Hardness	136-140 mg/L as $CaCO_3$
pH	8.0-8.1
Ca / Mg ratio	2.49
Na / K ratio	2.50
Oxygen content	7.9-8.4 mg/L
Conductance	300-325 µmhos/cm
Holding water different from dilution water	No

Table A7.4.3.2-2: Test organisms

Criteria	Details
Species/strain	*Pimephales promelas*
Source	Chesapeake cultures, Inc., USA
Wild caught	No
Age/size	Embryos < 24 h after fertilisation / Size not documented
Kind of food	Live brine shrimp nauplii (*Artemia* sp.)
Amount of food	Not documented
Feeding frequency	3 times/day during first 7 days, thereafter 2-3 times per day
Post-hatch transfer time	On day 5, once hatching has reached 90%
Time to first feeding	Within two days of hatching
Feeding of animals during test	Yes; Larvae were fed 3 times per day during the first 7 days. Thereafter, they were fed 3 times per day on weekdays and 2 per day on weekends. Fish were not fed for approximately 48 h prior to the termination of the test.
Treatment for disease within 2 weeks preceeding test	No

Table A7.4.3.2-3: Test system

Criteria	Details
Test Type	Flow-through
Renewal of test solution	Daily turnover of 10 volumes
Volume of test vessels	9 litres
Volume/animal	350 mL / fertilised egg
Number of animals/vessel	20 embryos / chamber
Number of vessels/ concentration	4 chambers / concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.2-4: Test conditions

Criteria	Details
Test temperature	24.1 – 25.7 °C
Dissolved oxygen	6.9-8.5 mg/L
pH	8.0 – 8.2
Adjustment of pH	Not documented
Aeration of dilution water	Yes
Quality/Intensity of irradiation	Fluorescent light bulbs/442 lux
Photoperiod	16 hour photoperiod daily

Table A7.4.3.2-5: Actual concentrations of test substance

Nominal concentrations (mg/L)	Mean measured concentration (mg/L)			
	Initial (Day 0)	Aged (Day 33)	Geometric mean (all time points)	
			mg/L	% nom
Control (untreated dilution water)	< LOQ*	< LOQ	-	-
Solvent control (0.0)	< LOQ	< LOQ	-	-
0.31	0.284	0.268	0.28	90
0.63	0.604	0.577	0.59	94
1.3	1.21	1.18	1.2	92
2.5	2.40	2.37	2.4	96
5.0	4.79	4.85	4.8	96

* LOQ = 0.100 mg/L

Table A7.4.3.2-6: Survival, growth and weight data

Mean measured Concentration (mg/L)	28 days post-hatch			
	Mean % Survivors	Length	Wet weight	Dry weight
		Mean ± SD (mm)	Mean ± SD (mg)	Mean ± SD (mg)
Negative control (0.0)	95	22.7 ± 0.22	84.7 ± 3.35	15.8 ± 0.52
Solvent control (0.0)	89	23.0 ± 0.30	93.8 ± 4.20	16.6 ± 0.67
0.28	88	22.9 ± 0.14	91.5 ± 3.10	16.8 ± 0.71
0.59	90	22.3 ± 0.20*	85.3 ± 1.05*	15.4 ± 0.28
1.2	84	22.8 ± 0.29**	88.5 ± 4.53**	16.6 ± 0.52**
2.4	55	22.1 ± 0.12**	81.3 ± 2.92**	15.6 ± 0.67**
4.8	37	21.1 ± 0.37**	67.7 ± 2.90**	12.7 ± 0.60**

* Significant difference from control by means of Williams' test ($p < 0.05$)
** Groups excluded from analyses of growth due to significant effects on larval survival

Table A7.4.3.2-7: Validity criteria for invertebrate reproduction test according to OECD Guideline 210

Criteria	Fulfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	Yes
Difference of water temperature < 1.5°C 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

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	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X].**	
Detailed justification:	It is proposed that this point is not relevant to BIT as significant exposure of the aquatic environment is unlikely to occur and based on the physico-chemical properties of BIT, there is little or no risk of bioaccumulation in aquatic species. According to the Technical Guidance Document (TGD) on Risk Assessment (ECB Part II, 2003), substances with a log K_{ow} < 3 are unlikely to bioaccumulate and the risk of secondary poisoning is minimal. BIT has an estimated log K_{ow} of 1.4, indicating little or no risk of secondary poisoning to mammalian species or bioaccumulation in the food chain. Furthermore, the TGD also states that substances with a log K_{ow} of < 4.5 are likely to have a bioconcentration factor (BCF) of < 2000. The BCF value estimated by the USES modelling system is only 3.09 L/kg which is significantly lower than this and would indicate that bioconcentration in fish tissues is highly unlikely. A risk assessment has been carried out to investigate the potential for secondary poisoning in birds and mammals as a result of the consumption of aquatic species exposed to BIT (Doc. II-C, 2.4). The results were found to be well below the trigger value of 1, indicating negligible risk of secondary poisoning in the aquatic food chain. The product is not intended for release to watercourses and is recommended not to be used on or near water. It can therefore be concluded that in the unlikely event that exposure of the aquatic environment to BIT should occur, there is little or no risk of bioaccumulation in fish species.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*August 2010*	
Evaluation of applicant's justification	*Applicant's justification accepted*	
Conclusion	*Applicant's justification is acceptable*	

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	**Official use only**
Remarks			

Section A7 **Subsection A7.4.3.4/1** **Annex Point IIIA XIII 2.4**		**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON REPRODUCTION AND GROWTH RATE WITH AN INVERTEBRATE SPECIES** ***Daphnia magna***	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] Dates of experimental work: October 23, 2006 – November 28, 2006	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Rohm and Hass	
1.2.2	Companies with letter of access	Troy Chemical Company B.V.	
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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test was carried out in accordance with OECD Guideline No. 211, "*Daphnia magna*, Reproduction Test", US EPA Ecological Effects Test Guidelines OPPTS 850.1300, "Daphnid Chronic Toxicity Test", and ASTM Standard E1193-97, "Standard Guide for Conducting *Daphnia magna* Life Cycle Toxicity Tests".	
2.2	**GLP**	Yes, self certified	
2.3	**Deviations**	**No**	
		3 METHOD	

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	**Official use only**
3.1 Test material	1,2-Benzisothiazolin-3-one	
3.1.1 Lot/Batch number	2005-051	
3.1.2 Specification	As given under point 3.1.3	
3.1.3 Purity	89.8%	
3.1.4 Composition of Product	Not applicable	
3.1.5 Further relevant properties	None	
3.1.6 Method of analysis	HPLC with UV detection	
3.2 Preparation of TS solution for poorly soluble or volatile test substances	Not applicable	
3.3 Reference substance	No	
3.2.1 Method of analysis for reference substance	Not applicable	
3.4 Testing procedure		
3.4.1 Dilution water	Details are given in Table A7.4.3.4-1	**X**
3.4.2 Test organisms	*Daphnia magna*, details are given in Table A7.4.3.4-2	
3.4.3 Handling of offspring	Three times weekly, at the times of renewal of the test solutions, newborn daphnids were counted, inspected for abnormalities and removed.	
3.4.4 Test system	Details are given in Table A7.4.3.4-3	
3.4.5 Test conditions	Details are given in Table A7.4.3.4-4	**X**
3.4.6 Duration of the test	21 days	

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	**Official use only**
3.4.7	Test parameter	Effects on survival, reproduction (the number of live young produced per reproductive day) and growth (length and dry weight).	
3.4.8	Examination / Sampling	Daphnids were observed daily for the mortality, immobility and any clinical signs of toxicity. The presence of eggs in the brood pouch, aborted eggs, males or ephippia also were recorded daily. Newborn daphnids were counted and discarded three times weekly. At study termination, the length and the dry weight of each surviving first-generation daphnid were measured.	
3.4.9	Monitoring of TS concentration	Yes; Water samples were collected from one test chamber of each treatment and control group two days prior to the start of the test after conditioning the diluter for 24 h. Samples were collected from alternating replicate test chambers at the beginning of the test, at weekly intervals during the test and at the end of the test to determine concentrations of the test substance in the test chambers.	
3.4.10	Statistics	Data from the negative and solvent control groups for each parameter were compared using an appropriate statistical test. Survival, considered to be discrete-variable data, was analyzed using the Chi-square and Fisher's Exact tests to identify treatment groups that showed a statistically significant difference ($p \leq 0.05$) from the pooled control. Growth data, considered as continuous, were evaluated for normality using the Shapiro-Wilk test, and for homogeneity of variance using Levene's or Bartlett's test. Analysis of variance (ANOVA) was used to determine whether or not statistically significant differences existed among the experimental groups ($p = 0.05$). Those treatments that were significantly different from the control means were identified using Bonferroni's t-test ($p \leq 0.05$). All statistical tests were performed using ToxStat or SAS software.	
		4 RESULTS	
4.1	**Range finding test**	Performed	
4.1.1	Concentrations	Test concentrations for the main study were based on a range-finding test. Concentrations from this study were not documented in the report.	
4.1.2	Number/ percentage of animals showing adverse effects	Not documented	
4.1.3	Nature of adverse effects	Not documented	

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	**Official use only**
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Please refer to Table A7.4.3.4-5	
4.2.2	Actual concentrations of test substance	Please refer to Table A7.4.3.4-5	
4.2.3	Effect data	Please refer to Tables A7.4.3.4-6 and A7.4.3.4-7 The NOEC for survival was 0.91 mg/L and the LOEC was 1.9 mg/L. Based on the mortalities observed, the 21-day EC_{50} value for adult mortality/immobility was calculated to be 2.5 mg/L, with a 95% confidence interval of 1.9 to 3.8 mg/L.	**X**
		The NOEC for reproduction was 0.91 mg/L and the LOEC was 1.9 mg/L.	
4.2.4	Concentration / response curve	Please refer to Figure A7.4.3.4-1	
4.2.5	Other effects	Daphnids that survived to test termination in the treatment groups at concentrations ≤ 1.9 mg/L generally appeared normal. A daphnid exhibiting a pale discoloration or immobility was observed occasionally in these groups, but the observations were not considered to be treatment related. In the 3.8 mg/L treatment group, there were more frequent observations of animals exhibiting a pale discoloration, immobility or lethargy; these observations were considered to be treatment related.	
4.3	**Results of controls**	Please refer to Tables A7.4.3.4-6 and A7.4.3.4-7.	
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	

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	**Official use only**
5.1 Materials and methods	The effects of 1,2-benzisothiazolin-3-one on the survival, reproduction and growth of *Daphnia magna* was determined under flow-through test conditions during a 21-day exposure period. The test was conducted according to OECD test guideline 211, *"Daphnia magna,* Reproduction Test", US EPA Ecological Effects Test Guidelines OPPTS 850.1300, "Daphnid Chronic Toxicity Test", and ASTM Standard E1193-97, "Standard Guide for Conducting *Daphnia magna* Reproduction life Cycle Toxicity Tests" with no deviations and is described under point 3.	
5.2 Results and discussion	Please refer to Tables A7.4.3.4-6 to A7.4.3.4-8. The mean measured test concentrations were 0.21, 0.46, 0.91, 1.9 and 3.8 mg/L, which represented 84, 92, 91, 95 and 95 % of nominal concentrations, respectively. The results of the study were based on the mean measured concentrations. Temperatures in the test chambers ranged from 19.6 to 20.2 ºC during the test, and the temperature measured continuously was approximately 20.0 º C. Dissolved oxygen concentrations remained ≥ 6.2 mg/L (≥ 69% of saturation) throughout the test. Measurements of pH ranged from 8.0 to 8.2. TOC in the dilution water at test initiation and termination was < 2 mg C/L. After 21 days of exposure, survival in the negative and solvent control groups was 95 and 100 %, respectively. The difference in survival between the control groups was not statistically significant. Therefore, the control data were pooled for comparison to the treatment groups data. Survival in the 0.21, 0.46, 0.91, 1.9 and 3.8 mg/L treatment groups was 95, 100, 95, 80 and 10%, respectively, at test termination. The decrease in survival in the 1.9 and 3.8 mg/L groups was statistically significant in comparison to the controls, consequently the NOEC for survival was determined to be 0.91 mg/l and the LOEC was determined to be 1.9 mg/L. Based on the mortalities observed, the 21-day EC_{50} value for adult mortality/immobility was calculated to be 2.5 mg/L, with a 95% confidence interval of 1.9 to 3.8 mg/L. Observations of *Daphnia* exhibiting a pale discoloration or immobility in the treatment groups at concentrations ≤ 1.9 mg/L were comparable to the control groups and therefore were not considered treatment-related. More frequent observations of animals exhibiting a pale discoloration, immobility or lethargy in the 3.8 mg/L treatment group were considered treatment-related. The first day of brood production in the negative and solvent control replicates and in all treated group replicates was day 8, indicating that there was no apparent delay in the onset of production at any test substance concentration tested. No males or ephippia were produced	

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	**Official use only**
	during the test. However, aborted or shed eggs were noted in the test compartments of the 1.9 and 3.8 mg/L treatment groups during the test. The difference in reproduction between the control groups was not statistically significant. Therefore, the control data were pooled for comparison to the treatment groups data. Daphnids in the 0.21, 0.46, 0.91, 1.9 and 3.8 mg/L treatment groups produced an average of 12.6, 11.3, 11.8, 10.5 and 7.0 neonates per reproductive day, respectively. The decrease in reproduction in the 3.8 mg/L group was statistically significant in comparison to the controls. Although reproduction in the 1.9 mg/L group was significantly lower than the controls, a treatment-related effect could not be precluded based on the slight decrease in mean reproduction and the observations of aborted or shed eggs at 1.9 and 3.8 mg/L. Consequently, the NOEC for reproduction was 0.91 mg/L and the LOEC was 1.9 mg/L. Differences in mean total length and dry weight between the control groups were not statistically significant. Therefore, the control data were pooled for comparison to the treatment groups data. In the 3.8 mg/L group only two daphnids survived to test termination, so this group was excluded from statistical analysis of growth. No statistically significant decreases from the control means were found in length or dry weight at concentrations from 0.21 to 1.9 mg/L. Consequently, the NOEC for growth was 1.9 mg/L and the LOEC was > 1.9 mg/L.	
5.2.1 NOEC	0.91 mg/L	
5.2.2 EC_{10}	-	
5.2.3 EC_{50}	2.5 mg/L (95% confidence interval 1.9-3.8 mg/L)	
5.3 Conclusion	The 21 d NOEC for reproduction in *Daphnia magna* was determined to be 0.91 mg/L, the LOEC was 1.9 mg/L, the 21-day EC_{50} value for adult mortality/immobility was calculated to be 2.5 mg/L, and the 21-day EC_{50} value for reproduction was determined to be > 3.8 mg/L, the highest concentration tested.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	**No**	

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	**Official use only**

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*March 2015.*
Materials and Methods	*Applicant's version is accepted with the following remarks:* ▪ *3.4.1: It is recommended by the OECD TG 211 to estimate the TOC levels in the medium* ▪ *3.4.5: The light intensity was lower than the recommended by the OECD Guideline (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:* o *Coefficient of variation for control fecundity (based of total number of living offspring per parent animal alive)* o *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	*Key study* *It is noted that the test concentrations were not corrected to take account of the purity of 89.8% BIT.*

Table A7.4.3.4-1: Dilution water

Criteria	Details
Source	Well water
Alkalinity	178-182 mg/L as $CaCO_3$
Hardness	136 mg/L as $CaCO_3$
pH	8.0-8.1

Ca / Mg ratio	2.49
Na / K ratio	2.50
Oxygen content	≥ 6.2 mg/L
Conductance	310-320 µmhos/cm
DOC	TOC < 2 mg C/L
Holding water different from dilution water	No

Table A7.4.3.4-2: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	Wildlife International, USA
Age	< 24 h old
Breeding method	Adults used to supply neonates for the test were held for 16 days prior to collection of the juveniles for testing. Adult daphnids were cultured in water from the same source and approximately the same temperature as used during the test. Juvenile Daphnia were collected from the cultures and indiscriminately transferred one and two at a time to transfer chambers until each chamber contained 5 daphnids. Each group then was transferred to an indiscriminately assigned test compartment. All transfers were made below the water surface using wide-bore pipettes.
Kind of food	Algal suspension (Pseudokirchneriella subcapitata) and YCT (mixture of yeast, cereal grass media and trout chow).
Amount of food	1.5-2 mL suspension and 0.75-1 mL YCT per 1.5 L Daphnia medium
Feeding frequency	3 times per day through day 7 4 times per day days 7-21
Pretreatment	Not documented
Feeding of animals during test	Yes

Table A7.4.3.4-3: Test system

Criteria	Details
Test Type	Flow-through
Renewal of test solution	Yes; daily turnover of 5-8 volumes
Volume of test vessels	Two 300 mL glass beakers in two 25 L aquaria
Volume/animal	1100 mL / daphnid
Number of animals/vessel	5 daphnids / vessel (2 replicates)
Number of vessels/ concentration	2 / concentration

Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.4-4: Test conditions

Criteria	Details
Test temperature	Ranged between 19.6 – 20.2 °C
Dissolved oxygen	Ranged between 6.2-8.7 mg/L
pH	Ranged between 8.0 – 8.2
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Fluorescent light bulbs (Colortone 50) / 219 lux
Photoperiod	16 h photoperiod daily

Table A7.4.3.4-5: Actual concentrations of test substance

Nominal concentrations (mg/L)	Mean measured concentration (mg/L)					
	Initial (day 0)		Aged (day 21)		Geometric mean	
	mg/L	% nom	mg/L	% nom	mg/L	% nom
Negative control (0.0)	< LOQ*	NA	< LOQ	NA	NA	NA
Solvent control (0.0)	< LOQ	NA	< LOQ	NA	NA	NA
0.25	0.230	92.0	0.205	81.9	0.21	84
0.50	0.485	97.0	0.445	88.9	0.46	92
1.0	0.923	92.3	0.945	94.5	0.91	91
2.0	1.94	96.9	1.92	95.9	1.9	95
4.0	3.85	96.3	3.91	97.7	3.8	95

* < LOQ calculated as LOQ = 0.100 mg/L
NA = Not applicable

Table A7.4.3.4-6: Survival and growth data

Mean measured Concentration [mg/L]	Survival (%)	Number of dead parent animals	Time of death (day)	Growth (length on day 21) Mean ± SD (mm)	Growth (dry weight on day 21) Mean ± SD (mg)
Negative control	95	0	-	5.7 ± 0.21	1.12 ± 0.16
Solvent control	100	0	-	5.7 ± 0.22	1.10 ± 0.05
0.21	95	1	18	5.8 ± 0.10	1.16 ± 0.07
0.46	100	0	-	5.7 ± 0.15	1.02 ± 0.03
0.91	95	1	20	5.8 ± 0.06	1.09 ± 0.14
1.9	80*	4	14-18	5.5 ± 0.00	0.99 ± 0.25
3.8	10*	18	8-20	5.3**	1.08**

* Statistically significant decrease in survival
** This group was excluded from analysis of growth since only two daphnids survived to test termination

Table A7.4.3.4-7: Reproduction data

Mean measured Concentration [mg/L]	Total number of living offspring per parent animal alive at day 21	Coefficient of variation for control fecundity (%)	First day of reproduction	Number of young produced per reproductive day
			(day)	Mean ± SD (#)
Negative control	9.35	100	8	11.2 ± 1.4
Solvent control	5.85	62.57	8	11.4 ± 3.5
0.21	28.74	307.38	8	12.6 ± 1.3
0.46	31.6	337.97	8	11.3 ± 1.4
0.91	15.89	169.95	8	11.8 ± 0.79
1.9	13.31	142.35	8	10.5 ± 1.1
3.8	0.5	5.35	8	7.0 ± 1.9*

* This group was excluded from analysis of growth since only two daphnids survived to test termination

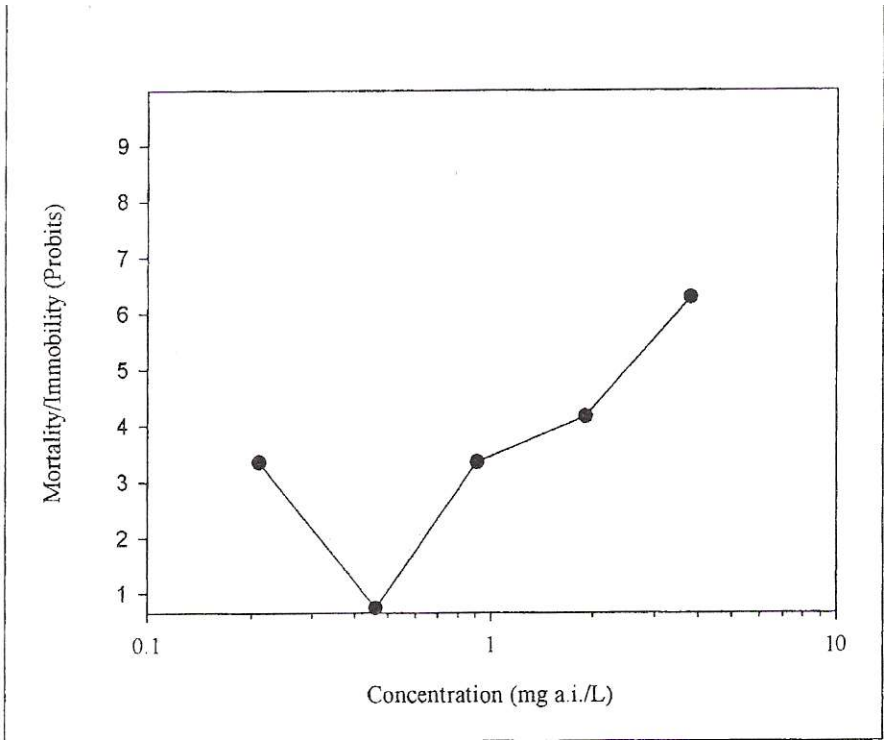


Figure A7.4.3.4-1: Concentration-response curve for first generation mortality/immobility at test termination.

Table A7.4.3.4-8: Validity criteria for invertebrate reproduction test according to OECD Guideline 211

Criteria	Fulfilled
Mortality of parent animals < 20% at test termination	Yes
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	Yes
Criteria for poorly soluble test substances	Not applicable

Section A7
Subsection A7.4.3.5.1/1
Annex Point IIIA, XIII.3.4

Ecotoxicological Profile Including Environmental Fate and Behaviour

EFFECTS ON SEDIMENT DWELLING ORGANISMS

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: November 7, 2006 – November 22, 2006	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Rohm and Haas	
1.2.2	Companies with letter of access	Troy Chemical Company B.V.	
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**	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test was carried out in accordance with US EPA Ecological Effects Test Guidelines OPPTS 850.1735, "Whole Sediment Acute Toxicity Invertebrates, Freshwater" and the ASTM E 1706-96b Guideline: Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Fresh Water Invertebrates. These are equivalent to the OECD test Guideline 218, "Sediment-Water Chironomid Toxicity Test Using Spiked Sediment".	
2.2	**GLP**	Yes, self certified	
2.3	**Deviations**	**No**	
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one	
3.1.1	Lot/Batch number	2005-051	
3.1.2	Specification	As given under point 3.1.3	
3.1.3	Purity	89.8%	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.3.5.1/1 EFFECTS ON SEDIMENT DWELLING ORGANISMS

Annex Point IIIA, XIII.3.4

		1 REFERENCE	Official use only
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	HPLC with UV detection	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Details are given in Table A7.4.3.5.1-1	
3.3	**Reference substance**	No	
3.2.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water, Test sediment	Details are given in Table A7.4.3.5.1-2	
3.4.2	Sediment parameters	Details are given in Table A7.4.3.5.1-3	
3.4.3	Test organisms	*Chironomus tentans*, details are given in Table A7.4.3.5.1-4	**X**
3.4.4	Test system	Details are given in Table A7.4.3.5.1-5	**X**
3.4.5	Test conditions	Details are given in Table A7.4.3.5.1-6	**X**
3.4.6	Duration of the test	10 days	
3.4.7	Test parameter	Mortality, clinical and behavioural signs.	
3.4.8	Sampling	Chironomids were observed daily for mortality and any signs of toxicity or abnormal behaviour. On day 10, chironomids were removed from the sediment, and the numbers of live and dead chironomids were enumerated.	
3.4.9	Monitoring of TS concentration	Yes; Prior to Day 0, samples were collected from the 0.63, 1.3, 2.5, 5.0 and 10.0 mg/mL stock solution after preparation. Overlying water, pore water and sediment samples were collected from the analytical	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.3.5.1/1 EFFECTS ON SEDIMENT DWELLING ORGANISMS

Annex Point IIIA, XIII.3.4

		1 REFERENCE	Official use only
		replicates from each test concentration and control shortly after the introduction of the chironomids on Day 0 and at test termination on Day 10.	
3.4.10	Control	Yes, negative control and solvent control	
3.4.11	Statistics	The test showed no effects on survival over the 10 day test period, therefore EC_{50} was determined to be greater than the highest concentration tested. The ash-free dry weight data were analyzed using the computer program TOXSTAT version 3.5. The no observed-effect-concentration (NOEC) and lowest-observed-effect –concentration (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 data were evaluated for normality (Chi-square) and homogeneity of variance (Levene's Test). After the data were determined to be normally distributed with homogeneous variance, the negative and solvent control data were compared using a two-tailed t-Test (p = 0.05). The treatment groups were compared to solvent control due to the significant differences between the negative control and solvent control.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentrations	Not applicable	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Please refer to Table A7.4.3.5.1-7	
4.2.2	Actual concentrations of test substance	Please refer to Table A7.4.3.5.1-7	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.3.5.1/1 EFFECTS ON SEDIMENT DWELLING ORGANISMS

Annex Point IIIA, XIII.3.4

		1 REFERENCE	Official use only
4.2.3	Effect data	Please refer to Tables A7.4.3.5.1-8 The LOEC and NOEC for survival were both determined to be > 100 mg BIT/kg dry sediment. The EC_{50} for percent survival was also determined to be > 100 mg BIT/kg dry sediment. The mean individual ash-free dry weight of chironomids in the negative control, solvent control, 6.3, 13, 25, 50 and 100 mg/kg treatment groups were 1.54, 1.76, 1.54, 1.95, 1.56, 1.63 and 1.39 mg, respectively, at test termination. There was a significant difference between the solvent control group and the 100 mg/kg group. The NOEC for mean individual ash-free dry weight was determined to be 50 mg BIT/kg dry sediment and the LOEC for mean individual ash-free dry weight was 100 mg BIT/kg dry sediment. The EC_{50} for mean individual ash-free dry weight was also determined to be > 100 mg BIT/kg dry sediment.	**X**
4.2.4	Concentration / response curve	Not applicable	
4.2.5	Other effects	A few observations of organisms of on the surface of the sediment or climbing the walls of the test compartments were made in all treatment groups and controls.	
4.3	**Results of controls**	Please refer to Tables A7.4.3.5.1-8	
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**	The effects of 1,2-benzisothiazolin-3-one on the survival, reproduction and growth of *Chironomus tentans* was determined under flow-through test conditions during a 10-day exposure period. The test was carried out in accordance with US EPA Ecological Effects Test Guidelines OPPTS 850.1735, "Whole Sediment Acute Toxicity Invertebrates, Freshwater" and the ASTM E 1706-96b Guideline: Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Fresh Water Invertebrates. The methods used are equivalent to the OECD test Guideline 218, "Sediment-Water Chironomid Toxicity Test Using Spiked Sediment" with no deviations and are described under point 3.	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.3.5.1/1 EFFECTS ON SEDIMENT DWELLING ORGANISMS

Annex Point IIIA, XIII.3.4

		1 REFERENCE	Official use only
5.2	**Results and discussion**	Please refer to Tables A7.4.3.5.1-7, A7.4.3.5.1-8 The measured concentrations of 1,2-Benzisothiazolin-3-one in the 10.0, 5.0, 2.5, 1.3 and 0.63 mg/mL stock solutions yielded 96, 95, 87, 90 and 31% of nominal values respectively. Mass balance of the test material was relatively good on Day 0 in the 6.3, 13, 25, 50 and 100 mg/kg treatment groups at 73, 62, 76, 84 and 70% of nominal, respectively. By Day 10, the mass balance of material was reduced in the 6.3, 13, 25, 50 and 100 mg/kg treatment groups to 2.3, 30, 29, 33 and 29% of nominal, respectively. Both sediment and pore water contained reduced concentrations of the test substance suggesting that degradation, transformation or adsorption was occurring The absence of test material in the overlying water was attributed to the exchange of overlying water that occurred twice daily during the equilibration period and during the test. Temperatures were within the 23 ± 1°C range. Dissolved oxygen concentrations were ≥ 66% (5.6 mg/L) of saturation throughout the test. Measurement of pH ranged from 7.9 to 8.2. The LOEC and NOEC for survival were both determined to be > 100 mg BIT/kg dry sediment. The EC_{50} for percent survival was also determined to be > 100 mg BIT/kg dry sediment. The mean individual ash-free dry weight of chironomids in the negative control, solvent control, 6.3, 13, 25, 50 and 100 mg/kg treatment groups were 1.54, 1.76, 1.54, 1.95, 1.56, 1.63 and 1.39 mg, respectively, at test termination. There was a significant difference between the solvent control group and the 100 mg/kg group. The NOEC for mean individual ash-free dry weight was determined to be 50 mg BIT/kg dry sediment and the LOEC for mean individual ash-free dry weight was 100 mg BIT/kg dry sediment. The EC_{50} for mean individual ash-free dry weight was also determined to be > 100 mg BIT/kg dry sediment. A few observations of organisms of on the surface of the sediment or climbing the walls of the test compartments were made in all treatment groups and controls.	
	$LOEC_0$	> 100 mg BIT/kg dry sediment for survival 100 mg BIT/kg dry sediment for mean ash-free dry weight	
	NOEC	100 mg BIT/kg dry sediment for survival 50 mg BIT/kg dry sediment for mean ash-free dry weight	
	EC_{50}	> 100 mg BIT/kg dry sediment for survival > 100 mg BIT/kg dry sediment for mean ash-free dry weight	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.3.5.1/1

Annex Point IIIA, XIII.3.4

EFFECTS ON SEDIMENT DWELLING ORGANISMS

		1 REFERENCE	Official use only
5.3	**Conclusion**	The 10-day NOEC for *Chironomus tentans* was determined to be 100 mg BIT/kg dry sediment, the LOEC was > 100 mg BIT/kg dry sediment and the EC_{50} value was calculated to be > 100 mg/kg dry sediment based on survival. The NOEC for mean individual ash-free dry weight was determined to be 50 mg BIT/kg dry sediment, the LOEC was 100 mg BIT/kg dry sediment and the EC_{50} value was calculated to be > 100 mg BIT/kg dry sediment.	**X**
5.3.1	Reliability	1	
5.3.2	Deficiencies	**No**	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

November 2012.

Materials and Methods

Accept the applicant's version with the following comments:

- *3.4.3. The OECD Guideline 218 recommends to use first instar larvae*
- *3.4.4 The OECD Guideline 218 recommends that the ratio of the depth of sediment: water must be 1:4 .and a static system is recommended by the Guideline*
- *3.4.5: The light intensity used in the study is lower than those recommended by the OECD*

Results and discussion

Accept the applicant's version with the following remarks:

- *Table A7.4.3.5.1-7 only give information about the total mass balance. Tables that consider recoveries for all compartments of the experiment (porewater, overlyingwater and sediment), can be obtained from the correspondent document IVA, and are included below:*

Measured BIT concentrations in sediment samples:

Nominal	***Measured Day 0***	***Measured Day 10***
Negative control	*< LOQ*	*< LOQ*
Solvent control	*< LOQ*	*< LOQ*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.3.5.1/1 EFFECTS ON SEDIMENT DWELLING ORGANISMS

Annex Point IIIA, XIII.3.4

1 REFERENCE | **Official use only**

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***
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*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.4.3.5.1/1

Annex Point IIIA, XIII.3.4

EFFECTS ON SEDIMENT DWELLING ORGANISMS

	1 REFERENCE	**Official use only**
	25 mg BIT/kg | *33.8* | *14.8* *50 mg BIT/kg* | *93.3* | *32.6* *100 mg BIT/kg* | *173* | *66.5* *ECx values should consider the measured concentration at the beginning of the test (as recommended by the guidances) and not be based on nominals. Therefore:* *EC_0 = 32.8 mg/kg; EC_{50} > 45.9 mg/kg and EC_{100}= Not applicable.*	
Conclusion	*Applicant's version is accepted.*	
Reliability	*2*	
Acceptability	*Acceptable*	
Remarks		

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

Criteria	Details
Dispersion	No
Vehicle	Yes, acetone
Concentration of vehicle	Not documented
Vehicle control performed	Yes, acetone
Other procedures	None

Table A7.4.3.5.1-2: Dilution water

Criteria	Details
Source	Well water
Alkalinity	178 – 180 mg/L as $CaCO_3$
Hardness	136 mg/L as $CaCO_3$
pH	8.0 – 8.2
Ca / Mg ratio	1/2.5
Na / K ratio	1/2.5
Oxygen content	Not documented
Conductance	300 – 320 µmhos/cm
DOC	5.6 – 8.1 mg/L
Holding water different from dilution water	No

Table A7.4.3.5.1-3: Sediment parameters

Criteria	Details
Nature	Formulated sediment
Humic acid and dolomite/alpha-cellulose/silt and clay/industrial quartz sand [% dry weight]	1/5/14/80
pH	7.0 ± 0.5
Reference of methods	OECD 207 and 218
Collection / storage of samples	The dry soil was stored under ambient conditions until used.

Table A7.4.3.5.1-4: Test organisms

Criteria	Details
Strain	*Chironomus tentans*
Source	Environmental Consulting and Testing, Superior, Wisconsin, USA
Age	10 days
Introduction to test system	The chironomids were collected for the culture and impartially added one and two at a time into transfer chambers until each container contained 10 chironomids. Each group was impartially assigned and transfered to a test compartment. All transfers were made below the air/water interface using wide-bore pipettes.
Kind of food	flake food
Amount of food	1.5 mL of a 4 g/L suspension of flake food
Feeding frequency	Not documented
Pretreatment	Acclimatization, chironomids were held for three days at approximately the same temperature as used during the test in water from the same source.
Feeding of animals during test	Yes

Table A7.4.3.5.1-5: Test system

Criteria	Details
Test Type	Flow-through
Renewal of test solution	Yes; daily turnover of 2 volumes
Volume of test vessels	300 mL
Volume/animal	30 mL / chironomid
Number of animals/vessel	10 chironomids / vessel
Number of vessels/ concentration	8 / concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.5.1-6: Test conditions

Criteria	Details
Test temperature	Ranged between 22.2 – 23.5 °C
Dissolved oxygen	Ranged between 5.6 – 8.1 mg/L
pH	Ranged between 7.9 – 8.2
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Fluorescent light bulbs (Colortone 50) / 219 lux
Photoperiod	16 h photoperiod daily

Table A7.4.3.5.1-7: Actual concentrations of test substance

Nominal test concentrations (mg BIT/kg dry sediment)	Mean measured concentration (mg/l)								
	Day 0			Day 10			Geometric mean		
	Nominal BIT in test system (mg)	Total BIT in test system (mg)	% nom	Nominal BIT in test system (mg)	Total BIT in test system (mg)	% nom	Nominal BIT in test system (mg)	Total BIT in test system (mg)	% nom
Negative control (0.0)	< LOQ*	< LOQ	N/A	< LOQ	< LOQ	N/A	< LOQ	< LOQ	N/A
Solvent control (0.0)	< LOQ	< LOQ	N/A	< LOQ	< LOQ	N/A	< LOQ	< LOQ	N/A
6.3	0.668	0.484	73	0.662	0.015	2.3	0.665	0.2495	37.65
13	1.433	0.890	62	1.508	0.455	30	1.4705	0.6725	46
25	2.550	1.943	76	2.875	0.842	29	2.7125	1.3925	52.5
50	5.150	4.311	84	5.350	1.750	33	5.250	6.061	58.5
100	10.100	7.105	70	10.500	2.996	29	10.300	5.0505	49.5

* LOQ was 1.00 mg/kg
N/A: Not applicable

Table A7.4.3.5.1-8: Effect data

Nominal concentration (mg BIT/kg dry sediment	Percent survival*	Mean individual ash-free dry weight (mg)
Negative control (0.0)	100	1.54
Solvent control (0.0)	9	1.76
6.3	100	1.54
13	100	1.95
25	103**	1.56
50	100	1.63
100	100	1.39***

* A total of 80 individuals were exposed per test concentration with 10 individuals in each of 8 replicate test chambers.
** Two extra organisms were inadvertently added to replicate G of this test level for a total of 82 individuals exposed.
*** There was a statistically significant difference ($p < 0.05$) from the solvent control using Dunnett's test.

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	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X].**	
Detailed justification:	It is proposed that this point is not relevant as the use of BIT is not likely to adversely impact aquatic organisms. BIT is not intended for direct release to waterbodies and is not recommended for use on or near surface waters. In the unlikely event that BIT were introduced to the aquatic compartment *via* accidental exposure, it is not believed that the active substance will pose any risk to plant species as its mode of action is not herbicidal in nature. Therefore, it is proposed that a study is not required to address this point.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*August 2010*	
Evaluation of applicant's justification	*Accept applicant's justification.*	
Conclusion	*Accept applicant's justification.*	
Remarks		

Section A7
Subsection A7.5.1.1/1
Annex Point IIA7.4

Ecotoxicological Profile Including Environmental Fate and Behaviour

INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: November 2, 2006 – December 2, 2006	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Rohm and Haas	
1.2.2	Companies with letter of access	Troy Chemical Company B.V.	
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.**	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	**Yes, the test was carried out in accordance with OECD Guideline No. 217: "Soil Microorganisms: Carbon Transformation Test".**	
2.2	**GLP**	Yes, self certified	
2.3	**Deviations**	Yes, the following deviations were noted: 1. Variation among the controls on day 28 was not within the acceptable range (±15%). 2. Carbon content of microbial biomass is not specified. These deviations are not considered to compromise the scientific validity of this study.	**X**
		3 MATERIALS AND METHODS	
3.1	**Test material**	1,2-Benzisothiazolin-3-one	
3.1.1	Lot/Batch number	2005-051	
3.1.2	Specification	As given under point 3.1.3	
3.1.3	Purity	89.8%	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.1/1 INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)

Annex Point IIA7.4

3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Not documented	
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	Please refer to Table A7.5.1.1-1	
3.3.2	Test system	Not applicable	
3.3.3	Application of TS	Please refer to Table A7.5.1.1-2	
3.3.4	Test conditions	Please refer to Table A7.5.1.1-3	
3.3.5	Test parameter	Inhibition of microbial carbon transformation	
3.3.6	Analytical parameter	Pressure drop due to O_2 consumption and CO_2 production	
3.3.7	Duration of the test	28 days	**X**
3.3.8	Sampling	Days 0, 7 and 28 after application.	
3.3.9	Monitoring of TS concentration	No	
3.3.10	Controls	Yes, three test chambers served as untreated controls	
3.3.11	Statistics	Respiration rates were statistically analyzed using analysis of variance (ANOVA). Bonferroni t-Test or Dunnett's Test were used to determine statistically significant differences from untreated controls at each sampling interval. All calculations were performed using full precision.	
		4 RESULTS	
4.1	**Range finding test**	Not performed	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.1/1 INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)

Annex Point IIA7.4

4.1.1	Concentration	Not applicable	
4.1.2	Effect data	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0, 10.7, 28.7, 100, 317 and 1000 mg/kg dry soil	**X**
4.2.2	Actual concentrations of test substance	Not documented	
4.2.3	Growth curves	Not applicable	
4.2.4	Cell concentration data	Not applicable	
4.2.5	Concentration/ response curve	Please refer to Figure A7.5.1.1-1	
4.2.6	Effect data	Please refer to Table A7.5.1.1-4	
4.2.7	Other observed effects	None	
4.3	**Results of controls**	Please refer to Table A7.5.1.1-4	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.1/1 INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)

Annex Point IIA7.4

	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The effects of 1,2-Benzisothiasolin-3-one on soil microflora were assessed in a test that measured short-term respiration following an application of 1,2-Benzisothiasolin-3-one to soil. The test was conducted according to OECD test guideline 217 and is described under point 3. The following deviations were noted: 1. Variation among the controls on day 28 was not within the acceptable range (±15%). 2. Carbon content of microbial biomass is not specified. However, these deviations are not considered to compromise the scientific validity of this study.
5.2	**Results and discussion**	Please refer to Table A7.5.1.1-4 for short-term respiration results. No statistically significant effect was observed for the short-term respiration (produced CO_2) at any of the concentrations of 1,2-Benzisothiasolin-3-one, 7 days after application. On day 28, the two highest concentrations showed statistically significant increases in respiration rates compared with the untreated controls. No significant adverse treatment-related effects were observed.
5.2.1	EC_{10}	> 1000 mg/kg
5.2.2	EC_{25}	> 1000 mg/kg
5.2.3	EC_{50}	> 1000 mg/kg
5.3	Conclusion	Based on the results of this study and in accordance with OECD guideline 217, 1,2-Benzisothiasolin-3-one was found to have no adverse effects on the short-term respiration of a microbial population in a field soil tested up to 1000 mg/kg.
5.3.1	Reliability	1
5.3.2	Deficiencies	Two deviations were noted and are outlined under point 5.1, however they are not expected to compromise the scientific validity of this study.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.1/1 INHIBITION TO MICROBIAL ACTIVITY (TERRESTRIAL)

Annex Point IIA7.4

Date	*December 2012.*
Materials and Methods	*Applicant's version is accepted with the following comments:* ▪ *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 guidelines 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.* ▪
Results and discussion	*Applicant's version is accepted with the following comments:* ▪ *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 an important lack of data: 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 CO2 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.*
Conclusion	*The test was considered valid. The NOEC, based on increase respiration rate as an effect, is 100 mg/kg (see **Table A7.5.1.1-4** below).*
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 A7.5.1.1-1: Soil parameters

Criteria	Details
Nature	Sandy loam
Sampling site:	
Geographical reference on the sampling site	Grand Forks County, North Dakota at coordinates N 47° 48.166 – W 97° 37.264.
Data on the history of the site	Pesticides and fertilizers were not used on site in the previous year.
Use pattern	Tree farm
Depth of sampling [cm]	0 – 20 cm
Sand / Silt / Clay content [% dry weight]	66/16/18
pH	7.1
Organic carbon content [% dry weight]	1.4
Total Nitrogen (mg/kg)	Not documented
Cation exchange capacity [mval/kg]	Not documented
Initial microbial biomass	330 µg/g
Reference of methods	OECD 217
Collection / storage of samples	Soil was collected and stored for a total of 80 days under refrigerated conditions.
Preparation of inoculum for exposure	Moisture content was measured and adjusted to approximately 50% WHC (water holding capacity).
Pretreatment	The soil was 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°C (19.7°C – 22.2°C).

Table A7.5.1.1-2: Application of test substance

Criteria	Details
Application procedure	The test substance was administered by direct weight addition to the soils in the test chambers. After dosing the soils were thoroughly homogenized using stainless steel spatulas.
Carrier	Not applicable
Concentration of liquid carrier [% v/v]	Not applicable
Liquid carrier control	Not applicable
Other procedures	None

Table A7.5.1.1-3: Test conditions

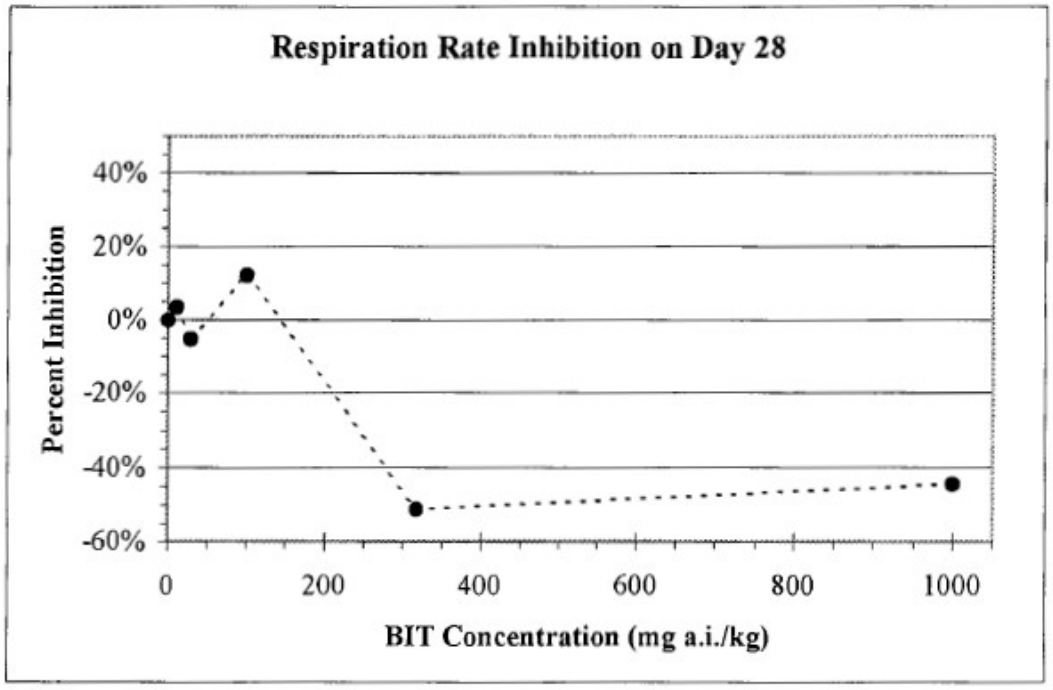
Criteria	Details
Organic substrate	Soil samples received 300 mg of glucose in a solution in water to induce respiration.
Incubation temperature	20°C (19.2°C – 22.2°C)
Soil moisture	Ranged from 19.5% to 23.5% (43.0% - 51.8% of WHC). Adjusted to 50% WHC.
Method of soil incubation	Test chambers were 11x7 inch Pyrex® glass baking dishes with plastid lids. The plastic lids were drilled with holes to allow circulation. All test chambers were incubated under aerobic conditions in the dark.
Aeration	No

Table A7.5.1.1-4: Results of short-term respiration test (mean mg CO_2/h/kg dry soil weight per hour ± standard deviation)

Time (days)	Control	10.7 mg/kg	28.7 mg/kg	100 mg/kg	317 mg/kg	1000 mg/kg
0	17.3 ± 0.0	18.3 ± 0.9	18.8 ± 0.0	23.3 ± 0.0	20.2 ± 2.0	20.2 ± 0.0
7	11.0 ± 0.9	14.3 ± 2.5	11.9 ± 0.8	12.9 ± 2.5	10.5 ± 2.2	10.5 ± 0.8
28	11.3 ± 2.6	10.9 ± 0.8	11.9 ± 0.8	10.0 ± 2.1	17.1* ± 1.3	16.4* ± 1.0

* Statistically significant differences from respective controls

Figure A7.5.1.1-1: Concentration response curve: respiration rate inhibition on day 28



Section A7
Subsection A7.5.1.2/1
Annex Point IIIA XIII 3.2

Ecotoxicological Profile Including Environmental Fate and Behaviour

EARTHWORM, ACUTE TOXICITY TEST

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: June 5, 2006 – June 23, 2006	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Rohm and Haas	
1.2.2	Companies with letter of access	Troy Chemical Company B.V.	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	**Yes, the test was carried out in accordance with OECD Guideline No. 207: "Earthworm, Acute Toxicity Tests".**	
2.2	**GLP**	Yes, self certified	
2.3	**Deviations**	**No**	
		3 METHOD	
3.1	**Test material**	1,2-Benzisothiazolin-3-one	
3.1.1	Lot/Batch number	2005-051	
3.1.2	Specification	As given under point 3.1.3	
3.1.3	Purity	89.8%	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	

Section A7 Subsection A7.5.1.2/1 Annex Point IIIA XIII 3.2	Ecotoxicological Profile Including Environmental Fate and Behaviour EARTHWORM, ACUTE TOXICITY TEST	
3.1.6 Method of analysis	**Not documented**	
3.2 Reference substance	Yes; Chloroacetamide	**X**
3.2.1 Method of analysis for reference substance	Not documented	
3.3 Testing procedure		
3.3.1 Preparation of the test substance	Details are provided in Table A7.5.1.2-1	
3.3.2 Application of the test substance	Different concentrations of 1,2-Benzisothiazolin-3-one were prepared in reverse osmosis water and added to 2167 g of artificial soil medium and mixed thoroughly to ensure homogeneity. The moistened soil samples were divided into test containers corresponding to the different test concentrations and controls (750 g per 1 litre test vessel).	
3.3.3 Test organisms	*Eisenia foetida* (Details are given in Table A7.5.1.2-2)	
3.3.4 Test system	Details are given in Table A7.5.1.2-3	
3.3.5 Test conditions	Details are given in Table A7.5.1.2-4	
3.3.6 Test duration	14 days	
3.3.7 Test parameter	Mortality, clinical symptoms and weight change	
3.3.8 Examination	Twice at test initiation for burrowing behavior or pathological abnormalities, and thereafter at days 7 and 14 of the 14-day exposure period. Observations were made on days 7 and 14 of the 14-day exposure period for mortality, and on days 0 and 14 for body weight.	
3.3.9 Monitoring of test substance concentration	No	
3.3.10 Statistics	The LC_{50} value and 95% confidence interval were calculated using the Stephan computer program, by nonlinear interpolation for the day 7 LC_{50} and probit analysis for the day 14 LC_{50}. Body weights and their changes were compared with Dunnett's 2-Tailed Test of Means using SAS version 8.	
	4 RESULTS	
4.1 Filter paper test	Not performed	
4.4.1 Concentration	Not applicable	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.2/1 EARTHWORM, ACUTE TOXICITY TEST

Annex Point IIIA XIII 3.2

4.4.2	Number/ percentage of animals showing adverse effects	Not applicable
4.4.3	Nature of adverse effects	Not applicable
4.2	**Soil test**	
4.2.1	Initial concentrations of test substance	28.06, 56.13, 112.25, 224.5, 449 and 898 mg/kg dry weight artificial soil.
4.2.2	Effect data (Mortality)	Details are provided in Tables A7.5.1.2-5 and A7.5.1.2-6.
4.2.3	Concentration / effect curve	Not documented
4.2.4	Other effects	In observations of burrowing behavior it was noted that the earthworms showed a strong aversion to the test soils. A slight reduction in weight of earthworms was noted in treated groups compared with the control.
4.3	**Results of controls**	
4.3.1	Mortality	Details are provided in Table A7.5.1.2-5
4.3.2	Number/ percentage of earthworms showing adverse effects	Details are provided in Table A7.5.1.2-5 and A7.5.1.2-7
4.3.3	Nature of adverse effects	Details are provided in Table A7.5.1.2-5 and A7.5.1.2-7
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	13, 25 and 50 mg Chloracetamide/kg dry soil
4.4.2	Results	The 14-day LC_{50} value for Chloracetamide was 24.5 mg/kg dry weight of soil with a 95 % confidence interval of 13 and 50 mg/kg.

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.2/1 EARTHWORM, ACUTE TOXICITY TEST

Annex Point IIIA XIII 3.2

	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	An acute toxicity test was carried out in order to assess the effects of 1,2-Benzisothiazol-3-(2*H*)-one on earthworms (*Eisenia foetida*), during a 14-day exposure period in an artificial soil substrate. The test was conducted according to OECD guideline 207 with no deviations and is described under point 3.
5.2	**Results and discussion**	No mortality was observed in the negative control group throughout the study. Following 7 days of exposure, mortality was 0, 18, 18, 20, 100 and 100% in the 28.06, 56.13, 112.25, 224.5, 449 and 898 mg/kg treatment groups respectively. On the 14th day of exposure, mortality was 0, 20, 33, and 93% in the 28.06, 56.13, 112.25, 224.5 mg/kg concentration group, respectively. All control worms were normal in appearance and behaviour throughout the test period. Following Day 7 observations, the 28.06 and 56.13 mg/kg groups burrowed when returned to the soil surface, but worms in all other groups remained on the soil surface or on the sides of the glass chambers. It was noted that worms in the 898 mg/kg group appeared to be very lethargic, and possibly some were dead, approximately ½ hour after test initiation. A slight treatment-related reduction in weight of earthworms was observed in all surviving animals from treatments compared with the control. The final body weight and weight change values were normally distributed. The 7-day LC_{50} for Eisenia foetida was determined to be 278 mg/kg dry weight soil, with 95% confidence limits of 224.5 and 449 mg/kg. The 14-day LC_{50} for Eisenia foetida was determined to be 114 mg/kg dry weight soil, with 95% confidence limits of 98.1 and 132 mg/kg.
5.2.1	NOEC	Not documented
5.2.2	LC_0	114 mg/kg (with 95% confidence limits of 98.1 and 132 mg/kg)
5.2.3	LC_{50}	Not documented
5.2.4	LC_{100}	The validity criteria for acute earthworm test according to OECD 207 were fulfilled. The 14-day LC_{50} for *Eisenia foetida* was determined to be 114 mg/kg dry weight soil.
5.3	**Conclusion**	None
5.3.1	Other Conclusions	1
5.3.2	Reliability	**No**

Section A7 **Subsection A7.5.1.2/1** **Annex Point IIIA XIII 3.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EARTHWORM, ACUTE TOXICITY TEST**	
5.3.3 Deficiencies	An acute toxicity test was carried out in order to assess the effects of 1,2-Benzisothiazol-3-(2*H*)-one on earthworms (*Eisenia foetida*), during a 14-day exposure period in an artificial soil substrate. The test was conducted according to OECD guideline 207 with no deviations and is described under point 3.	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*November 2012.*
Materials and Methods	*Applicant's version is accepted with the following remarks:* ▪ *3.3.9. Concentration of test substance was not monitored. Terrestrial tests are normally performed without confirmatory analysis but BIT is photolytically unstable and the concentrations in the test could be minor that the recommended in the test.*
Results and discussion	*Applicant's version is accepted with the following comments:* - *The lack of measured concentrations of BIT may affect the reliability of results, as LC_{50} is calculated using nominal concentrations instead of real concentrations.* - *NOEC value corresponds to 28.06 mg BIT/kg dry soil.*
Conclusion	*The validity criteria for acute earthworm test according to OECD 207 were fulfilled. The 14-day LC_{50} for Eisenia fetida was determined to be 114 mg/kg dry weight soil.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*Key study*

Table A7.5.1.2-1: Preparation of TS solution

Criteria	Details
Type and source of dilution water	Reverse osmosis water
Alkalinity / Salinity	Not documented
Hardness	Not documented
pH	Not documented
Oxygen content	Not documented
Conductance	Not documented
Holding water different from dilution water	No

Table A7.5.1.2-2: Test organisms

Criteria	Details
Species/strain	*Eisenia foetida*
Source of the initial stock	University of Maryland, Wye Research & Education Center, USA
Culturing techniques	Not documented
Age/weight	Adult worms (with clitellum) Details of worm weight before treatment are provided in Table A7.5.1.2-7.
Pre-treatment	A 24 h acclimation period was observed. On the day of test initiation, worms were rinsed briefly with reverse osmosis water.

Table A7.5.1.2-3: Test system

Criteria	Details
Artificial soil test substrate	The artificial soil contains sand, kaolin clay and Sphagnum peat in the ratio 70:20:10 (weight:weight) respectively and was prepared in bulk. The pH was adjusted to 5.9 using calcium carbonate.
Test mixture	Test solutions of BIT were mixed with 2167 g of artificial soil medium.
Size, volume and material of test container	One-liter glass beakers
Amount of artificial soil (kg)/ container	0.75 kg
Nominal levels of test concentrations	28.06, 56.13, 112.25, 224.5, 449 and 898 mg/kg dry weight artificial soil.
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Overhead fluorescent bulbs
Test performed in closed vessels due to significant volatility of TS	The glass trays were covered with perforated plastic film to prevent the test medium from drying out although this is not due to volatility of the test substance

Table A7.5.1.2-4: Test conditions

Criteria	Details
Test temperature	20.2-21.5 °C
Moisture content	33.8 - 34.8% at test initiation 32.8 – 34.0% at test termination
pH	7.0 – 7.4 at test initiation 7.2– 7.5 at test termination
Adjustment of pH	pH of bulk soil was adjusted prior to hydration
Light intensity / photoperiod	400-800 lux / 24 hours
Relevant degradation products	Not documented

Table A7.5.1.2-5: Mortality data (test substance)

Test Substance Concentration (nominal) [mg/kg artificial soil]	Mortality (based on 40 worms/concentration)			
	Number		Percentage	
	7 d	14 d	7 d	14 d
negative control	0	0	0	0
28.06	0	0	0	0
56.13	7	8	17.5	20
112.25	8	13	17.5	32.5
224.5	8	37	20	92.5
449	40	40	100	100
898	40	40	100	100
Temperature [°C]	ND	20.2-21.0		
pH	ND	7.2-7.5		
Moisture content	ND	32.8-34.0		

ND = not documented

Table A7.5.1.2-6: Effect data

Time	LC_{50} (mg/kg soil)[1]	95 % c.l.
7 d	278	224.5 and 449
14 d	114	98.1 and 132

[1] nominal concentrations

Table A7.5.1.2-7: Group mean bodyweight changes

Test Substance Concentration (nominal) [mg/kg artificial soil]	Average Weight of Earthworm (g)*		Mean bodyweight change
	Before Treatment	14th day	
Negative Control	0.63	0.55	-0.08
28.06	0.63	0.52	-0.11
56.13	0.63	0.51	-0.12
112.25	0.61	0.42	-0.19
224.5	0.64	0.47	-0.18
449	0.63	-	-
898	0.61	-	-

* - Mean of 4 replications

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

	fulfilled
Mortality of control animals < 10%	Yes

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.3/1 TERRESTRIAL PLANT TOXICITY

Annex Point IIIA XIII 3.4 **Seedling emergence test**

		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] Dates of experimental work: August 23, 2006 – November 3, 2006	
1.2	**Data protection**	**Yes**	
1.2.1	Data owner	Rohm and Hass	
1.2.2	Companies with letter of access	Troy Chemical Company B.V.	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test was carried out in accordance with OECD Guideline No. 208: "Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test".	
2.2	**GLP**	Yes, self certified	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	1,2-Benzisothiazolin-3-one	
3.1.1	Lot/Batch number	2005-051	
3.1.2	Specification	As given under point 3.1.3	
3.1.3	Purity	89.8%	
3.1.4	Composition of Product	Not applicable	

Section A7	Ecotoxicological Profile Including Environmental Fate and Behaviour	
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Annex Point IIIA XIII 3.4	Seedling emergence test	

3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	HPLC with UV detection.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
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	Details not documented	
3.4.2	Test plants	Please refer to Table A7.5.1.3-1.	
3.4.3	Test system	Please refer to Table A7.5.1.3-2.	
3.4.4	Test conditions	Please refer to Table A7.5.1.3-3.	
3.4.5	Test duration	21 days	
3.4.6	Test parameter	Seedling emergence, survival, plant condition and growth (shoot dry weight).	
3.4.7	Sampling	Sampling of plant material for dry weight analysis took place on the final assessment day (day 21). Observations of emergence were made on days 7, 14 and 21. Observations of plant condition and shoot fresh weight were made on day 21.	

Section A7	Ecotoxicological Profile Including Environmental Fate and Behaviour	
Subsection A7.5.1.3/1	TERRESTRIAL PLANT TOXICITY	
Annex Point IIIA XIII 3.4	Seedling emergence test	

3.4.8	Method of analysis of the plant material	For each replicate, the plants were clipped to soil level and placed in a labelled bag. Total dry weight of the replicate was determined after oven-drying.	
3.4.9	Quality control	Negative control group which received no test substance and no carrier solvent Solvent control which received acetone.	
3.4.10	Statistics	For the purpose of statistical analysis, emergence was defined as "the number of emerged plants per ten planted seeds in each pot", survival as "the number of emerged seedlings in each pot which were living at test termination per ten planted seeds", and shoot dry weight was defined as "the weight of shoots collected from all living emerged seedlings in each replicate". Data from control and treatment groups were compared with a one-tailed Dunnett's t-test, using SAS version 8 software. Dunnett's test was used to establish the LOEC and NOEC by determining treatment groups differed significantly ($p < 0.05$) from the control group. Effect concentrations estimates were made using the non-linear regression analysis of Bruce and Versteeg using the SAS version 8.	
		4 RESULTS	
4.1	**Results test substance**		
4.1.1	Applied initial concentration	Please refer to Table A7.5.1.3-4.	
4.1.2	Phytotoxicity rating	Data are presented in Table A7.5.1.3-5.	
4.1.3	Plant height	Not documented	
4.1.4	Plant dry weights	Data are presented in Table A7.5.1.3-5.	
4.1.5	Root dry weights	Not documented	
4.1.6	Root length	Not documented	

Section A7		**Ecotoxicological Profile Including Environmental Fate and Behaviour**	
Subsection A7.5.1.3/1		**TERRESTRIAL PLANT TOXICITY**	
Annex Point IIIA XIII 3.4		**Seedling emergence test**	
4.1.7	Number of dead plants	Data are presented in Table A7.5.1.3-5.	
4.1.8	Effect data	Data are presented in Tables A7.5.1.3-6 to A7.5.1.3-8.	
4.1.9	Concentration / response curve	Not documented	
4.1.10	Other effects	Effects on the condition of emerged seedlings such as chlorosis, leaf curl, stem curl, stunting and mortality were observed in all species tested at the test concentrations of 99.8 mg/kg and higher.	
4.2	**Results of controls**		
4.2.1	Number/ percentage of plants showing adverse effects	Data are presented in Table A7.5.1.3-5.	
4.2.2	Nature of adverse effects	Data are presented in Table A7.5.1.3-5.	
4.3	**Test with reference substance**	Not performed	
4.3.1	Concentrations	Not applicable	
4.3.2	Results	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The effects of 1,2-Benzisothiazol-3-(2*H*)-one on seedling emergence and growth was determined in a multi-rate test with six species of non-target terrestrial plants, four dicotyledonae and two monocotyledonae. The study was conducted according to OECD Guideline 208, "Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test".	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.5.1.3/1	**TERRESTRIAL PLANT TOXICITY**
Annex Point IIIA XIII 3.4	**Seedling emergence test**

5.2	**Results and discussion**	The solvent control sample (acetone) showed no indication of the presence of the test substance or of the presence of a co-eluting substance at the characteristic retention time of the test substance. The environmental conditions in the greenhouse were determined to be appropriate for normal seedling emergence and growth of the species tested. The results of the test satisfy the test guideline validity criteria, in that among the control groups: the seedling emergence was greater than 70%, the seedlings did not exhibit signs of phytotoxicity other than what is considered incidental and normal and mean control survival was greater than 90% for the duration of the study. There was an apparent solvent effect on *A. cepa* shoot dry weight, therefore the negative and solvent control mean shoot dry weights were not pooled for comparison to the treatment groups, which were compared to the solvent control only. Apparent treatment-related effects on the emergence, survival and/or growth were observed on all six species. Statistical results supported the observations of effects on emergence, survival and dry weight. In the initial test, significant effects on the dry weight of lettuce (*L. sativa*) were observed in all treatment groups. Since it was the most sensitive species, the test was repeated to determine the NOEC. Please refer to Tables A7.5.1.3-6 to A7.5.1.3-8 for the full details of the results.	
5.2.1	LOEC	Please refer to Tables A7.5.1.3-6 to A7.5.1.3-8	
5.2.2	NOEC	Please refer to Tables A7.5.1.3-6 to A7.5.1.3-8	
5.2.3	EC_{50}	Please refer to Tables A7.5.1.3-6 to A7.5.1.3-8	
5.3	**Conclusion**	Based on the results of this study (conducted under greenhouse conditions), it can be concluded that 1,2-Benzisothiazol-3-(2*H*)-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, with EC_{50} values ranging from 18.4 mg/kg for lettuce (*L. sativa*) to 166 mg/kg for oat (*A. sativa*). The lowest NOEC was observed for tomato (*L. esculentum*) dry weight and was determined to be less than 11.1 mg/kg, which was the lowest test concentration.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
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Annex Point IIIA XIII 3.4	**Seedling emergence test**

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
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.*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.1.3/1 TERRESTRIAL PLANT TOXICITY

Annex Point IIIA XIII 3.4 **Seedling emergence test**

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.*

	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*
Lycopersicon esculentum	*8.13±0.99*	*8.50±0.76*	*8.50±0.76*	*8.25±1.04*	*2.69±0.351*

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 A7.5.1.3-1: Test plants

	Family	Species	Common name	Source of seeds	Seed germination potential (%)
Dicotyledonae	Brassicaceae	*Brassica rapa*	Turnip	Park Seed Wholesale Inc.	90
	Cucurbitaceae	*Cucumis sativa*	Cucumber	The Meyer Seed Co.	85
	Asteraceae	*Lactuca sativa*	Lettuce	Johnny's Selected Seeds	99
	Solanaceae	*Lycopersicon esculentum*	Tomato	The Meyer Seed Co.	90
Monocotyledonae	Liliaceae	*Allium cepa*	Onion	Wannamaker Seeds	> 80
	Poaceae	Avena sativa	Oat	Johnny's Selected Seeds	95

Table A7.5.1.3-2: Test system

Criteria	Details
Test type	Greenhouse
Container type	Plastic pots, 16 cm diameter and 12 cm deep
Seed germination potential	Please refer to Table A7.5.1.3-1
Identification of the plant species	Please refer to Table A7.5.1.3-1
Number of replicates	4 (1 pot per replicate in each case)
Numbers of plants per replicate per dose	10 plants / replicate / dose
Date of planting	25th August 2006
Plant density	10 seeds per pot
Date of test substance application	Soil was treated on 25th August 2006
High of plants at application	Soil application: plants were at seed stage
Date of phytotoxicity rating	Day 21
Dates of analysis	15th September 2006

Table A7.5.1.3-3: Test conditions

Criteria	Details
Test type	The stock solution was mixed with sand and stirred. This was then mixed with soil and stirred to make pre-mixtures for the treatment groups. Each premixture was then added to bulk soil and mixed, then placed in pots.
Method of application	Soil incorporated, one application
Application levels	Not documented
Dose rates	Please refer to Table A7.5.1.3-4
Substrate characteristics	Mixture of kaolinite clay, industrial quartz sand and peat, with limestone added to buffer the pH, measured to be pH 7.3 (TOC of 1.3%) Soil composition: Sand – 65% Silt – 18% Clay – 17%
Watering of the plants	In order to minimize the potential for the leaching of the test substance through the soil, water lost was replaced by sub-irrigation, using well water from the greenhouse facility. Sub-irrigation trays were filled to a predetermined depth to help standarise the amount of water delivered to each tray.
Temperature	17.20 – 37.76°C
Thermoperiod	Not documented
Light regime	Artificial lightning was used to supplement natural sunlight in order to provide a minimum 16 h photoperiod
Relative humidity	13.63-91.60%
Wind volatility	Not applicable
Observation periods and duration of test	Observations were made at days 7, 14 and 21
Pest control	No plant protection measures were performed
Any other treatments and procedures	Not documented

Table A7.5.1.3-4: Test concentrations

Treatment group (mg/kg dry soil)	Nominal Concentrations (ppm)	Measured concentrations (ppm)	% of nominal concentration
Solvent control	0.0	< LOD*	-
11.1	4,038	4,131	102
33.3	12,114	11,900	98
99.8	36,343	34,550	95
299	109,030	91,962	84
898	327,090	262,052	80
Solvent control	0.0	< LOD	-
0.411	72.4	80.6	111
1.23	217	246	113
3.70	653	703	108
11.1	1,954	2,116	108
33.3	5,861	6,219	106

*The limit of detection (LOD) at the instrument was set at the lowest calibration standard analyzed (1.00 µg/mL)

Table A7.5.1.3-5: Effective phytotoxicity after test termination

Test Substance Concentration (nominal) [mg/kg]	Absolute Numbers		Per cent relative to control	
	Plant mean dry weight (g)	Dead plants	Plant dry weights	Dead plants
Allium cepa				
Negative control (0.0)	0.185	3	100.00	100.00
Solvent control (0.0)	0.132	7	71.35	233.33
11.1	0.152	7	82.16	233.33
33.3	0.106	11	57.30	366.67
99.8	0.008	35	4.32	1166.67
299	0.005	34	2.70	1133.33
898	0.000	36	0.00	1200.00
Avena sativa				
Negative control (0.0)	-	1	-	-
Solvent control (0.0)	2.25	3	100.00	100.00
11.1	2.32	0	103.11	0.00
33.3	2.37	1	105.33	33.33
99.8	1.81	4	80.44	133.33
299	0.46	4	20.44	133.33
898	0.05	15	2.22	500.00
Brassica rapa				
Negative control (0.0)	-	3	-	-
Solvent control (0.0)	4.87	2	100.00	100.00
11.1	4.73	3	97.13	150.00
33.3	3.18	2	65.30	100.00
99.8	0.04	28	0.82	1400.00
299	0.01	38	0.21	1900.00
898	0.00	40	0.00	2000.00

Test Substance Concentration (nominal) [mg/kg]	Absolute Numbers		Per cent relative to control	
	Plant mean dry weight (g)	Dead plants	Plant dry weights	Dead plants
Cucumis sativa				
Negative control (0.0)	-	0	-	-
Solvent control (0.0)	10.7	1	100.00	100.00
11.1	11.0	1	102.80	100.00
33.3	9.9	0	92.52	0.00
99.8	2.6	5	24.30	500.00
299	0.2	21	1.87	2100.00
898	0.0	40	0.00	4000.00
Lactuca sativa				
Negative control (0.0)	-	6	-	-
Solvent control (0.0)	1.54	10	100.00	100.00
11.1	0.88	13	57.14	130.00
33.3	0.39	18	25.32	180.00
99.8	0.01	38	0.65	380.00
299	0.00	40	0.00	400.00
898	0.00	40	0.00	400.00
***Lactuca sativa* (final trial). Temperature [°C]: 17.20-27.92, Relative humidity: 19.83-70.40 %**				
Negative control (0.0)	-	3	-	-
Solvent control (0.0)	1.12	3	100.00	100.00
0.411	0.90	7	80.36	233.33
1.23	1.02	2	91.07	66.67
3.70	0.89	2	79.46	66.67
11.1	0.58	1	51.79	33.33
33.3	0.45	2	40.18	66.67
Lycopersicon esculentum				

Test Substance Concentration (nominal) [mg/kg]	Absolute Numbers		Per cent relative to control	
	Plant mean dry weight (g)	Dead plants	Plant dry weights	Dead plants
Negative control (0.0)	-	8	-	-
Solvent control (0.0)	2.69	6	100.00	100.00
11.1	2.18	7	81.04	116.67
33.3	1.73	10	64.31	166.67
99.8	0.05	26	1.86	433.33
299	0.01	31	0.37	516.67
898	0.00	40	0.00	666.67
Temperature [°C]	20.79-26.80			
Relative humidity %	65.12-87.80			

Table A7.5.1.3-6: Effect data – effects on emergence (mg/kg dry soil)

Species	21-day Emergence			
	NOEC	LOEC	EC_{25}	EC_{50}
Allium cepa	33.3	99.8	26.9	67.7
Avena sativa	299	898	825	> 898
Brassica rapa	33.3	99.8	59.7	102
Cucumis sativa	99.8	299	297	585
Lactuca sativa[1]	33.3	> 33.3	> 33.3	> 33.3
Lycopersicon esculentum	33.3	99.8	87.8	166

[1] Based on results of second test

Table A7.5.1.3-7: Effect data – effects on survival (mg/kg dry soil)

Species	21-day Survival			
	NOEC	LOEC	EC_{25}	EC_{50}
Allium cepa	33.3	99.8	24.3	55.7
Avena sativa	299	898	756	> 898
Brassica rapa	33.3	99.8	45.3	79.3
Cucumis sativa	33.3	99.8	272	294
Lactuca sativa[1]	33.3	> 33.3	> 33.3	> 33.3
Lycopersicon esculentum	33.3	99.8	53.0	110

[1] Based on results of second test

Table A7.5.1.3-8: Effect data – effects on growth (mg/kg dry soil)

Species	21-day Dry weight			
	NOEC	LOEC	EC_{25}	EC_{50}
Allium cepa	33.3[1]	99.8[1]	25.1[1]	42.7[1]
Avena sativa	33.3	99.8	98.5	166
Brassica rapa	11.1	33.3	29.3	39.0
Cucumis sativa	11.1	33.3	40.9	65.1
Lactuca sativa[2]	3.70	11.1	3.70	18.4
Lycopersicon esculentum	< 11.1	11.1	28.3	40.0

[1] Based on comparison to the Solvent Control only
[2] Based on results of second test

Table A7.5.1.3-9: Validity criteria for terrestrial plant toxicity according to Seedling Emergence proposed test guideline 208

	Fulfilled
The seedling emergence should be at least 80% for crop and 65% for non-crop species	Yes
The mean control seedling growth does not exhibit visible phytotoxic effects	Yes
The mean control survival is at least 90% for the duration of the study	Yes
For any species, all organisms in a test must be from the same source	Yes
All test chambers or rooms used for a particular species should be identical and should have same conditions and contain same amount of soil matrix, support media, or substrate from the same source.	Yes

Section A7 **Subsection A7.5.2.1** **Annex Point IIIA XIII.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **TERRESTRIAL TESTS, LONG-TERM TESTS** **Reproduction Study with Other Soil Non-Target Macro-Organisms**	
	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:	This test may be required if the risk assessment for the terrestrial compartment (based on the results from acute toxicity tests) still indicates a concern for the terrestrial compartment. A risk assessment to determine the potential risk of BIT to the terrestrial compartment has been carried out (Doc. II-C, 2.2). This assessment indicates little or no risk to the terrestrial compartment through the use of BIT and as a result no acute terrestrial testing is required. Long-term testing is therefore also waived as no significant or prolonged exposure is expected in the terrestrial compartment as a result of the use of BIT according to recommended practices. Furthermore, as BIT has a DT_{50} in soil of < 1 day and is inherently biodegradable, the active substance is not expected to persist in soil, consequently long-term exposure is highly unlikely to occur. On this basis therefore, it is proposed that long-term terrestrial tests are not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*May 2011*	
Evaluation of applicant's justification	*Applicant's justification is accepted.*	
Conclusion	*Applicant's justification is acceptable.*	
Remarks		

Section A7 **Subsection A7.5.2.2** **Annex Point IIIA 13.3**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **TERRESTRIAL TESTS, LONG-TERM TESTS** **Long-term tests with terrestrial plants**	
	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:	It is proposed that this point is not relevant as BIT is not intended for direct application to plants or to soil. A risk assessment to determine the potential risk of BIT to the terrestrial compartment was carried out (Doc. II-C, 2.3). The risk quotient was calculated to be below the trigger value of 1, indicating that there is no significant risk expected to the terrestrial environment. Furthermore, as BIT has a DT_{50} in soil of < 1 day, the active substance is not expected to persist in soil, consequently long-term exposure is highly unlikely to occur. It should also be noted that there are no set guidelines for long-term tests on plants. On this basis therefore, it is proposed that long-term terrestrial tests are not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*May 2011*	
Evaluation of applicant's justification	*Applicant's justification is accepted.*	
Conclusion	*Applicant's justification is acceptable.*	
Remarks		

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.3.1.1/1 ACUTE ORAL TOXICITY ON BIRDS

Annex Point IIIA, XIII 1.1 **Bobwhite quail (*Colinus virginianus*)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: June 27, 1997 – July 18, 1997.	
1.2	**Data protection**	Yes	
1.2.4	Data owner	Troy Chemical Company B.V.	
1.2.5	Companies with letter of access	Not applicable	
1.2.6	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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test method was based on U.S. EPA FIFRA Guideline Subdivision E, No. 71-1 "Avian Single-Dose Oral LD_{50} Test", which is equivalent to OECD Draft Guideline 223, "Avian Acute Oral Toxicity Test".	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	No	**X**
		3 METHOD	
3.1	**Test material**	[redacted]® (1,2-benzisothiazol-3-(2*H*)-one)	
3.1.1	Lot/Batch number	060793	
3.1.2	Specification	Please refer to Doc. III-A, 2/2	
3.1.3	Purity	99.29%	
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 A7.5.3.1.1/1 ACUTE ORAL TOXICITY ON BIRDS

Annex Point IIIA, XIII 1.1 **Bobwhite quail (*Colinus virginianus*)**

3.1.6	Method of analysis	Not applicable	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Single oral administration by gelatine capsule, details are given in Table A7.5.3.1.1-1.	**X**
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	Bobwhite quail *(Colinus virginianus),* details are given in Table A7.5.3.1.1-2.	
3.4.2	Test plants	Details are given in Table A7.5.3.1.1-3.	
3.4.3	Test system	M.S. Egg Crumbles 18%. Composition: crude protein 18%, crude fat 2.5%, crude fibre 5.0%, Calcium 3.5-4%, Phosphorus 0.6% and Salt (NaCl) 0.2-0.4%.	**X**
3.4.4	Test conditions	Details are given in Table A7.5.3.1.1-4.	
3.4.5	Test duration	Single dose, birds were observed for 21 days	
3.4.6	Test parameter	Mortality and clinical signs	
3.4.7	Sampling	The animals were individually weighed just prior to dosing, and on Days 3, 7, 14 and 21. Group feed consumption values were recorded on Days 3, 7, 14 and 21. Examinations were made daily for mortalities, abundance of feed and water, and feed spillage. Clinical signs observations were carried out on each bird at least once daily. All birds that died during the study were subjected to gross necropsy examinations. Two animals per sex were selected from the control, 68.1, 147, 316 and 681 mg/kg bw dose level groups and subjected to complete necropsy examinations at the end of the study. The gastrointestinal tract, liver, kidneys, heart, spleen, muscle and subcutaneous fat were examined.	
3.4.8	Method of analysis of the plant material	At the end of the 21-day test, the acute dietary median lethal dose (LD_{50}) was calculated by employing the simplified method of Litchfield and Wilcoxon. Statistical analyses were performed on body weights using one-way analysis of variance.	

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.3.1.1/1 ACUTE ORAL TOXICITY ON BIRDS

Annex Point IIIA, XIII 1.1 **Bobwhite quail (*Colinus virginianus*)**

		4 RESULTS
4.1	**Range finding test**	Performed
4.1.1	Concentration	First range-finding: 464, 681, 1000, 1470 and 2150 mg/kg bw Second range-finding: 215, 464 and 681 mg/kg bw
4.1.2	Number/ percentage of animals showing adverse effects	First range-finding: the test involved 1 bird/sex/dose. Birds dosed at 1000, 1470 and 2150 mg/kg bw died. The male birds dosed at 464 and 681 mg/kg bw died. Second range-finding: the test involved 1 bird/sex/dose. The female bird dosed at 464 mg/kg bw died.
4.1.3	Nature of adverse effects	Mortality
4.2	**Results test substance**	
4.2.1	Applied concentrations	0, 68.1, 147, 316, 681 and 1470 mg/kg bw
4.2.2	Effect data (Mortality)	Data are provided in Table A7.5.3.1.1-5.
4.2.3	Body weight	Data are provided in Table A7.5.3.1.1-6.
4.2.4	Feed consumption	Data are provided in Table A7.5.3.1.1-7.
4.2.5	Concentration / response curve	Slope = 1.80 (with 95% confidence limits of 1.44 – 2.24) Please refer to Figure A7.5.3.1.1-1.
4.2.6	Other effects	Abnormal excreta, lethargy, piloerection, inability to walk and muscle spasms were also observed in the 681 and 1470 mg/kg bw treatment groups. Complete remission of clinical signs was noted in survivors on Day 13. Gross pathological examinations of the birds that died during the study revealed the following observations: void intestines, dark-coloured gizzard contents, clear to tan- or reddish-brown-coloured gel covering the crop and/or breast area, no subcutaneous fat present, pale lungs and bent legs. Gross examinations of the selected survivors revealed an area of red-coloured irritation in the crop area of seven birds from the 147, 316 and 681 mg/kg bw groups and three birds from the 681 and 1470 mg/kg bw groups had calloused spots on the pectoral muscles.
4.3	**Results of controls**	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**	
Subsection A7.5.3.1.1/1	**ACUTE ORAL TOXICITY ON BIRDS**	
Annex Point IIIA, XIII 1.1	**Bobwhite quail (*Colinus virginianus*)**	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were observed in the control group
4.3.2	Nature of adverse effects	Not applicable
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**	1,2-benzisothiazol-3-(2*H*)-one was administered as a single dose to six groups of 5 bobwhite quails/sex/dose at dose levels of 0, 68.1, 147, 316, 681 and 1470 mg/kg bw. This study was conducted according to U.S. EPA FIFRA Guideline Subdivision E, No. 71-1 "Avian Single-Dose Oral LD_{50} Test", which is equivalent to OECD Draft Guideline 223, "Avian Acute Oral Toxicity Test" with no deviations and is described under point 3.
5.2	**Results and discussion**	No mortality was observed in the control group. Four mortalities (2 males and 2 females) in the 681 mg/kg bw group and nine mortalities (5 males and 4 females) in the 1470 mg/kg bw group were recorded between Days 1 and 12. Slightly reduced feeding during the first days of the test was observed in the 147, 316 and 681 mg/kg bw groups. Anorexia during the first seven test days was observed in the 1470 mg/kg bw group. Abnormal excreta, lethargy, piloerection, inability to walk and muscle spasms were also observed in the 681 and 1470 mg/kg bw groups. Complete remission of clinical signs was noted in survivors on Day 13. Significant decreases in body weights were observed on Days 3 and 7 in the 316 mg/kg bw group and in the 681 mg/kg bw group on Days 3, 7 and 14. Gross pathological examinations of the birds that died during the study revealed the following observations: void intestines, dark-coloured gizzard contents, clear to tan- or reddish-brown-coloured gel covering the crop and/or breast area, no subcutaneous fat present, pale lungs and bent legs. Gross examinations of the selected survivors revealed an area of red-coloured irritation in the crop area of seven birds from the 147, 316 and 681 mg/kg bw groups and three birds from the 681 and 1470 mg/kg bw groups had calloused spots on the pectoral muscles.
5.2.1	LD_{50}	790 mg/kg bw (95% confidence limits of 549 and 1135 mg/kg bw)

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.5.3.1.1/1	**ACUTE ORAL TOXICITY ON BIRDS**
Annex Point IIIA, XIII 1.1	**Bobwhite quail (*Colinus virginianus*)**

5.3	**Conclusion**	The test met the validity criteria established in the recommended Guideline. The results of the 21-day acute oral toxicity study conducted with [redacted]® in bobwhite quail showed the acute oral median lethal dose (LD_{50}) to be 790 mg/kg bw with 95% confidence limits of 549 and 1135 mg/kg bw. The No Observed Effect Level (NOEL) was 68.1 mg/kg bw with regard to sub-lethal effects and 316 mg/kg bw with regard to mortality.	
5.3.1	Reliability	1	
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 comments:* ▪ *2.3. There is a protocol deviation reported in Doc. IV-A, but not in the present document. All deviations from the protocol must be reported in the summary test report.* ▪ *The deviation corresponds to increases of temperature and relative humidity, with regard to protocol conditions. The protocol states that temperature and relative humidity shall be maintained at 21ºC and 55% respectively. However, during parts of the quarantine period, humidity was found to be between 77 and 82%, and during test, the temperature raised up to 27- 30ºC.* ▪ *3.4.3. According to EPA guidelines, antibiotics or other medication should not be used in the diet during the acclimation period or the test.* ▪ *But in this study, during the quarantine period animals' feed were supplemented with 1lb VIP BMD 10 (Bacitracin Methylene Disalicilate) per 100 lbs. During test, untreated feed was offered.* ▪ *3.2. Dosing should be done preferably in early morning hours, and in this test, it was made between 11:50 a.m. to 12:30 p.m.*
Results and discussion	*Applicant's version is accepted*

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.3.1.1/1 ACUTE ORAL TOXICITY ON BIRDS

Annex Point IIIA, XIII 1.1 **Bobwhite quail (*Colinus virginianus*)**

Conclusion	*The test met the validity criteria established in the recommended Guideline. The results of the 21-day acute oral toxicity study conducted with [redacted]® in bobwhite quail showed the acute oral median lethal dose (LD_{50}) to be 790 mg/kg bw with 95% confidence limits of 549 and 1135 mg/kg bw. The No Observed Effect Level (NOEL) was 68.1 mg/kg bw with regard to sub-lethal effects and 316 mg/kg bw with regard to mortality.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*Key study.*

Table A7.5.3.1.1-1: Method of administration of the test substance

Carrier / Vehicle	Details
Water	No
Organic carrier	No
Concentration of the carrier [% v/v]	Not applicable
Other vehicle	Gelatine capsule
Function of the carrier / vehicle	Not applicable

Table A7.5.3.1.1-2: Test animals

Criteria	Details
Species/strain	Bobwhite quail (*Colinus virginianus*)
Source	Rice Family Farm, Wisconsin, USA
Age (in weeks), sex and initial body weight (bw)	18 weeks old Male and female Males: 170.1 – 225.3 g (Day 0) Females: 170.2 – 217.2 g (Day 0)
Breeding population	Not documented
Amount of food	Not documented
Age at time of first dosing	24 weeks old
Health condition / medication	At the supplier´s facility, the birds were fed VitaPlus Custom Mix feed medicated with BMD-60. During the first three days of quarantine, water was treated with L-S 50 Water Soluble Powder.

Table A7.5.3.1.1-3: Test system

Criteria	Details
Test location	Indoor in holding pens
Holding pens	Steel wire pens, 51 cm (L) x 51 cm (W) x 25 cm (H), maintained over steel shelves.
Number of animals	60 birds (30 males, 30 females)
Number of animals per pen [cm²/bird]	10 birds per pen [260 cm^2/bird]
Number of animals per dose	5/sex
Pre-treatment / acclimation	Birds were placed on a 42-day quarantine period. All birds were fed M.S. Egg Crumbles 18% containing approximately 450 g VIP BMD 10 per 45 kg mixed feed throughout the quarantine period.
Diet during test	M.S. Egg Crumbles 18%, Smith feed Service, P.O. Box 277, Loyal, WI 54446, USA
Dosage levels (of test substance)	0, 68.1, 147, 316, 681 and 1470 mg/kg bw; single dose
Replicate/dosage level	No replicates
Feed dosing method	Not applicable, the treatment was administered in a gelatine capsule.
Dosing volume per application	Not applicable
Frequency, duration and method of animal monitoring after dosing	Group feed consumption values were recorded on Days 3, 7, 14 and 21. Examinations were made daily for mortalities, abundance of feed and water, and feed spillage. Clinical observations were carried out on each bird at least once daily. All birds that died during the study were subjected to gross necropsy examinations. Two animals per sex were selected from the control, 68.1, 147, 316 and 681 mg/kg bw dose level groups and subjected to complete necropsy examinations at the end of the study.
Time and intervals of body weight determination	The animals were individually weighed just prior to dosing, and on Days 3, 7, 14 and 21.

Table A7.5.3.1.1-4: Test conditions (housing)

Criteria	Details
Test temperature	23.3 ºC
Shielding of the animals	No
Ventilation	Not documented
Relative humidity	55%
Photoperiod and lighting	Natural daylight spectrum lights which were on 10 hours/day

Table A7.5.3.1.1-5: Mortality data of bobwhite quail in an acute oral LD_{50} study with 1,2-benzisothiazol-3-(2*H*)-one

Dosage level [mg/kg bw]	Mortality	
	Males	Females
Control	0/5	0/5
68.1	0/5	0/5
147	0/5	0/5
316	0/5	0/5
681	2/5 (Days 3 and 12)	2/5 (Days 3 and 12)
1470	5/5 (Day 1, 2, 3, 5, 8)	4/5 (Days 1 and 2)

Table A7.5.3.1.1-6: Average body weights (g) of bobwhite quail (both sexes) in an acute oral LD_{50} study with 1,2-benzisothiazol-3 (2*H*)-one

Day of study	Mean body weights (Mean ± SD)					
	Control	68.1 mg/kg bw	147 mg/kg bw	316 mg/kg bw	681 mg/kg bw	1470 mg/kg bw
0	195.55 ± 14.32	199.85 ± 12.32	194.55 ± 13.40	193.46 ± 10.54	195.07 ± 11.72	196.11 ± 12.92
3	196.27 ± 14.22	199.26 ± 11.93	191.18 ± 15.31	179.29* ± 13.00	168.68** ± 8.99	178.40 ± 5.84
7	194.78 ± 13.82	197.40 ± 11.35	188.27 ± 17.07	169.98 ** ± 19.54	156.11** ± 22.49	128.75 ± 21.14
14	197.61 ± 14.38	203.71 ± 10.89	196.30 ± 15.21	182.27 ± 22.05	180.62* ± 13.03	-
21	201.33 ± 14.60	206.40 ± 10.66	201.18 ± 14.71	191.24 ± 22.20	190.37 ± 6.73	-

* A statistically significant difference was noted at the 95% confidence level, using one-way analysis of variance
** A statitically significant difference was noted at the 99% confidence level, using one-way analysis of variance

Table A7.5.3.1.1-7: Average feed consumption of bobwhite quail (both sexes) in an acute oral LD_{50} study with 1,2-benzisothiazol-3-(2*H*)-one

Test days	Feed consumption (g/bird/day)					
	Control	68.1 mg/kg bw	147 mg/kg bw	316 mg/kg bw	681 mg/kg bw	1470 mg/kg bw
1-3	13.1	11.9	9.1	4.2	1.3	0.0
4-7	12.0	12.3	11.3	8.3	6.8	0.0
8-14	12.9	13.5	13.8	13.8	16.1	21.1
15-21	12.4	12.6	12.9	13.9	13.9	21.0
1-21	12.6	12.6	11.8	10.1	9.5	10.5

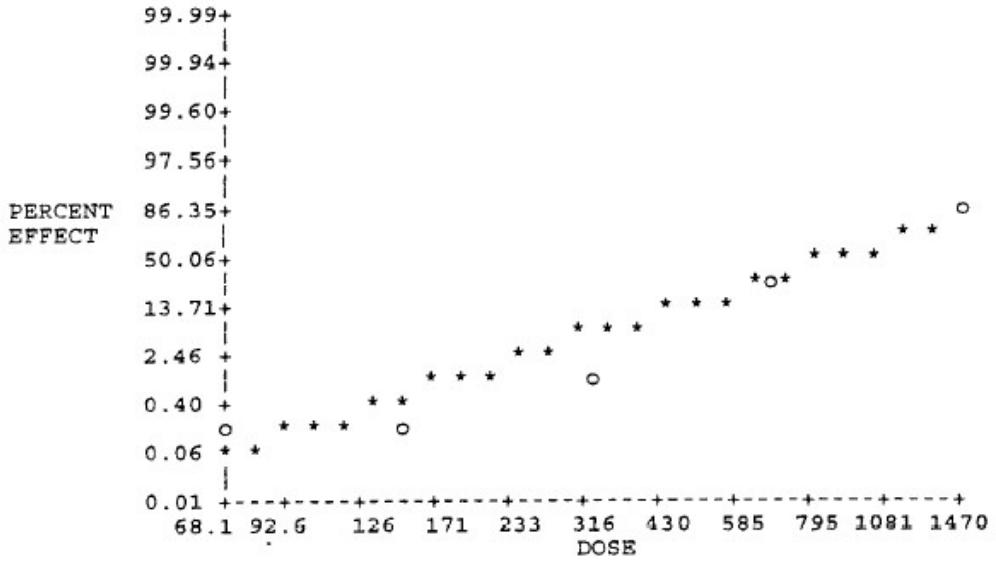

Figure A7.5.3.1.1-1: Acute avian oral toxicity study with 1,2-benzisothiazol-3-(2*H*)-one: LD_{50} calculation

Table A7.5.3.1.1-8: Validity criteria for avian acute oral toxicity test

	Fulfilled
Mortality of control animals < 10%	Yes

Section A7 Ecotoxicological Profile Including Environmental Fate and Behaviour

Subsection A7.5.3.1.2/1 SHORT-TERM TOXICITY ON BIRDS

Annex Point IIIA, XIII 1.2 **Bobwhite quail (*Colinus virginianus*)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] Dates of experimental work: October 23, 1997 – October 31, 1997.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Troy Chemical Company BV	
1.2.2	Companies with letter of access		
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the test method was based on U.S. EPA FIFRA Guideline Subdivision E, No. 71-2(a) "Acute Dietary LC_{50} Test for Waterfowl and Upland Game Birds", which is equivalent to OECD test Guideline 205, "Avian Dietary Toxicity Test".	
2.2	**GLP**	Yes (self-certified)	
2.3	**Deviations**	No	**X**
		3 METHOD	
3.1	**Test material**	[redacted]® (1,2-benzisothiazol-3-(2*H*)-one)	
3.1.1	Lot/Batch number	060793	
3.1.2	Specification	Please refer to Doc. III-A, 2/2	
3.1.3	Purity	99.29%	
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 A7.5.3.1.2/1 SHORT-TERM TOXICITY ON BIRDS

Annex Point IIIA, XIII 1.2 Bobwhite quail (*Colinus virginianus*)

3.1.6	Method of analysis	Not applicable
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	In food. Details are given in Table A7.5.3.1.2-1.
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	Bobwhite quail *(Colinus virginianus),* details are given in Table A7.5.3.1.2-2.
3.4.2	Test plants	Details are given in Table A7.5.3.1.2-3.
3.4.3	Test system	Purina® Game Bird Startena® mixed with nominal concentrations of [redacted] and delivered *ad libitum.* Composition of stock diet: crude protein 30%, crude fat 2.5% and crude fibre 3.5%. Details are given in Table A7.5.3.1.2-3.
3.4.4	Test conditions	Details are given in Table A7.5.3.1.2-4.
3.4.5	Test duration	Treated diets were fed to the birds for five consecutive days. After this, birds were offered untreated feed for a three-day recovery period.
3.4.6	Test parameter	Mortality
3.4.7	Sampling	Birds were weighed individually during the quarantine period, and on Days 0, 5 and 8. Feed consumption was recorded for each group during the last two days of the quarantine period, at the end of the five-day test period, and at the end of the three-day recovery period. Clinical observations were made at least once daily. Inspections were made daily for mortalities, abundance of feed and water and feed spillage. Four indiscriminately selected birds from the control groups and from each of the treated groups were subjected to gross pathological examinations at the termination of the study. Inspections of the GI tract, liver, kidneys, heart and spleen were made. Muscle and subcutaneous fat were examined for evidence of deterioration.

Section A7	Ecotoxicological Profile Including Environmental Fate and Behaviour
Subsection A7.5.3.1.2/1	SHORT-TERM TOXICITY ON BIRDS
Annex Point IIIA, XIII 1.2	Bobwhite quail (*Colinus virginianus*)

3.4.8	Method of analysis of the plant material	Statistical analysis of the body weights were performed using one-way analysis of variance.
		4 RESULTS
4.1	**Limit Test / Range finding test**	Not performed
4.1.1	Concentration / dose	Not applicable
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
4.1.3	Nature of adverse effects	Not applicable
4.2	**Results test substance**	
4.2.1	Applied concentrations	0 (control 1), 0 (control 2), 156, 312, 625, 1250, 2500 and 5000 mg/kg
4.2.2	Effect data (Mortality)	No mortalities were recorded during the study.
4.2.3	Body weight	Statistically significant decreases were observed in the 5000 mg/kg group at Days 5 and 8. Details are given in Table A7.5.3.1.2-5.
4.2.4	Food consumption	Reduced food consumption was observed in the 312, 625, 1250, 2500 and 5000 mg/kg groups during the test period. Food consumption in the 156 mg/kg group during the test period and all recovery periods in the test groups were comparable to the controls. Details are given in Table A7.5.3.1.2-6.
4.2.5	Concentration / response curve	Not applicable
4.2.6	Other effects	No clinical signs of toxicity were observed during the study or recovery periods. No gross abnormalities were observed in any of the 28 birds examined.
4.3	**Results of controls**	

Section A7 Subsection A7.5.3.1.2/1 Annex Point IIIA, XIII 1.2	Ecotoxicological Profile Including Environmental Fate and Behaviour SHORT-TERM TOXICITY ON BIRDS Bobwhite quail (*Colinus virginianus*)	
4.3.1 Number/ percentage of animals showing adverse effects	No adverse effects were observed in the control birds.	
4.3.2 Nature of adverse effects	Not applicable	
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	1,2-benzisothiazol-3-(2*H*)-one was administered in the diet of eight groups of 10 bobwhite quails/dose, for five consecutive days, at dose levels of 0 (control 1), 0 (control 2), 156, 312, 625, 1250, 2500 and 5000 mg/kg. This study was conducted according to U.S. EPA FIFRA Guideline Subdivision E, No. 71-2(a) "Acute Dietary LC_{50} Test for Waterfowl and Upland Game Birds" which is equivalent to OECD test Guideline 205, "Avian Dietary Toxicity Test", with no deviations and is described under point 3.	
5.2 Results and discussion	The test substance was stable in the diets. The dose verification corrected recovery results for the definitive study ranged from 101 to 125% of nominal; homogeneity corrected recovery results for the pre-test diets averaged 122% of nominal (156 mg/kg) and 102% of nominal (5000 mg/kg). No mortalities or clinical signs of toxicity were recorded during the study. Statistically significant decreases were observed in body weights in the 5000 mg/kg group at Days 5 and 8. No gross abnormalities were observed in any of the 28 birds examined. Reduced food consumption was observed in the 312, 625, 1250, 2500 and 5000 mg/kg groups during the test period. Food consumption in the 156 mg/kg group during the test period and all recovery periods in the test groups were comparable to the controls. There was no mortality in the control. The average percentage of the substance present in the diet was > 80% of initial concentration. The lowest treatment level did not result in compound-related mortality or other observable toxic effects.	
5.2.2 LC_0	> 5000 mg/kg (approximately > 940 mg/kg bw)	
5.2.3 LC_{50}	> 5000 mg/kg (approximately > 940 mg/kg bw)	

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.5.3.1.2/1	**SHORT-TERM TOXICITY ON BIRDS**
Annex Point IIIA, XIII 1.2	**Bobwhite quail (*Colinus virginianus*)**

5.2.4	LC_{100}	> 5000 mg/kg (approximately > 940 mg/kg bw)	
5.2.5	NOEC	156 mg/kg (approximately 30 mg/kg bw)	
5.3	**Conclusion**	The test met the validity criteria established in the recommended Guideline. The results of the acute dietary LC_{50} study conducted with 1,2-benzisothiazol-3-(2*H*)-one in bobwhite quail showed the median lethal concentration to be greater than 5000 mg/kg (approximately > 940 mg/kg bw).	
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2012.*
Materials and Methods	*Applicant's version is accepted with the following comments:* *2.3. There is a protocol deviation reported in Doc. IV-A, but not in the present document. All deviations from the protocol must be reported also in the summary test report. The deviation is the following:* *- During a portion of the quarantine period, the birds received a 14h/10h photoperiod instead of 16h/8h photoperiod recommended in the guidelines.*
Results and discussion	*Applicant's version is accepted with the following comments:* *LC_{50} could not be calculated because no mortality or clinical symptoms were observed during the test. In order to perform the test at more appropriate test substance concentrations, it is recommended to conduct a preliminary range-finding test.*
Conclusion	*The test met the validity criteria established in the recommended Guideline. The results of the acute dietary LC_{50} study conducted with 1,2-benzisothiazol-3-(2H)-one in bobwhite quail showed the median lethal concentration to be greater than 5000 mg/kg (approximately > 940 mg/kg bw). The NOEC was determined to be 156 mg/kg.*
Reliability	*1*
Acceptability	*Acceptable*

Section A7	**Ecotoxicological Profile Including Environmental Fate and Behaviour**
Subsection A7.5.3.1.2/1	**SHORT-TERM TOXICITY ON BIRDS**
Annex Point IIIA, XIII 1.2	**Bobwhite quail (*Colinus virginianus*)**
Remarks	*Key Study.*

Table A7.5.3.1.2-1: Method of administration of the test substance

Carrier / Vehicle	Details
Water	No
Organic carrier	No
Concentration of the carrier [% v/v]	Not applicable
Other vehicle	In food
Function of the carrier / vehicle	Not applicable

Table A7.5.3.1.2-2: Test animals

Criteria	Details
Species/strain	Bobwhite quail (*Colinus virginianus*)
Source	Sand Prairie Quail Farm, Iowa, USA
Age, sex and initial body weight (bw)	1-day old Sex not determined 18.7-30.9 g
Age range within the test	11-days old (first dosing) 16-days old (end of dosing)
Breeding population	Not documented
Age at time of first dosing	11-days old
Health condition / medication	Prior to the experimental start date, all birds were examined and found to be suitable for testing, based on general physical condition.

Table A7.5.3.1.2-3: Test system

Criteria	Details
Test location	Indoor in holding pens
Holding pens	Brooders, 91 cm long x 71 cm wide x 28 cm high
Number of animals	80 birds
Number of animals per pen [cm²/bird]	10 birds per brooder [646 cm^2/bird]
Number of animals per dose	10 birds
Pre-treatment / acclimation	The birds were placed on a ten-day quarantine period. All birds were fed Purina® Game Bird Startena® and natural well water, ad libitum.
Diet during test	Purina® Game Bird Startena®, ad libitum sourced from Purina Mills Inc., Missouri, USA. For each test level mix, the appropriate amounts of test material and stock diet were mixed to form a premix. This premix was then added to the appropriate amount of stock diet and mixed together. An additional amount of stock diet was then added and the entire batch was mixed. The test diets prepared for the definitive test were employed throughout the five-day test period. After this period, birds were offered untreated feed for a three-day recovery period.
Dosage levels (of test substance)	Two control groups, 156, 312, 625, 1250, 2500 and 5000 mg/kg. Test diets were fed to the bobwhite quail for five consecutive days.
Replicate/dosage level	No
Dosing method	Mixed in stock diet
Dosing volume per application	Not applicable
Frequency, duration and method of animal monitoring after dosing	Feed consumption was recorded for each group during the last two days of the quarantine period, at the end of the five-day test period, and at the end of the three-day recovery period. Clinical observations were made at least once daily. Inspections were made daily for mortalities, abundance of feed and water and feed spillage. Four indiscriminately selected birds from the control groups and from each of the treated groups were subjected to gross pathological examinations at the termination of the study.
Time and intervals of body weight determination	Birds were weighed individually during the quarantine period, and on Days 0, 5 and 8.

Table A7.5.3.1.2-4: Test conditions (housing)

Criteria	Details
Test temperature	22.8 ºC (mean)
Shielding of the animals	No
Ventilation	Not documented
Relative humidity	45%
Photoperiod and lighting	Natural daylight spectrum lighting, 14 hours per day during the first approximate 8 days of the quarantine period and 16 hours per day thereafter.

Table A7.5.3.1.2-5: Average body weights (g) of bobwhite quail in an acute dietary LC_{50} study with 1,2-benzisothiazol-3-(2*H*)-one

Day of study	Mean body weights (Mean ± SD)							
	Control 1	Control 2	156 mg/kg	312 mg/kg	625 mg/kg	1250 mg/kg	2500 mg/kg	5000 mg/kg
0	24.96 ± 2.78	24.38 ± 2.77	25.63 ± 2.35	25.19 ± 2.70	24.67 ± 2.67	24.35 ± 3.18	24.72 ± 3.28	24.34 ± 2.55
5	40.29 ± 3.91	38.53 ± 4.42	40.74 ± 3.71	39.75 ± 4.26	39.36 ± 2.72	38.35 ± 3.98	37.83 ± 4.19	32.15* ± 3.34
8	50.0 ± 4.44	48.67 ± 5.66	50.4 ± 4.38	50.27 ± 4.87	49.25 ± 3.02	47.67 ± 4.72	48.65 ± 5.50	43.55* ± 4.25

* A statistically significant difference was observed ($p < 0.05$), using one-way analysis of variance

Table A7.5.3.1.2-6: Average feed consumption of bobwhite quail in an acute dietary LC_{50} study with 1,2-benzisothiazol-3-(2*H*)-one

Test Days	Average feed consumption (g/bird/day)							
	Control 1	Control 2	156 mg/kg	312 mg/kg	625 mg/kg	1250 mg/kg	2500 mg/kg	5000 mg/kg
0-5	11.6	11.5	11.8	6.5	6.3	6.0	6.1	4.7
6-8	8.1	8.0	8.2	8.3	8.7	8.0	9.1	7.7

Table A7.5.3.1.2-7: Validity criteria for short-term toxicity test according to OECD 205

	Fulfilled
Mortality of control animals < 10%	Yes
Test substance concentration > 80 % of nominal concentration throughout the dosing period	Yes
Lowest treatment level causing no compound-related mortality or other observable toxic effects	Yes

Section A7 **Subsection A7.5.3.1.3** **Annex Point IIIA 13.1.3**	**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:	Although the results obtained in the acute avian toxicity testing (please refer to point IIIA 7.5.3.1.1/1) demonstrate slight toxicity to birds as well as several recorded mortalities, the results of the short-term avian dietary toxicity test (please refer to point IIIA 7.5.3.1.2/1) indicate low toxicity and no mortalities. This would suggest that any effect of the active substance on birds is transient and consequently it is proposed that a study to address this point is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*August 2010*	
Evaluation of applicant's justification	*Accept applicant's justification*	
Conclusion	*Accept applicant's justification*	
Remarks		

Section A7 **Subsection A7.5.4.1** **Annex Point IIIA 13.3.1**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **ACUTE TOXICITY TO HONEYBEES AND OTHER BENEFICIAL ARTHROPODS, FOR EXAMPLE PREDATORS**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [X]**	
Detailed justification:	According to the "Data requirements for biocidal product types, Version 4.3.2 (October 2000)", this test may only be required for certain product types, e.g. insecticides, acaricides and substance in products to control other arthropods. As BIT is not used for this purpose, nor is it intended for direct application to plants or flowers where bees may be actively foraging, it is proposed that a study to address this point is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE FI	
Date	*August 2010*	
Evaluation of applicant's justification	*Accept applicant's justification*	
Conclusion	*Accept applicant's justification*	
Remarks		

Section A7 **Subsection A7.5.5** **Annex Point IIA 7.5**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **BIOCONCENTRATION, TERRESTRIAL** **Bioconcentration, further studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	It is proposed that this point is not relevant to BIT as it is not applied directly to the soil or to watercourses and based on the physico-chemical properties of BIT, there is little or no risk of bioconcentration in terrestrial species. According to the Technical Guidance Document (TGD) on Risk Assessment (ECB Part II, 2003), substances with a log K_{ow} < 3 are unlikely to bioaccumulate and the risk of secondary poisoning is minimal. BIT has an estimated log K_{ow} of 1.4, indicating little or no risk of secondary poisoning to mammalian species or bioaccumulation in the food chain. The bioconcentration factor (BCF) for earthworms estimated by the USES modelling system is only 1.81 kg/kg which indicates that bioconcentration in the terrestrial compartment is highly unlikely. However, in the unlikely event that the product enters the terrestrial compartment by accidental discharge, an investigation into the effects on the terrestrial environment indicated minimal risk. Furthermore, a risk assessment has been carried out to investigate the potential for secondary poisoning in birds and mammals as a result of the consumption of earthworms exposed to BIT (Doc. II-C, 2.4). The results were found to be well below the trigger value of 1, indicating negligible risk of secondary poisoning in the terrestrial food chain. As minimal direct exposure of the terrestrial compartment is expected to occur and BIT is expected to degrade rapidly in the soil environment (DT_{50} in soil < 1 day) it can be assumed that there is little risk of bioconcentration in the terrestrial compartment as a result of exposure to BIT. Based on the information provided above, it can be concluded that further studies are not required in this case.	
Undertaking of intended data submission [ ]	Not applicable	

Section A7 **Subsection A7.5.5** **Annex Point IIA 7.5**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **BIOCONCENTRATION, TERRESTRIAL** **Bioconcentration, further studies**
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2010*
Evaluation of applicant's justification	*Accept the applicant's justification with the following comment:* *The bioconcentration factor (BCF) for earthworms estimated by the EUSES 2.1 modelling system is only 1.14 kg/kg which indicates that bioconcentration in the terrestrial compartment is highly unlikely.*
Conclusion	*Acceptable.*
Remarks	

Section A7 Subsection A7.5.6 Annex Point IIIA 13.3	Ecotoxicological Profile Including Environmental Fate and Behaviour EFFECTS ON OTHER TERRESTRIAL NON-TARGET ORGANISMS	
	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:	Field testing is only required where the risk assessment based on long-term terrestrial tests shows that there is still a concern for the terrestrial compartment. A risk assessment has been carried out (Doc. II-C), which indicates little or no risk to the terrestrial compartment through the use of BIT and as a result no acute terrestrial testing is required. Long-term and field testing are therefore also waived as no significant or prolonged exposure is expected in the terrestrial compartment as a result of the use of BIT according to recommended practices. BIT is applied indoors and not directly to plants or soil or to watercourses and based on the physico-chemical properties of BIT, there is little or no risk of bioconcentration in terrestrial species. As direct exposure of the terrestrial compartment does not occur and BIT is expected to degrade rapidly in the soil environment (DT_{50} in soil < 1 day, inherently biodegradable) it can be assumed that there is little risk of adverse effects in the terrestrial compartment as a result of exposure to BIT. Based on the information above, it is therefore proposed that further studies on non-target organisms are not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*May 2011*	
Evaluation of applicant's justification	*Applicant's justification is accepted.*	
Conclusion	*Applicant's justification is acceptable.*	
Remarks		

Section A7 **Subsection A7.5.7.1.1** **Annex Point IIIA 13.3.4**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON MAMMALS** **Acute oral toxicity**	
	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:	Data from the studies, "Acute oral toxicity study with [redacted] in rats", [redacted] (1993) and "Acute oral toxicity study of BIT in rats", [redacted] (2003a), are presented under points IIIA 6.1.1-1 and IIIA 6.1.1-2, respectively. The acute oral LD_{50} of BIT in rats was found to be 1010 mg/kg bw (for combined sexes) and 597.40 mg/kg bw, in each study respectively. It would be expected that terrestrial vertebrates would exhibit similar toxic responses on exposure to BIT as the mammals tested in the above named studies.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*August 2010*	
Evaluation of applicant's justification	*Accept applicant's justification*	
Conclusion	*Accept applicant's justification*	
Remarks		

Section A7 **Subsection A7.5.7.1.2** **Annex Point IIIA 13.3.4**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** **EFFECTS ON MAMMALS** **SHORT TERM TOXICITY**	
	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:	Data from the studies, "Repeated dose 90-day oral toxicity study of BIT in rats", [redacted] (2003) and "A 90-day dietary toxicity study of 1,2-Benzisothiazolin-3-one in beagle dogs", [redacted] (2007), are presented under points IIIA 6.4.1-1 and IIIA 6.4.1-2, respectively. The NOAELs of BIT in rats and dogs were found to be 27.5 mg/kg bw and 37.5 mg/kg bw (combined sexes), respectively. It would be expected that terrestrial vertebrates would exhibit similar toxic responses on exposure to BIT as the mammals tested in the above named studies.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*August 2010*	
Evaluation of applicant's justification	*Accept applicant's justification*	
Conclusion	*Accept applicant's justification*	
Remarks		

Section A7.6 Annex Point IIA 7.8	Ecotoxicological Profile Including Environmental Fate and Behaviour SUMMARY OF ECOTOXICOLOGICAL EFFECTS AND FATE AND BEHAVIOUR IN THE ENVIRONMENT	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Please refer to Doc. II-A.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*August 2010*	
Evaluation of applicant's justification	*Accept applicant's justification*	
Conclusion	*Accept applicant's justification*	
Remarks		

Section A8	Measures necessary to protect man, animals and the environment	
Subsection (Annex Point)		**Official use only**
8.1	**Recommended methods and precautions concerning handling, use, storage, transport or fire** **(IIA8.1)**	

Section A8 Measures necessary to protect man, animals and the environment

8.1.0	Methods and precautions concerning placing on the market	Professional users of the product are assumed as trained, skilled, healthy adults with workplace risk assessments and controls for residual risk, with access to personal protective equipment.
8.1.1	Methods and precautions concerning production, handling and use of the active substance and its formulations	Put on appropriate personal protective equipment. Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Do no get in eyes or on skin or clothing. Do not breathe vapour or mist. Do not ingest. Use only with adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Keep in the original container or an approved alternative made from a compatible material, kept tightly closed when not in use. Keep away from acids. Empty containers retain product residue and can be hazardous. Do not re-use container.
8.1.2	Methods and precautions concerning storage of the active substance and its formulations	Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials and food and drink. Separate from acids. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.
8.1.3	Methods and precautions concerning transport of the active substance and its formulations	UN/NA Number: UN3266 Class: 8 Packing Group: III Proper Shipping Name: Corrosive liquid, basic, inorganic, n.o.s. (Contains: Sodium hydroxide)

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8.1.4	Methods and precautions concerning fire of the active substance and its formulations	Suitable Extinguishing Media:	An extinguishing agent suitable for the surrounding fire
		Specific Hazards:	In a fire, or if heated, a pressure increase will occur and the container may burst. Decomposition products may include carbon oxides, nitrogen oxides, sulfur oxides and metal oxide/oxides.
		Fire fighting advice:	Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. No action shall be taken involving any personal risk or without suitable training. Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.
	8.2	**Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment if available; emergency measures to protect the environment (IIB8.2)**	
8.2.1	Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment if available	If in eyes:	Get medical attention immediately. Immediately flush eyes with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Continue to rinse for at least 10 minutes. Chemical burns must be treated promptly by a physician.
		If on skin:	Get medical attention immediately. Flush contaminated skin with plenty of water. Remove contaminated clothing and shoes. Wash contaminated clothing thoroughly with water before removing or wear gloves. Continue to rinse for at least 10 minutes. Chemical burns must be treated promptly by a physician. Wash clothing before reuse. Clean shoes thoroughly before reuse.
		If swallowed:	Get medical attention immediately. Wash out mouth with water. Remove dentures if any. Move exposed person to fresh air. Keep person warm and at rest. It material has been swallowed and the exposed person is conscious, give small quantities of water to drink. Stop if the exposed person feels sick as vomiting may be dangerous. Do not induce vomiting unless directed to do so by medical personnel. If vomiting occurs, the head should be kept low so that vomit does not enter the lungs. Chemical burns must be treated promptly by a physician. Never give anything by mouth to an unconscious person. If unconscious,

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		place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband. If inhaled: Get medical attention immediately. Move exposed person to fresh air. If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus. Keep person warm and at rest. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. It may be dangerous to the person providing aid to give mouth-to mouth resuscitation. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie belt or waistband. In case of inhalation of decomposition products in a fire, symptoms may be delayed. The exposed person may need to be kept under medical surveillance for 48 hours.
8.2.2	Emergency measures to protect the environment	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewer, waterways, soil or air). Stop leak if without risk. Move containers from spill area. Approach release from upwind. Prevent entry into sewers, water courses, basements or confined areas. Wash spillages into an effluent treatment plant or proceed as follows. Contain and collect spillage with non-combustible, absorbent material e.g. sand, earth, vermiculite or diatomaceous earth and place in container for disposal according to local regulations. Dispose of via a licensed waste disposal contractor. Contaminated absorbent material may pose the same hazard as the spilled product.
	8.3	**Procedures, if any, for cleaning application equipment (IIB8.3)**
		Not applicable, as no application equipment is required.
	8.4	**Identity of relevant combustion products in cases of fire (IIB8.4)**
		Decomposition products may include carbon oxides, nitrogen oxides, sulfur oxides and metal oxide/oxides.
	8.5	**Procedures for waste management of the active substance for industry or professional users e.g. possibility of re-use or recycling, neutralisation, conditions for controlled discharge, and incineration (IIA8.5)**

Section A8		Measures necessary to protect man, animals and the environment	
		Waste must be disposed of in accordance with federal, state and local environmental control regulations. When being disposed of in its unused and uncontaminated state it should be treated as a hazardous waste according to EC Directive 91/689/EEC.	
	8.6	**Possibility of destruction or decontamination following release in or on the following: (a) Air; (b) Water, including drinking water; (c) Soil (IIB8.6)**	
		According to the data presented in Section A 7.5, undesired and unintended side-effects for non-target organisms and vertebrates are not expected to be caused by 1,2-Benzisothiazol-3-(2*H*)-one.	
8.6.1	Possibility of destruction or decontamination following release in the air	[redacted] is used primarily indoors. In addition the active substance has a vapour pressure of 1.5×10^{-4} Pa and is not considered volatile. Therefore, contamination following release into air is unlikely.	
8.6.2	Possibility of destruction or decontamination following release in water, including drinking water	[redacted] is used primarily indoors. Therefore, contamination following release into water is unlikely. If contamination does occur, transfer the material to a convenient waste disposal container.	
8.6.3	Possibility of destruction or decontamination following release in or on soil	[redacted] is used primarily indoors. Therefore, contamination following release in or on soil is unlikely. If contamination does occur, use a shovel to transfer the material to a convenient waste disposal container.	
	8.7	**Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms (IIB8.7)**	
		According to the data presented in Section B 7.8, undesired and unintended side-effects for non-target organisms and vertebrates are not expected to be caused by [redacted].	
	8.8	**Specify any repellents or poison control measures included in the preparation that are present to prevent action against non-target organisms (IIB8.8*)***	
		None.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Section A8 Measures necessary to protect man, animals and the environment

Date	*October 2011.*
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	*Accepted.*
Remarks	

Section A9 Classification and Labelling

Annex Point IIA, IX

Subsection (Annex point)			Official use only
	9.1	**Current classification**	
Classification		as in Directive 67/548/EEC	
Class of danger		Xi, Xn, N	
R phrases		R22, R38, R41, R43, R50	
S phrases		S24, S26, S37/38, S61	
	9.2	**Proposed classification**	
Symbol		Xn Xi N	
Risk phrase(s)		R22: Harmful if swallowed R41: Risk of serious damage to eyes R43: May cause sensitisation by skin contact R50: Very toxic to aquatic organisms	
Safety phrase(s)		S24: Avoid contact with skin S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice S29: Do not empty into drains S37/39: Wear suitable gloves and eye/face protection S45: In case of accident or if you feel unwell seek medical advice immediately (show the label where possible) S61: Avoid release to the environment. Refer to special instructions/safety data sheets	

Classification	as in Regulation (EC) No 1272/2008	X
Pictograms		
Hazard class and category: Hazard statement	Danger, Acute Toxicity 4, Eye Damage 1, Skin sensitization 1 Aquatic Acute 1 H302, H318, H317; H400, H410	

Section A9 Classification and Labelling

Annex Point IIA, IX

Subsection (Annex point)	Official use only

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*May 2020*
Materials and Methods	
Results and discussion	
Conclusion	*Acute Inhalation Toxicity (H330) and Aquztic Chronic should be added. M-factor = 1 should be added. Skin sensitization should becategorized as 1B. GHS06 should be added.*
Reliability	
Acceptability	
Remarks	

Section A10	Summary and Evaluation of Sections 2 to 9	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	A summary of IIIA 2 to IIIA 9 is provided in IIA.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*March 2015*	
Evaluation of applicant's justification		
Conclusion	*accepted*	
Remarks		