Section A7.1.1.1.1 Annex Point IIA7.6.2.1	**Hydrolysis as a function of pH and identification of breakdown products**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [X]** **Other justification [ ]**	
Reference	[redacted] (1999) Degradation - Abiotic degradation: Hydrolysis as a function of pH. [redacted] 1st Amendment to Analytical Report [redacted], unpublished, [redacted] 1999, BPD ID A7.01.1.1.1_01	
Detailed justification:	Hydrolysis as a function of pH According to [redacted] (1999), the required pH values for the hydrolysis test are pH 4.0, 7.0 and 9.0. At these pH values, dialdehydes such as glyoxal are stable against hydrolysis. Therefore no tests were carried out. The author reported that Dialdehydes, especially glyoxal and methylglyoxal, were intensively examined regarding their hydrolysis behaviour already in the years 1920 – 1930. It was observed that glyoxal is stable against hydrolysis at pH values < 7.5 [2, 3, 4]. In aqueous glyoxal solutions, stable oligomeric or polymeric glyoxal species are in equilibrium with monomeric glyoxal, and accordingly with its hydrated form (ethane bis-gemdiol) [4, 5]. With increasing alkalinity glycolic acid is formed, which becomes first detectable at pH 11, and is rapidly formed at pH 12. This alkali influence is interpreted as the shift of the equilibrium between polyglyoxal and monoglyoxal and the increasing tendency towards enolization with increasing pH value [4]. The mechanism of the internal disproportionation of glyoxal to glycolic acid at high pH values was investigated in D_2O [6]. It was found that also at the internal Cannizzaro reaction the hydrogen is transferred directly from one carbon to the other. Glyoxal does not exchange with slightly alkaline D_2O, since a possible exchange (i.e. hydrolysis) should be manifested in a deuterium content of the glycolic acid. Thus, since the conduct of a further hydrolysis study is not expected to bring further informations on the hydrolysis of glyoxal as a function of pH, no such study was attempted. References: [1] [redacted] (1999) Degradation - Abiotic degradation: Hydrolysis as a function of pH. [redacted] 1st Amendment to Analytical Report [redacted], unpublished, [redacted] 1999, BPD ID A7.01.1.1.1_01 [2] Ariyama N (1928) Studies on glyoxals. J. Biol. Chem. 77, 359-394 [3] Friedemann TE (1927) The action of alkali and hydrogen peroxide on glyoxals. J. Biol. Chem. 73, 331-334 [4] Sakuma F (1931) The fate of the glyoxals in the animal body. J. Biochem. Tokyo 13(8), 423-440 [5] Shaffer PA, Friedemann TE (1924) Antiketogenesis - V. The ketolytic reaction; action of glycol aldehyde and of glyoxal. J. Biol. Chem. 61, 585-623 [6] Whipple EB (1970) The structure of glyoxal in water. J. Am. Chem. Soc. 92(24), 7183-7186 [7] Fredenhagen H, Bonhoeffer KF (1938) Untersuchungen über die CANNIZZAROsche Reaktion in schwerem Wasser. Z. Physik. Chem. [A] 180, 379-391	
Undertaking of intended data submission [ ]	Not relevant	

Section A7.1.1.1.1 Annex Point IIA7.6.2.1	**Hydrolysis as a function of pH and identification of breakdown products**
	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date	08/02/2018
Evaluation of applicant's justification	Literature data underline the stability of glyoxal against hydrolysis due to its chemical reactive properties in water at environmentally relevant pH.
Conclusion	Applicant's justification is acceptable.
Remarks	
	Comments from other member state *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.1.1.2 **Annex Point IIA, VII.7.6.2.2.**	**Phototransformation in water including identity of transformation products**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Direct photolysis in water can be a relevant process for removal of light absorbing, non biodegradable organic substances [1]. Glyoxal is readily biodegradable according to OECD criteria [2]. Following the Technical Guidance Document [TGD, 1], a first order rate constant for microbial mineralization in surface water of 4.7 x 10^{-2} [d^{-1}] is assigned to a substance which is readily biodegradable. The relevance of direct photodegradation in water can be discussed as well as the photochemical transformation mechanism in principle. Since glyoxal absorbs light slightly in the visible spectra (>290 nm), such a degradation mechanism is basically conceivable and leads at least to the formation of formaldehyde [3]. Under illumination conditions at 20 °C it was shown that glyoxal spontaneously oxidizes to glyoxylic acid and supposedly to formaldehyde [4]. Glyoxylic acid functions as a central metabolite of the anaplerotic sequence in the tricarboxylic acid cycle. Formaldehyde itself is also readily biodegradable [5]. Under environmental conditions in surface water the hydrated monomer (ethane bis-gemdiol) is the main form of glyoxal in water. This gemdiol tends to polymerize to acetals-semiacetals. However, in the environment at low realistic concentrations it can be assumed that only the monomer is present [6]. This mechanism is also in line with the opinion of the European Commission [7]. Light absorption of organic compounds in the wavelength range of 290 – 600 nm is in most cases associated with the presence of a delocalized л–electron system. Hence, aromatic rings and conjugated double bonds may form a chromophore structural moiety. For this reason, a gemdiol functional group is not a chromophore and does not adsorb light in the vis absorption spectrum. The gemdiol monomers are the main products of glyoxal in aqueous solutions as well as the potentially formed formaldehyde which is in equilibrium with the aldehyde functional group. Therefore, photodegradation processes are of less importance under these chemical aspects. In conclusion, glyoxal and its potential photolytic degradation product formaldehyde are both readily biodegradable. In aqueous solution, the main forms of glyoxal or formaldehyde are the hydrated monomers, which have no delocalized л–electron system and therefore do not essentially absorb light above 290 nm. For these reasons, it can be assumed that photodegradation processes in water are of low relevance [8]. <u>References:</u> [1] EC (European Commission, 2003) Technical Guidance Document on Risk Assessment, Part III, EUR 20418 EN/3, ECB, Ispra, Italy [2] [redacted] (1996) Determination of the Biodegradability of [redacted] in the DOC Die-Away Test. [redacted] (unpublished), BPD ID A7.1.1.2.1_01 [3] Jarret M, Bermond A, Ducauze CJ (1986) Elimination du glyoxal et de l'acide glyoxylique par filtration sur charbon actif en grains. Sciences de L'eau 5, 377-400 [4] BUA Report 187: Glyoxal (Ethanedial), GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance (BUA), Feb 1996; Publisher: S. Hirzel Wissenschaftliche Verlagsgesellschaft, ISBN 3-7776-0824-6, Stuttgart (1998); BPD ID A4_01	

Section A7.1.1.1.2 **Annex Point IIA, VII.7.6.2.2.**	**Phototransformation in water including identity of transformation products**
	[5] Gerike P, Gode P (1990) The biodegradability and inhibitory threshold concentration of some disinfectants. Chemosphere 21(6), 799-812; BPD ID A7.1.1.2.1_02 [6] OECD (2001), SIDS Dossier on Glyoxal, SIAM 11 [7] EU (2005) Scientific Committee on Consumer Products (SCCP) of the European Commission, Opinion on glyoxal, SCCP/0881/05 [8] [redacted] (2007) Justification for non-submission of photodegradation in water. [redacted] 2007, BPD ID A7.1.1.1.2_01
Undertaking of intended data submission [ ]	Not relevant
	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date*08/02/2018*	
Evaluation of applicant's justification	Agree
Conclusion	Applicant's justification is acceptable
Remarks	
	Comments from other member state *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.1.2.1_01 Biodegradability (ready)

Annex Point IIA7.6.1.1

		Official use only
	1 REFERENCE	
1.1 Reference	[redacted] (1996) Determination of the Biodegradability of [redacted] in the DOC Die-Away Test. [redacted] (unpublished), BPD ID A7.1.1.2.1_01	
1.2 Data protection	Yes	
1.1 Data owner	[redacted]	
1.2 Companies with letter of access	[redacted]	
1.3 Criteria for data protection	Data on new a.s. for first entry to Annex I/IA	
	2 GUIDELINES AND QUALITY ASSURANCE	
1.3 Guideline study	Yes, Annex method C.4-A of Directive 92/69/EEC corresponding to OECD TG 301 A and ISO 7827:1992.	
1.4 GLP	Yes	
1.5 Deviations	No	
	3 MATERIALS AND METHODS	

Section A7.1.1.2.1_01 Biodegradability (ready)

Annex Point IIA7.6.1.1

1.6 Test material	[redacted] (1,2-ethanedial)	
3.1 Lot/Batch number	[redacted]	
3.2 Specification	As given in section 2	
3.3 Purity	[redacted]	
3.4 Further relevant properties	Not relevant	
3.5 Composition of Product	Additive: water, [redacted]	
3.6 TS inhibitory to microorganisms	No	
3.7 Specific chemical analysis	No compound specific analytical technique was applied	
1.7 Reference substance	Yes, aniline	
3.8 Initial concentration of reference substance	20 mg DOC/L	
1.8 Testing procedure		
3.9 Inoculum / test species	For details of inoculum see table A7_1_1_2-2	
3.10 Test system	For details on test type, laboratory equipment etc. see table A7_1_1_2-3	
3.11 Test conditions	For relevant test conditions see table A7_1_1_2-4	
3.12 Method of preparation of test solution	Not appropriate	
3.13 Initial TS concentration	121 mg/L equivalent to 20 mg DOC/L	X
3.14 Duration of test	19 days	
3.15 Analytical parameter	DOC removal	
3.16 Sampling	0, 1, 3, 5, 6, 8, 11, 12, 14, 18, and 19 days	
3.17 Intermediates/ degradation products	Not identified	
3.18 Nitrate/nitrite measurement	No	
3.19 Controls	Blank control (BC), abiotic control (PC), inhibition control (IH), adsorption control (AC)	
3.20 Statistics	The percentage degradation at each sampling time was calculated separately for both replicates taking into account the blank control for the respective sampling time. Results refer to the initial concentration (DOC removal).	

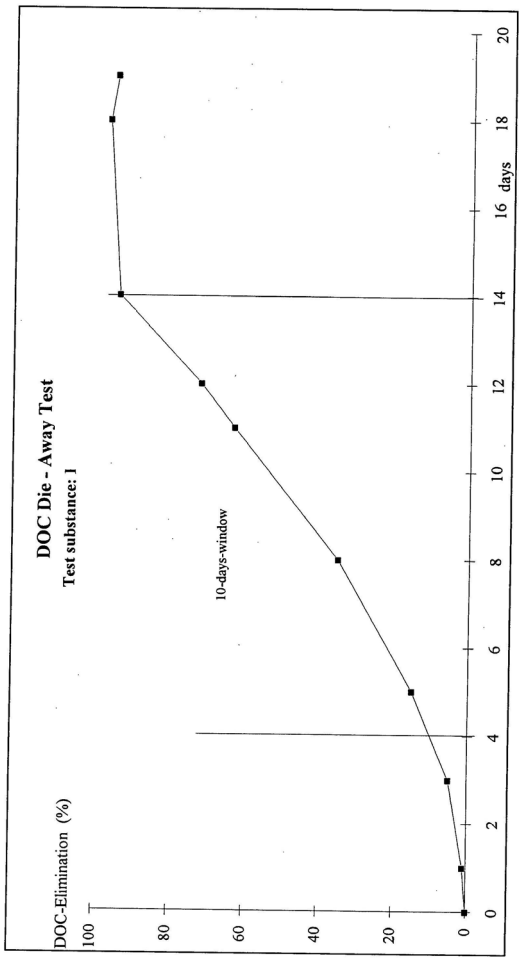
4 RESULTS

1.9 Degradation of test substance

Section A7.1.1.2.1_01 Biodegradability (ready)

Annex Point IIA7.6.1.1

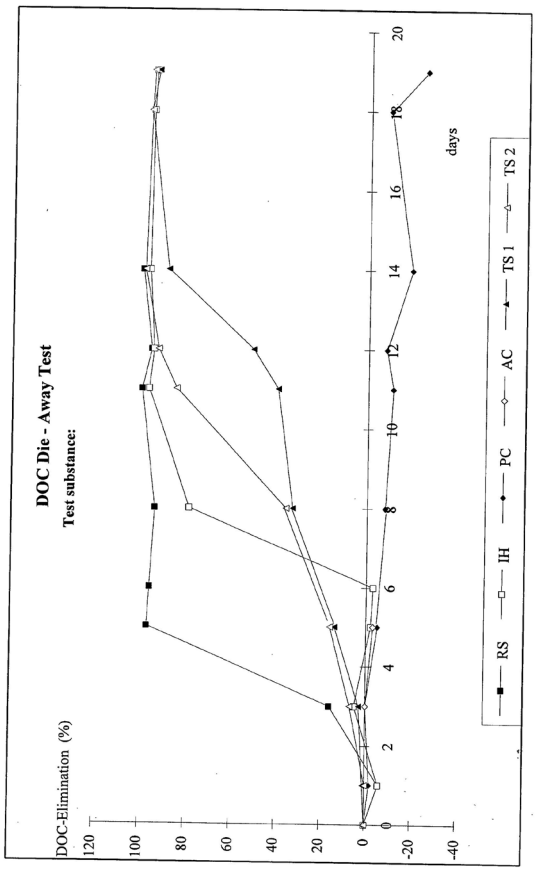
4.1 Graph



Section A7.1.1.2.1_01 Biodegradability (ready)

Annex Point IIA7.6.1.1

4.2 Degradation	> 90% degradation at plateau, after 10-d window (both reached on day 14) and at the end of incubation (day 19)
4.3 Other observations	No inhibitory effects were observed.
4.4 Degradation of TS in abiotic control	< 10 % after 19 days
4.5 Degradation of reference substance	



RS = reference substance; IH = inhibition control; PC = abiotic control; AC = adsorption control; TS = test substance

Section A7.1.1.2.1_01 Biodegradability (ready)

Annex Point IIA7.6.1.1

4.6	Intermediates/ degradation products	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
1.10	**Materials and methods**	The aim of the present study was to investigate the biodegradability of glyoxal in the DOC Die-Away Test, which is a static method for the determination of the ultimate aerobic biodegradation of a test substance in water. Test substance: [redacted] (1,2-ethanedial), purity [redacted] The test was performed according to OECD TG 301 A (1993) under GLP conditions. The test substance as well as the reference substance aniline was tested at a concentration of 20 mg DOC/L. Activated sludge of laboratory waste water treatment plants fed with municipal and synthetic sewage (80:20 mixture) was used as inoculum (30 mg/L dry weight). The test or reference substance and the inoculum were mixed together and aerated for 19 days at 20-25°C. Samples were taken on days 0, 1, 3, 5, 6, 8, 11, 12, 14, 18, and 19 to measure the DOC concentrations with a DOC analyser. A blank control, an inhibition control, an abiotic control and an adsorption control were included in the test. Measurements of the DOC concentration taken from the two replicates per test unit were done separately from each other and were averaged to the mean percentage of degradation.	X
1.11	**Results and discussion**	The adaptation phase lasted for 3 days and the degradation phase lasted for 11 days. On day 14, 94 % of the initial glyoxal was eliminated from water. The 10-days window was met. At the end of the test, after 19 days, the test substance was degraded to 97 %. The degradation of the reference substance aniline was 97 % after 5 days. Elimination of the test substance by abiotic processes or adsorption was less than 10 % after 19 and 5 days, respectively. The report states no deviations from the test guideline and the validity criteria for testing of ready biodegradability were fulfilled.	X
1.12	**Conclusion**	More than 90 % of the initial glyoxal (20 mg/L DOC) was eliminated from water after 19 days. The 10-days window was met. As neither toxicity nor abiotic degradation was observed in the controls at the concentrations tested, and as the reference substance proofed the validity of the test system, glyoxal can be regarded as readily biodegradable in this test system.	
5.1	Reliability	[redacted]	
5.2	Deficiencies	No	

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date	15/02/2018

Section A7.1.1.2.1_01 Biodegradability (ready)

Annex Point IIA7.6.1.1

Materials and Methods	No information is provided on pH and the darkness conditions. pH is know to impact the oligomer equilibrium. However, at low environmentally realistic concentrations, it can be assumed that only the hydrated monomer II is present (see DocV_2). Then, the e-fate and ecotoxicological studies (conducted at high dilution rate) can be considered as relevant in spite of unknow pH. Furthermore, a QSAR analysis (BIOWIN 4.10) with the hydrates was performed showing that all compounds which are in equilibrium with Glyoxal are also readily biodegradable. Photolytic processes are of less importance since the structural properties of the gemdiol, as stated in A7.1.1.1.2. "a gemdiol functional group is not a chromophore and does not adsorb light in the vis absorption spectrum. Consequently, the darkness conditions shouldn't have an impact on the result. In line with the OECD 301A requirements.
Results and discussion	1.10: According to the OECD TG 301, measurements of the DOC concentration in samples from each flask have to be done in duplicate. Additionally, at least 2 flasks containing the test substance plus inoculum, and at least 2 containing inoculum only (blank control) should be used. In the study report, DOC in the blank control was measured in duplicate in only one flask. 1.11: Note that variations higher than 20%, 53% at day 11 and 45% day 12 were observed between the test substance duplicates. However, since this variation is below 20% at the end of the study, eCA considers the criteria is fulfilled.
Conclusion	Agree, Glyoxal can be regarded as readily biodegradable in this test system
Reliability	[redacted]
Acceptability	
Remarks	Considering the hydrated monomeric form II of glyoxal, the TS initial concentration of 121 mg/L is equivalent to 12 mg DOC.
	Comments from …
Date	*Give date of 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*
Remarks	

Table A7_1_1_2_01-1: 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_01-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Source	Laboratory waste water treatment plants, fed with municipal and synthetic sewage
Laboratory culture	Yes
Method of cultivation	Laboratory waste water treatment plant
Preparation of inoculum for exposure	Washing and centrifugation in accordance with guideline; 80:20 (% v/v) mixture of municipal and synthetic sewage
Pretreatment	Not performed
Initial cell concentration	30 mg suspended solids/L (dry weight)

Table A7_1_1_2_01-3: Test system

Criteria	Details
Culturing apparatus	Shaken flasks cultured under aerobic conditions and constant temperature; DOC analyser
Number of culture flasks/concentration	2 replicates/concentration
Aeration device	Aerated up to 28 days as prescribed by the guideline
Measuring equipment	DOC-analyzer: [redacted]
Test performed in closed vessels due to significant volatility of TS	Not indicated due to the low volatility of glyoxal.

Table A7_1_1_2_01-4: Test conditions

Criteria	Details
Composition of medium	In accordance with guideline
Additional substrate	No
Test temperature	In accordance with guideline (20-25°C)
pH	Not specified
Aeration of dilution water	Not specified
Suspended solids concentration	30 mg/L
Other relevant citeria	No

Table A7_1_1_2_01-5: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC	X	
Pass values reached within 10-d window (within 28-d test period)	X	
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%	X	
Percentage of removal of reference substance in the toxicity control reaches pass level 35% by day 14	X	

Section A7.1.1.2.1_02 & _03 Biodegradability (ready)

Annex Point IIA7.6.1.1

Official use only

		1 REFERENCE
1.1	**Reference**	NITE (1982). Biodegradation and Bioconcentration of Existing Chemical Substances under the Chemical Substances Control Law. MITI (I) test with glyoxal. National Institute of Technology and Evaluation (NITE), Japan; database available online, URL: http://www.safe.nite.go.jp/english/kizon/KIZON_start_hazkizon.html; results first published 28 Dec 1982; BPD ID A7.1.1.2.1_02 Chemicals Inspection & Testing Institute Japan (1992). Biodegradation and Bioaccumulation Data of Existing Chemicals based on the CSCL Japan, compiled under the Supervision of Chemical Products Safety Division, Basic Industries Bureau MITI, Japan Chemical Industry Ecology-Toxicology Information Center; BPD ID A7.1.1.2.1_03
1.2	**Data protection**	No
1.1	Data owner	Data published
1.2	Companies with letter of access	Not applicable as data published
1.3	Criteria for data protection	Data on new a.s. for first entry to Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
1.3	**Guideline study**	Yes; MITI (I)-test following OECD TG 301C
1.4	**GLP**	No, GLP was not compulsory at the time the study was performed
1.5	**Deviations**	No
		3 MATERIALS AND METHODS

Section A7.1.1.2.1_02 & _03 Biodegradability (ready)

Annex Point IIA7.6.1.1

1.6	**Test material**	Glyoxal
3.1	Lot/Batch number	Not applicable
3.2	Specification	No data
3.3	Purity	No data
3.4	Further relevant properties	-
3.5	Composition of Product	-
3.6	TS inhibitory to microorganisms	No
3.7	Specific chemical analysis	-
1.7	**Reference substance**	Yes, aniline (reagent grade)
3.8	Initial concentration of reference substance	100 mg/L
1.8	**Testing procedure**	
3.9	Inoculum / test species	see table A7_1_1_2-2
3.10	Test system	see table A7_1_1_2-3
3.11	Test conditions	see table A7_1_1_2-4
3.12	Method of preparation of test solution	No data
3.13	Initial TS concentration	100 mg/L
3.14	Duration of test	14 days
3.15	Analytical parameter	BOD, TOC removal
3.16	Sampling	No data
3.17	Intermediates/ degradation products	Not identified
3.18	Nitrate/nitrite measurement	Not applicable
3.19	Controls	- blank control (inoculated) - abiotic sterile control - toxicity control (reference substance: aniline)
3.20	Statistics	None

4 RESULTS

Section A7.1.1.2.1_02 & _03 Biodegradability (ready)

Annex Point IIA7.6.1.1

1.9	**Degradation of test substance**		
4.1	Graph	None available	
4.2	Degradation	Test end (after 14 days): 65% related to BOD/ThOD 98% related to TOC	
4.3	Other observations	None reported	
4.4	Degradation of TS in abiotic control	Not reported	
4.5	Degradation of reference substance	None available	
4.6	Intermediates/ degradation products	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
1.10	**Materials and methods**	The ready biodegradability of glyoxal was tested in a MITI (I) – Test following OECD TG 301C. Activated sludge was used as inoculum in a concentration of 30 mg/L. The test substance concentration was 100 mg/L. A blank control, an abiotic sterile control and a toxicity control with aniline as reference substance were also set up. The test temperature was 25 ± 1 °C and the pH was adjusted to pH 7. Degradation of glyoxal was recorded over a period of 14 days by analyzing BOD and TOC removal.	X
1.11	**Results and discussion**	After 14 days, glyoxal was degraded by 65 % related to BOD/ThOD and by 98 % related to TOC.	X
1.12	**Conclusion**	Although not indicated in detail, the validity criteria can be considered as fulfilled. The test result is indicative for the ready biodegradability of glyoxal.	X
5.1	Reliability	[redacted]	
5.2	Deficiencies	Yes (no full report available; therefore, only short description of methods and results, but the source is considered reliable)	

Section A7.1.1.2.1_02 & _03 **Biodegradability (ready)**
Annex Point IIA7.6.1.1

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
*Date*19/02/2018	
Materials and Methods	1.10: The description is that of the MITI guideline procedure and not the experimental test performed with glyoxal.
Results and discussion	1.11: No data for the substance specifications, as well as for the blank, the abiotic sterile and the toxicity controls are given. Results for the test substance are not detailed.
Conclusion	1.12: The validity criteria cannot be controlled since data are not available. This test can only be used as supportive information for the ready biodegradability of glyoxal.
Reliability	[redacted]
Acceptability	
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_1_1_2_02-1: Guidline-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_02-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Not applicable
Strain	Not applicable
Source	Municipal STPs, industrial STPs, lakes, rivers and bays
Sampling site	10 different sites in Japan
Laboratory culture	Yes
Method of cultivation	Fresh and old sludge was mixed at regular intervals under sufficient aeration; about 30 minutes after ceasing the aeration the supernatant corresponding to about 1/3 of whole volume was removed. Then the equal volume of dechlorination water was added to the remaining portion and aerated again, followed by addition of synthetic sewage* (0.1% w/v). This procedure was repeated once every day. The culturing was carried out at 25 °C. * Synthetic sewage: each 5% (w/v) glucose, peptone and monopotassium phosphate were dissolved in dechlorinatian water, adjusted to pH 7.0 ± 1.0 with sodium hydroxide.
Preparation of inoculum for exposure	See above
Pretreatment	See above
Initial cell concentration	30 mg suspended solids/L

Table A7_1_1_2_02-3: Test system

Criteria	Details
Culturing apparatus	300 mL vessels
Number of culture flasks/concentration	According to guideline (3 per concentration)
Aeration device	Not specified
Measuring equipment	Closed system oxygen consumption measuring apparatus
Test performed in closed vessels due to significant volatility of TS	Not specified

Table A7_1_1_2_02-4: Test conditions

Criteria	Details
Composition of medium	Basal mineral culture medium according to the guideline
Additional substrate	No
Test temperature	25 °C
pH	7
Aeration of dilution water	Pre-filtered air was used for aeration
Suspended solids concentration	30 mg/L
Other relevant criteria	Stirring of test solution by magnetic stirrer

Table A7_1_1_2_02-5: 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 $ThCO_2$	Yes	
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 applicable	
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%	no data	
Percentage of removal of reference substance reaches pass level by day 14	no data	
Oxygen uptake of the inoculum blank <60 mg O_2/L in 28d	no data	

Section A7.1.1.2.1_04 **Biodegradability (ready)**
Annex Point IIA7.6.1.1 **Closed Bottle Test**

Official use only

6 REFERENCE

1.1	**Reference**	Gerike P, Gode P (1990) The biodegradability and inhibitory threshold concentration of some disinfectants. Chemosphere 21(6), 799-812 (published), BPD ID A7.1.1.2.1_04
1.2	**Data protection**	No
6.1	Data owner	Data published
6.2	Companies with letter of access	Not applicable as data published
6.3	Criteria for data protection	Data on new a.s. for first entry to Annex I/IA

7 GUIDELINES AND QUALITY ASSURANCE

1.3	**Guideline study**	The test was described as a “ready biodegradability test” of the OECD and the European Chemicals legislation.
1.4	**GLP**	Not specified; however, GLP was not compulsory at the time the study was performed
1.5	**Deviations**	Not applicable

8 MATERIALS AND METHODS

Section A7.1.1.2.1_04 **Biodegradability (ready)**
Annex Point IIA7.6.1.1 **Closed Bottle Test**

1.6	**Test material**	Glyoxal
8.1	Lot/Batch number	No data
8.2	Specification	No data
8.3	Purity	No data
8.4	Further relevant properties	No data
8.5	Composition of Product	No data
8.6	TS inhibitory to microorganisms	At high concentrations
8.7	Specific chemical analysis	No data
1.7	**Reference substance**	None
8.8	Initial concentration of reference substance	Not applicable
1.8	**Testing procedure**	
8.9	Inoculum / test species	Bacteria preacclimatized in the Zahn-Wellens Test
8.10	Test conditions	Closed Bottle Test
8.11	Method of preparation of test solution	No data
8.12	Initial TS concentration	2 to 5 mg/L
8.13	Duration of test	Not specified
8.14	Analytical parameter	Percentage of theoretical oxygen demand (ThOD)
8.15	Sampling	No data
8.16	Intermediates/ degradation products	Not identified
8.17	Nitrate/nitrite measurement	Not applicable
8.18	Controls	Mentioned but no details
8.19	Testing for inhibition	The inhibitory effect of the test material was measured (1), in an oxygen consumption inhibitory test (OCIT) according to ISO 8192, and OECD 209 but with Pseudomonas putida instead of activated sludge; DIN 38412/27, which was in preparation at the time the study was conducted, and (2), by comparing the degradation performance of an OECD Confirmatory Test unit (OCT) with disinfectant added to the effluent with that of a control, according to Guhl and Gode (Vom Wasser 72: 165, 1989).

9 RESULTS

Section A7.1.1.2.1_04 **Biodegradability (ready)**
Annex Point IIA7.6.1.1 **Closed Bottle Test**

1.9	**Degradation of test substance**	
9.1	Graph	None
9.2	Degradation	A biodegradability of 90% of ThOD was reported for glyoxal tested in the Closed Bottle Test.
9.3	Other observations	Both approaches for measuring the inhibitory potential of glyoxal (OCIT and OCT) revealed that sewage treatment plant performance in impaired at only rather high effluent concentrations; a limit concentration of 500 mg/L was reported for both test approaches.
9.4	Degradation of TS in abiotic control	No data
9.5	Degradation of reference substance	No data
9.6	Intermediates/ degradation products	No data

10 APPLICANT'S SUMMARY AND CONCLUSION

1.10	**Materials and methods**	In the present publication, a series of substance including glyoxal was tested for biodegradability and inhibitory potential according to acknowledged test methods. The biodegradability of glyoxal was measured in the Closed Bottle Test as percentage of theoretical oxygen demand (ThOD), and the inhibitory effect was measured in the oxygen consumption inhibitory test (OCIT) and in the OECD Confirmatory Test (OCT) by comparing the degradation performance when the disinfectant was added to the effluent with that of a control. Test substance: Glyoxal, no further details given.
1.11	**Results and discussion**	In the Closed Bottle test, the biodegradability of glyoxal was 90% of ThOD. The inhibitory potential of glyoxal measured in the OCIT and OCT revealed that sewage treatment plant performance in impaired at only rather high effluent concentrations; a limit concentration of 500 mg/L was reported for both test approaches.
1.12	**Conclusion**	Glyoxal tested in the Closed Bottle Test was described as readily biodegradable in present study. The method reported in the present publication is in accordance with the ready biodegradability methods of the OECD 301 guideline series. The results confirm those obtained in the Die-Away Test and the MITI tests reported under BPD ID A7.1.1.2.1_01 and BPD ID A7.1.1.2.1_02 & 03, respectively. In the Die-Away Test no abiotic degradation including volatilization was observed. Therefore the present publication is suitable for the purpose of supporting.
10.1	Reliability	[redacted]
10.2	Deficiencies	Details on test conduct and test substance were few.

Section A7.1.1.2.1_04 **Biodegradability (ready)**
Annex Point IIA7.6.1.1 **Closed Bottle Test**

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date	19/02/2018
Materials and Methods	No details on test conduct and test substance.
Results and discussion	Agree
Conclusion	Agree
Reliability	[redacted]
Acceptability	
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Section A7.1.1.2.2 **Annex Point 7.1**	**Inherent Biodegradation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The endpoint is not of concern as glyoxal was shown to be readily biodegradable [1]. Moreover a simulation test was conducted, which gave no hint on adsorption or other abiotic elimination processes and confirmed that glyoxal is biodegradable [2]. [1] [redacted] (1996) Determination of the Biodegradability or Eliminability of [redacted] in the DOC Die-Away Test. [redacted] (unpublished), BPD ID A7.1.1.2.1_01 [2] [redacted] (1996) Determination of the Biodegradability or Eliminability of [redacted] in the Activated Sludge Simulation Test. [redacted] (unpublished), BPD ID A7.1.2.1.1_01	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	23/02/2018	
Evaluation of applicant's justification	Agree	
Conclusion	Agree	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A7_1_1_2_2_04-1: Guidline-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

Section A7.1.1.2.3_01 Biodegradability in the marine environment
Annex Point IIA7.6.1.1

Official use only

11 REFERENCE

1.1	**Reference**	[redacted] (2009) Glyoxal [redacted] - Biodegradability in Seawater – Shake Flask Method. B[redacted] (Unpublished), [redacted] 2009, BPD ID A7. 1.1.2.3_01
1.2	**Data protection**	Yes
11.1	Data owner	[redacted]
11.2	Companies with letter of access	[redacted]
11.3	Criteria for data protection	Data on new a.s. for first entry to Annex I/IA

12 GUIDELINES AND QUALITY ASSURANCE

1.3	**Guideline study**	Yes, OECD 306 (1992)
1.4	**GLP**	Yes
1.5	**Deviations**	No

13 MATERIALS AND METHODS

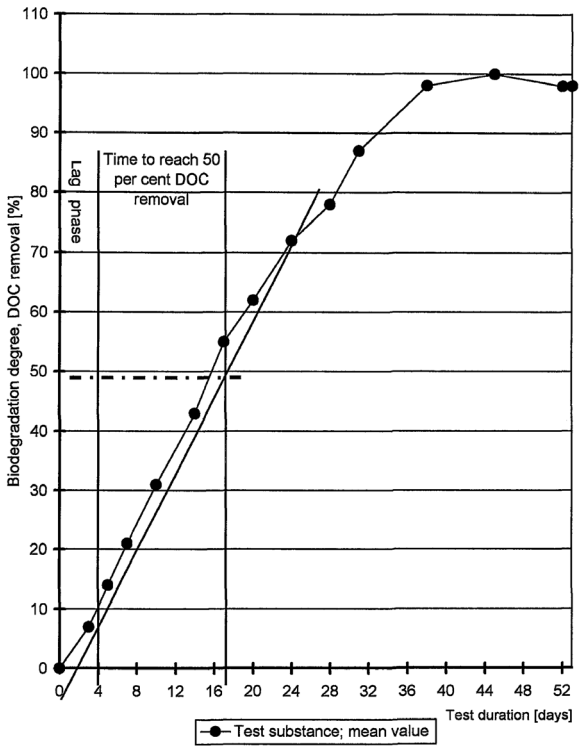
1.6	**Test material**	Glyoxal [redacted] (aqueous solution)
13.1	Lot/Batch number	[redacted]
13.2	Specification	[redacted]
13.3	Purity	[redacted]
13.4	Further relevant properties	Stable under storage conditions (at room temperature under nitrogen)
13.5	Composition of Product	Active ingredient: [redacted]
13.6	TS inhibitory to microorganisms	It is anticipated that the test concentration causes no toxic effects to the microorganisms.
13.7	Specific chemical analysis	TOC: 172 mg/g
1.7	**Reference substance**	Sodium benzoate
13.8	Initial concentration of reference substance	20 mg/L DOC

Section A7.1.1.2.3_01 Biodegradability in the marine environment
Annex Point IIA7.6.1.1

1.8	**Testing procedure**	
13.9	Inoculum / test species	Filtered (50 µm) natural sea water; for details on inoculum see table A7_1_1_2-2
13.10	Test system	For details on test type, laboratory equipment etc. see table A7_1_1_2-3
13.11	Test conditions	For relevant test conditions see table A7_1_1_2-4
13.12	Method of preparation of test solution	1163.0 mg of Glyoxal [redacted] were weighed in a 100 mL volumetric flask, dissolved in demineralised water and made up to the mark.
13.13	Initial TS concentration	A test concentration of 20 mg/L DOC was used. The selected test concentration corresponds to approximately 116 mg/L test material. X The selected test concentration was tested in an additional inhibition control test assay and no toxic effects to the microorganism were observed.
13.14	Duration of test	53 days
13.15	Analytical parameter	Removal of Dissolved Organic Carbon (DOC)
13.16	Sampling	The DOC measurement was performed twice per week (days: 0, 3, 5, 7, 10, 14, 17, 20, 24, 28, 31, 38, 45, 52, and 53)
13.17	Intermediates/ degradation products	Not identified
13.18	Nitrate/nitrite measurement	No, not applicable
13.19	Controls	Control without test substance (sea water and inorganic medium = blank control); Inhibition control (reference substance and test substance); Control of abiotic elimination (test substance and 50 mg/L mercury chloride).
13.20	Statistics	The biodegradation in percentage DOC removal at time (D_t) was calculated and the results were represented graphically in a diagram, showing the lag phase, slope and time (starting from the end of the lag phase) to reach 50% removal (t_{50}). The lag phase was estimated as the time needed for 10% biodegradation.
	14 RESULTS	
1.9	**Degradation of test substance**	

Section A7.1.1.2.3_01 Biodegradability in the marine environment
Annex Point IIA7.6.1.1

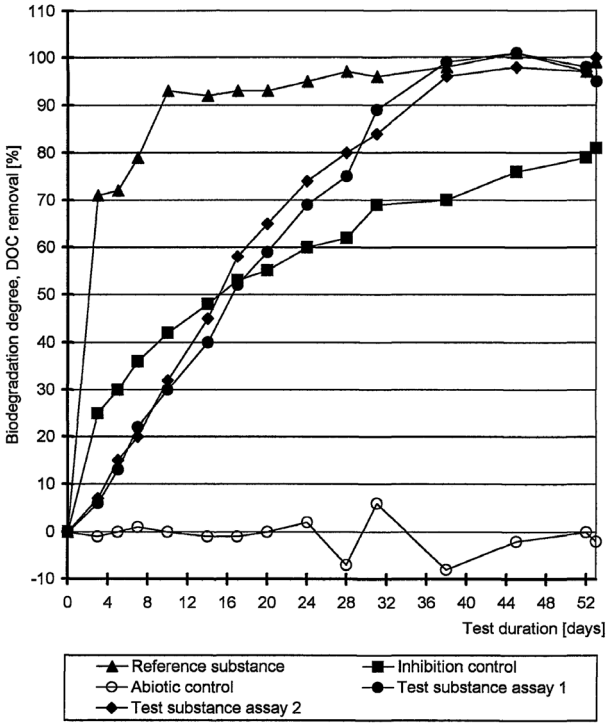
14.1 Graph



14.2	Degradation	Percentage degradation of Glyoxal [redacted] with the initial test concentration of 20 mg/l DOC was 90-100% at the end of exposure (53 days).
14.3	Other observations	The lag phase to reach 10% DOC removal is about 4 days; the time from the end of the lag phase to reach 50% removal (t_{50}) is graphically estimated to be about 12 days.
14.4	Degradation of TS in abiotic control	The abiotic elimination of the test substance (% DOC) is smaller than 10% at the end of exposure.

Section A7.1.1.2.3_01 Biodegradability in the marine environment
Annex Point IIA7.6.1.1

14.5 Degradation of reference substance



The reference substance was degraded to 79% after 7 days.

14.6 Intermediates/ degradation products

Not identified

15 APPLICANT'S SUMMARY AND CONCLUSION

1.10 Materials and methods

The objective of the study is to assess the biodegradability of Glyoxal in sea water by determination of the removal of Dissolved Organic Carbon (DOC).

Test material: Glyoxal [redacted]

The test was performed according to OECD 306 (1992) under GLP conditions.

The biodegradation of Glyoxal was evaluated at a concentration of 116 mg test material/L (corresponding to 20 mg/L DOC). A blank control (seawater and inorganic medium without test substance), a reference substance (sodium benzoate), a toxicity control (for both, the reference and the test substance) and a control for abiotic elimination (test substance and mercury chloride) were considered in this test.

To ensure that the salinity of sea water is not altered, the loss of water by evaporation was compensated with demineralised water before sampling for DOC-measurement.

DOC-samples about 10 mL were taken twice per week (day: 0, 3, 5, 7, 10, 14, 17, 20, 24, 28, 31, 38, 45, 52, and 53) and centrifuged at 4000

Section A7.1.1.2.3_01 Biodegradability in the marine environment
Annex Point IIA7.6.1.1

		rpm for about 15 minutes. The DOC-analyses were performed on the day of sampling using a TOC-analyser equipped with an auto sampler. For calibration standard samples were measured before start of measurements to prove the conformity with the calibration line. The DOC-results were converted in the biodegradation in per cent at time (D_t) and represented graphically in a diagram.
1.11	**Results and discussion**	The percentage of degradation of Glyoxal at 20 mg/L DOC was determined to be 90-100% at the end of exposure (53 days). Biodegradation of the reference substance was 79% measured on day 7. There were no indications for other abiotic elimination processes (<10% DOC).
1.12	**Conclusion**	Glyoxal [redacted] can be regarded as biodegradable in this test system. The validity criteria for the testing of marine biodegradability were fulfilled.
15.1	Reliability	[redacted]
15.2	Deficiencies	[redacted]

Section A7.1.1.2.3_01 Biodegradability in the marine environment
Annex Point IIA7.6.1.1

EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date	20/02/2018
Materials and Methods	13.13: The selected test concentration corresponds to approximately 12 mg/L DOC considering the hydrated monomers of glyoxal. It could be precised that after filtration, centrifugation and 2d pre-aeration, the coastal seawater has a DOC value of 2,1 mg/L which is less than 20% of the total DOC concentration after addition of test material.
Results and discussion	4.1 and 5.2: the degradation rate observed in the test substance assays reaches 7% at day 3, 43% at day 14 and 72 % at day 24. At the end of the test, 98% of DOC is removed. For sodium benzoate reference substance, the lag phase (t_L) is about 1 day and time to achieve 50 per cent degradation (t_{50}), excluding the lag phase, is about 2 days, then the validity criteria of marine biodegradability were fulfilled. It could be precised that the substance biodegradation has been corrected with the blank control.
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_1_1_2_3-1: Guidline-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	C.4-C	301B	ready

(Modified Sturm Test)			
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_3-2: Inoculum / Test organism

Criteria	**Details**
Nature	natural seawater
Species	Not applicable
Strain	Not applicable
Source	Not specified according to OECD 306
Sampling site	oast of North Sea of the island Sylt, Westerland Brandenburger Strand, Germany
Laboratory culture	No
Method of cultivation	Not specified
Preparation of inoculum for exposure	Aerated for 2 days prior to exposure in a dark room at 20±2 °C
Pretreatment	No
Initial cell concentration	Not relevant

Table A7_1_1_2_3-3: Test system

Criteria	Details
Culturing apparatus	2-L conical flasks
Number of culture flasks/concentration	two
Aeration device	test vessels were shaken using an orbital shaker
Measuring equipment	DOC
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2_3-4: Test conditions

Criteria	Details
Composition of medium	According to OECD 306
Additional substrate	No
Test temperature	20±2 °C
pH	7.4±0.2
Aeration of dilution water	No
Suspended solids concentration	Not applicable
Other relevant criteria	Not relevant

Table A7_1_1_2_3-5: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
Criteria for validity		
Degradation of the reference substance: - the lag phase (t_L) is 1 to 4 days - time (starting from the end of the lag phase) to reach 50% removal (t_{50}) is 1 to 7 days	X	

Section A7.1.2.1.1_01 Aerobic biodegradation
Activated Sludge Simulation Test

Annex Point IIA7.6.1.1

			Official use only
		16 REFERENCE	
1.1	**Reference**	[redacted] (1996) Determination of the Biodegradability or Eliminability of [redacted] in the Activated Sludge Simulation Test. [redacted] (unpublished), BPD ID A7.1.2.1.1_01	
1.2	**Data protection**	Yes	
16.1	Data owner	[redacted]	
16.2	Companies with letter of access		
16.3	Criteria for data protection	Data on new a.s. for first entry to Annex I/IA	
		17 GUIDELINES AND QUALITY ASSURANCE	
1.3	**Guideline study**	Yes, Annex of Directive 88/302/EEC corresponding to OECD TG 303 A and ISO 11733.	
1.4	**GLP**	Yes	
1.5	**Deviations**	No	
		18 MATERIALS AND METHODS	
1.6	**Test material**	[redacted] (1,2 ethanedial)	
18.1	Lot/Batch number	[redacted]	
18.2	Specification	As given in section 2	
18.3	Purity	[redacted]	
18.4	Further relevant properties	Not relevant	
18.5	Composition of Product	Additive: water, [redacted]	
18.6	TS inhibitory to microorganisms	No	
18.7	Specific chemical analysis	No compound specific analytical technique was applied	
1.7	**Reference substance**	No, not required	
18.8	Initial concentration of reference substance	Not applicable	
1.8	**Testing procedure**		
18.9	Inoculum / test species	For details of inoculum see table A7_1_2_1_1-2	
18.10	Test system	For details on test type, laboratory equipment etc. see table A7_1_2_1_1-3	
18.11	Test conditions	For relevant test conditions see table A7_1_2_1_1-4	
18.12	Method of preparation of test solution	No data	
18.13	Initial TS concentration	20 mg DOC/L	

Section A7.1.2.1.1_01 **Aerobic biodegradation**
Activated Sludge Simulation Test

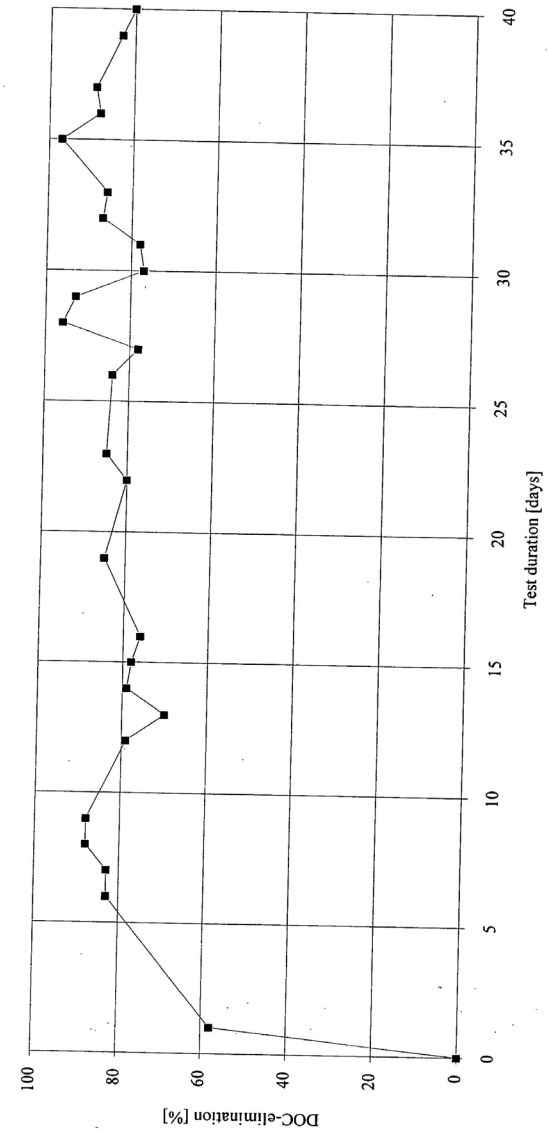
Annex Point IIA7.6.1.1

18.14	Duration of test	41 days	
18.15	Analytical parameter	DOC removal	
18.16	Sampling	Sampling on days: 0, 1, 6, 7, 8, 9, 12, 13, 14, 15, 16, 19, 22, 23, 26, 27, 28, 29, 30, 31, 32, 33, 35, 36, 37, 39, 40	
18.17	Intermediates/ degradation products	Not identified	
18.18	Nitrate/nitrite measurement	Yes, according to guideline	X
18.19	Controls	Two continuously operated test units were run in parallel under identical conditions. The test substance was added only to one unit, the second unit was used as control to determine the biodegradation of the organic medium.	
18.20	Statistics	The difference of the corresponding influent and effluent DOC values is the measure of the biodegradation. Mean values and standard deviations were determined without considering outliers as determined by current statistical methods (95% confidence intervals).	
		19 RESULTS	
1.9	**Degradation of test substance**		

Section A7.1.2.1.1_01 **Aerobic biodegradation**
Activated Sludge Simulation Test

Annex Point IIA7.6.1.1

19.1 Graph



19.2 Degradation

Duration of the adaptation phase was 14 days. Duration of the evaluation phase was 27 days. The mean value of DOC removal was

X

Section A7.1.2.1.1_01 **Aerobic biodegradation**
Activated Sludge Simulation Test

Annex Point IIA7.6.1.1

		81.6 % with a standard deviation of 4.0 % (95% confidence intervals: ± 2.1 %). Following the guideline the number of determinations was 15 (without outliers); 4 outliers were detected by the Grubbs method.	
19.3	Other observations	There were no indications for adsorption or DOC elimination due to other abiotic processes.	
19.4	Degradation of TS in abiotic control	Not relevant (see above)	
19.5	Degradation of reference substance	Not required	
19.6	Intermediates/ degradation products	Not required	

20 APPLICANT'S SUMMARY AND CONCLUSION

1.10	**Materials and methods**	The aim of the present study was to investigate the biodegradability of glyoxal in the Activated Sludge Simulation Test. Test substance: [redacted] (1,2 ethanedial), [redacted] The test was performed under GLP conditions according to the method stated in the Annex of Directive 88/302/EEC which is corresponding to OECD TG 303 A and ISO 11733. The test system consisted of a test unit and a control unit. The inoculum was activated sludge from a laboratory wastewater treatment plant fed with municipal and synthetic sewage. The test duration was 41 days, the DOC concentration of the test substance in the influent was 20 mg/L; the nutrient solution was 117 mg/L DOC in the influent (mean value), the dry weight of the added inoculum was 2.5 g/l, the mean retention period was 6 hours, the volume of the test units were 3 litres. The influent with test substance and synthetic medium was dosed by 12 L/day. Samples were taken from the influent and effluent of the two units. DOC values were measured via a DOC analyser (Shimadzu TOC 500 and TOC 5000) on days 0, 1, 6, 7, 8, 9, 12, 13, 14, 15, 16, 19, 22, 23, 26, 27, 28, 29, 30, 31, 32, 33, 35, 36, 37, 39, 40. The difference of the corresponding influent and effluent DOC values is indicative of the biodegradation. Mean values and standard deviations were determined without considering outliers which were determined by current statistical methods.	
1.11	**Results and discussion**	The adaptation phase was 14 days, followed by a plateau phase of 27 days. The mean value for biodegradation was 81.6 % after 40 days with a corresponding standard deviation of 4.0 % (n = 15). The 95% confidence intervals of the mean value were ± 2.1 %. Since there were no hints on adsorption, major loss due to volatility or other abiotic degradation processes, the test substance can be regarded as biodegradable in this test system. The report stated no deviations from the test guideline. The test substance was removed by > 80 %, related to the DOC. The DOC elimination of the synthetic medium as measured in the control unit was > 80 % after 28 days (94 %). Therefore, the validity criteria for the testing of biodegradability in the activated sludge simulation test were fulfilled.	X X

Section A7.1.2.1.1_01 Aerobic biodegradation
Activated Sludge Simulation Test

Annex Point IIA7.6.1.1

1.12	**Conclusion**	The test substance was degraded by more than 80 % related to DOC. The results of the present study gave no hint on adsorption or other abiotic elimination processes; therefore the test substance can be regarded as biodegradable in this test.
20.1	Reliability	[redacted]
20.2	Deficiencies	[redacted]

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date	22/02/2018
Materials and Methods	
Results and discussion	19.2: According the guidance, the adaptation phase ends and the degradation phase is taken to begin when about 10% of the initial amount of test substance is removed (after allowing for adsorption, if it occurs). Results show very high DOC elimination (58%) from the first day. Then, duration of the adaptation phase was not 14 days but less than 1 day. 1.11: No hints on adsorption and major loss due to volatility or other abiotic degradation processes are considered in spite of high DOC removing the first day of the test. Based on physico-chemical properties of glyoxal, it could be hypothesized low adsorption and volatilisation. However, this was not proved by specific analysis and the absence of justification to explain the high initial removal the first day gives rise to uncertainties.
Conclusion	These data give indication on the biodegradability of glyoxal under these specific conditions.
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from
Date	*Give date of 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*
Remarks	

Table A7_1_2_1_1-1: 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_2_1_1-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Source	From laboratory wastewater treatment plants fed with municipal and/or synthetic sewage.
Laboratory culture	Yes
Method of cultivation	Laboratory waste water treatment plant
Preparation of inoculum for exposure	No data
Pretreatment	Not applicable
Initial cell concentration	Not applicable (2.5 g /L activated sludge dry weight)

Table A7_1_2_1_1-3: Test system

Criteria	Details
Culturing apparatus	Two continuously operating activated sludge plants
Number of culture flasks/concentration	Not applicable; two test units in parallel: one unit was fed with the test substance and organic medium, whereas the other unit served as control (organic medium only)
Aeration device	Air blast: 1 s on, 30 min off
Measuring equipment	DOC analyser
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_2_1_1-4: Test conditions

Criteria	Details
Composition of medium	According to guideline
Additional substrate	No
Test temperature	Not available in the subtitted report
pH	Not available in the subtitted report
Aeration of dilution water	Not available in the subtitted report
Suspended solids concentration	2.5 g activated sludge/L (dry weight)
Other relevant criteria	No

Table A7_1_2_1_1-5: Pass levels and validity criteria for tests on biodegradability in the activated sludge simulation test

	fulfilled	not fulfilled
Criteria for validity		
Percentage of DOC removal of organic medium in the control unit is >80% after two weeks		X

Section 7.1.2.1.2_01 Anaerobic biodegradation

Annex Point IIIA XII 2.1

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21 REFERENCE

1.1	**Reference**	[redacted]., 2009, Glyoxal [redacted] - Determination of the ultimate anaerobic biodegradability in the anaerobic biodegradation test - [redacted] 2009 (unpublished), BPD ID A7.1.2.1.2_01
1.2	**Data protection**	Yes
21.1	**Data owner**	[redacted]
21.2	Companies with letter of access	[redacted]
21.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation

22 GUIDELINES AND QUALITY ASSURANCE

1.3	**Guideline study**	Yes, according to OECD-Guideline 311, (Adopted 23 March 2006)
1.4	**GLP**	Yes
1.5	**Deviations**	Yes. After the second washing step of the anaerobic sludge the inorganic carbon concentration was 21.2 mg/L instead of less than 10 mg/L which OECD-Guideline 311 requires. No further washing step was conducted to avoid loss of anaerobic bacteria. The deviation from OECD-Guideline 311 is not expected to have a significant impact on the outcome of the test.

23 METHOD

1.6	**Test material**	Glyoxal [redacted]
23.1	Lot/Batch number	[redacted]
23.2	Specification	See below
23.3	Purity	[redacted]
23.4	Further relevant properties	Homogeneity: homogeneous Physical state: liquid Appearance: colourless, clear Molecular weight: 58.04 g/mol Molecular formula: $C_2H_2O_2$ Water solubility: miscible (20°C) Storage conditions: storage at room temperature under nitrogen Total organic carbon: 175 mg/g

Section 7.1.2.1.2_01 Anaerobic biodegradation

Annex Point IIIA XII 2.1

23.5	Composition of Product	[redacted]% aqueous solution
23.6	TS inhibitory to microorganisms	Inhibitory effects were shown concerning total degradation but pressure development was as in the reference control assays (see section 4.1.2 and 4.1.4 for details).
23.7	Specific chemical analysis	Not applicable.
1.7	**Reference substance**	Yes, sodium benzoate (CAS No.: 532-32-1; [redacted].
23.8	Initial concentration of reference substance	20 mg/L TOC
1.8	**Testing procedure**	
23.9	Inoculum / test species	see table A7_1_2_1_2-1
23.10	Test system	see table A7_1_2_1_2-2
23.11	Test conditions	see table A7_1_2_1_2-3
23.12	Method of preparation of test solution	Test material stock solution: 750 mg of the test material were weighed into 100 mL volumetric flask which than was filled to the mark with demineralised water. Dilution was verified by visual inspection. Reference substance stock solution: 215 mg of the reference substance were weighed into 100 mL volumetric flask which than was filled to the mark with demineralised water. After short stirring time the reference substance appeared completely dissolved.
23.13	Initial TS concentration	20 mg/L TOC corresponds to ca. 114 mg/L of test material.
23.14	Duration of test	62 days
23.15	Analytical parameter	CH_4 and CO_2 evolution via pressure measurement in headspace and total carbon determination in liquid phase.
23.16	Sampling	Pressure measurements in all test vessels on day 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 45, 49, 52, 56 and 62. Total inorganic carbon analysis was performed in all test vessels at the end of the test. pH values were measured at the beginning and end of the test.
23.17	Intermediates/ degradation products	Amount of degradation products was calculated according to OECD-Guideline 311.

Section 7.1.2.1.2_01 Anaerobic biodegradation

Annex Point IIIA XII 2.1

23.18 Controls

Blank control without test substance:
(inoculum and demineralised water)

Reference substance:
(inoculum, sodium benzoate and demineralised water)

Inhibition control:
(inoculum, sodium benzoate and test material)

23.19 Statistics

Calculations were made according to OECD-Guideline 311.

24 RESULTS

1.9 Degradation of test substance

24.1 Degradation of TS in abiotic control

No abiotic control was set up.

24.2 Degradation

Biological degradation (D_T), by biogas formation (D_B)

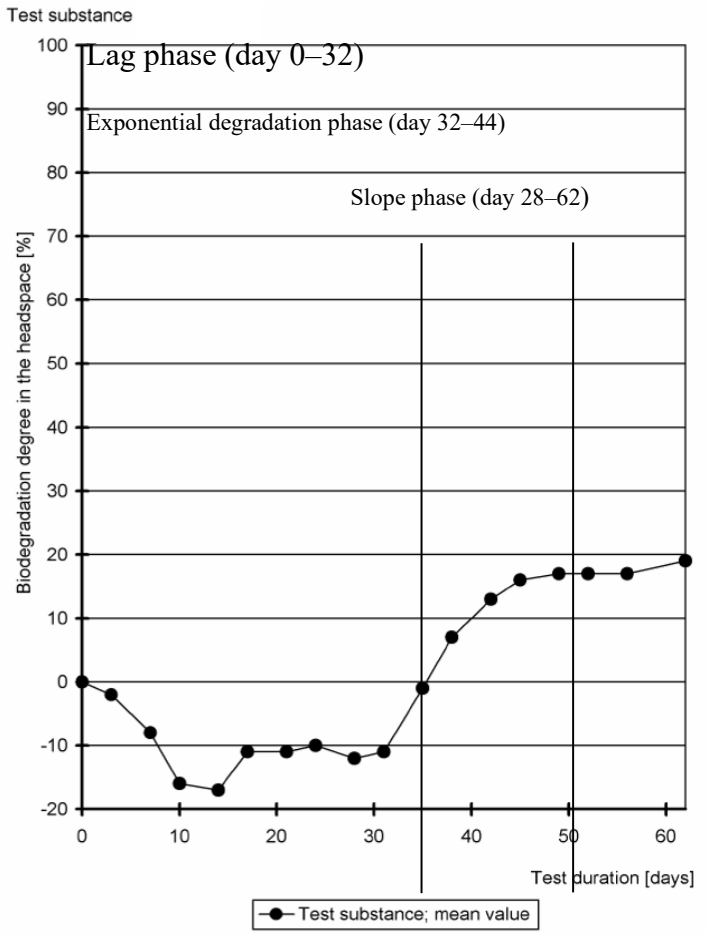
		RS1	RS2	RS3	IH1	IH2	TS1	TS2	TS3	TS4	TS mv
Added test subst.	[mg/L]	-	-	-	114.4	114.4	114.4	114.4	114.4	114.4	
TOC(start)	[mg/L]	20	20	20	40	40	20	20	20	20	
V_L	[mL]	125	125	125	125	125	125	125	125	125	
C_{Pr}	[mg]	1.23	1.10	1.23	1.07	1.46	0.59	0.54	0.22	0.47	
C_L	[mg]	0.7	0.7	0.7	-0.2	-0.1	-0.2	-0.1	-0.1	0.2	
C_T	[mg]	1.93	1.8	1.93	0.87	1.36	0.39	0.44	0.12	0.67	
C_V	[mg]	2.50	2.50	2.50	5.00	5.00	2.50	2.50	2.50	2.50	
D_B	[%]	49	44	49	21	29	24	22	9	19	
D_T	**[%]**	77	72	77	17	27	16	18	5	27	**17**

Legend : RS mv = reference substance mean value, IH = inhibition control,
TS mv = test substance mean value
TOC(start) = TOC value at the start of exposure calc. by added test substance
C_{Pr} = total carbon production
V_L = volume of liquid in the test vessel
C_V = total organic carbon content in the test vessel

Mean degradation of the reference substance (RS 1–3) was 75% after 62 days of exposure.
Mean degradation of the test material (TS1–4) was 17% after 62 days of exposure.

Section 7.1.2.1.2_01 Anaerobic biodegradation
Annex Point IIIA XII
2.1

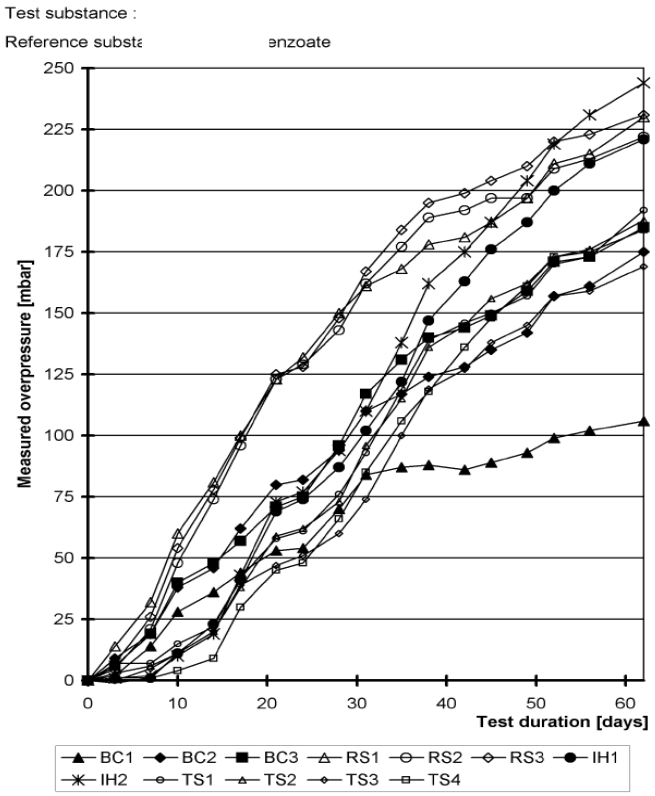
24.3 Graph



Section 7.1.2.1.2_01 Anaerobic biodegradation

Annex Point IIIA XII 2.1

24.4 Other observations

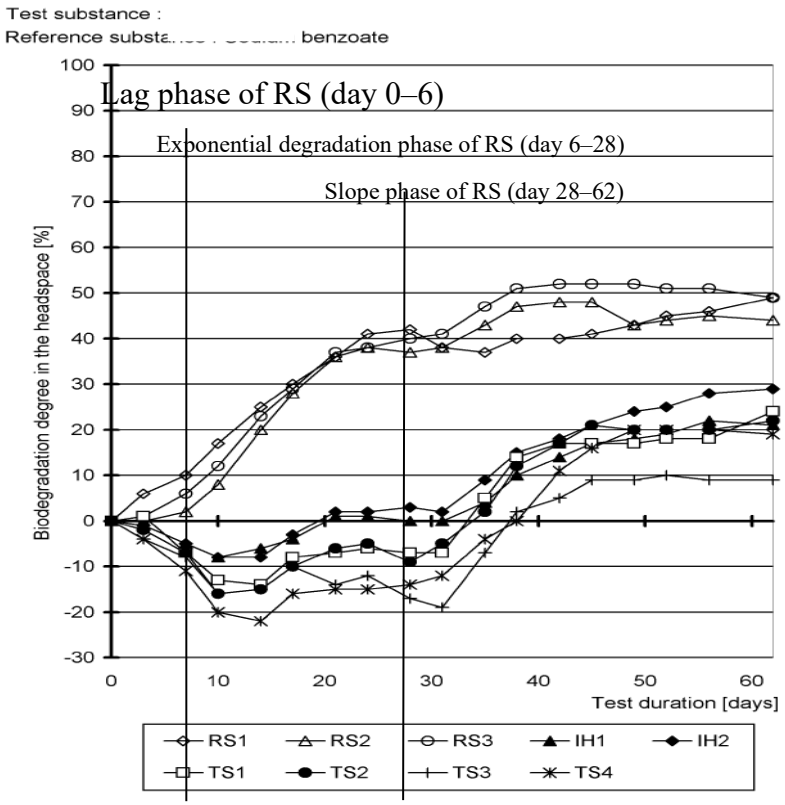


BC1–3: Blank control assays
RS1–3: Reference substance assays
IH1–2: Inhibition control assays
TS1–4: Test material assays

The pressure measurements of the inhibition controls (IH1–2; mean value 233 mbar) indicate comparable biodegradation to the reference substance (RS 1–3; mean value 228 mbar) after 62 days of exposure.

Section 7.1.2.1.2_01 Anaerobic biodegradation
Annex Point IIIA XII 2.1

24.5 Degradation of reference substance



RS1–3: Reference substance assays
IH1–2: Inhibition control assays
TS1–4: Test material assays

24.6 Intermediates/ degradation products

No data

25 APPLICANT'S SUMMARY AND CONCLUSION

1.10 Materials and methods

The test was performed in ca. 160 mL pressure tight incubation bottles at 35 ± 2°C for 62 days according to OECD Guideline 311.
The test preparations were carried out on a workbench with a nitrogen, hydrogen and carbon dioxide atmosphere (ratio: 80/10/10) to ensure anaerobic conditions.
121 mL of the inoculum solution were added to each vessel.
The liquid volume of each vessel was made up to 125 mL using demineralised water (blank control) or/and the corresponding stock solutions.
Vessels for the test material and reference substance assays contained 2.5 mg total organic carbon. Vessels for the inhibition control assays contained 5 mg total organic carbon.
A small magnetic stirrer was added to all vessels which were than closed with gas-tight rubber plugs.
An injection needle with a three way valve with Luer lock adapter was

Section 7.1.2.1.2_01 Anaerobic biodegradation

Annex Point IIIA XII 2.1

		inserted through the rubber plugs of all vessels. Pressure measurements in all test vessels were conducted, after short stirring time, by connecting each vessel to a pressure measuring device (Co. Schlee, Type V-D2) on day 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 45, 49, 52, 56 and 62. Total inorganic carbon analysis were performed in all test vessels with a total organic carbon analyser with an autos ampler (Shimadzu, TOC-5000A) at the end of the test. pH values were measured at the beginning and end of the test.
1.11	**Results and discussion**	The reference substance was degraded to 75% (mean value of three replicates) after 62 days of exposure. The inhibition control assays showed a reduced degradation of 22% (mean value of two replicates) after 62 days of exposure. The test material was degraded to 17% (mean value of four replicates) after 62 days of exposure. Validity criteria: fulfilled (see following details) No oxygen contamination: fulfilled; All vessels were completely colourless after two days of exposure indicating a strictly anaerobic environment. More than 60% biodegradation of reference substance: fulfilled; Mean degradation (triplicates) of the reference substance was 75%. pH value 7.0 ± 1.0: fulfilled; pH value range was 6.8–7.0 in all vessels.
1.12	**Conclusion**	The results show that the test material (concentration of 114 mg/L with a carbon contend of 20 mg/L) is poorly biodegradable (10–20%) after 62 days under anaerobic test conditions according to OECD-Guideline 311. Since the total degradation in the inhibition control was reduced compared to the reference substance batch, inhibitory effects of the test substance on anaerobic bacteria cannot be excluded. The results are not applicable for assessment of anaerobic biodegradability of the test material under different environmental conditions.
25.1	Reliability	[redacted]
25.2	Deficiencies	[redacted]

Section 7.1.2.1.2_01 Anaerobic biodegradation

Annex Point IIIA XII 2.1

EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	26/02/2018
Materials and Methods	Agree
Results and discussion	Agree
Conclusion	Agree
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from ... (specify)
Date	*Give date of 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*
Remarks	

Table A7_1_2_1_2-1: Inoculum / Test organism

Criteria	Details
Nature	Anaerobic digester sludge
Species	-
Strain	-
Source	5 L of anaerobic sludge were collected from a digester at a municipal wastewater treatment plant on [redacted] 2009.
Sampling site	[redacted]
Laboratory culture	No
Method of cultivation	The sludge was stored for 6 days under a nitrogen atmosphere at 35 ± 2°C without addition of a carbon source on an anaerobic workbench.
Preparation of inoculum for exposure	The sludge was washed twice with synthetic medium according to OECD-Guideline 311.
Pretreatment	-
Initial cell concentration	-

Table A7_1_2_1_2-2: Test system

Criteria	Details
Culturing apparatus	Not applicable: 160 mL incubation bottles
Number of culture flasks/concentration	Four per concentration
Aeration device	Not applicable
Measuring equipment	Pressure measurement
Test performed in closed vessels due to significant volatility of TS	No, due to maintain anaerobic conditions

Table A7_1_2_1_2-3: Test conditions

Criteria	Details
Composition of medium	According to guideline
Additional substrate	No
Test temperature	33-37 °C
pH	6.8 - 7.0
Aeration of dilution water	Not applicable
Suspended solids concentration	2.0 g activated sludge/L (dry weight)
Other relevant criteria	No

Table A7_1_2_1_2-4: Pass levels and validity criteria for tests on biodegradability in the activated sludge simulation test

	fulfilled	not fulfilled
Criteria for validity		
Degradation degree of the reference substance (>60%) by day 62	X	
The pH-values at the end of exposure is in the range of 6.9 – 7.1	X	
The complete net pressure in the inhibition control should be equal or higher compared to the reference substance	X	
Compliance of strict anaerobic operation (no pink colour of resazurin)	X	

Section A7.1.2.2.1 **Annex Point IIIA, XII.2.1.**	**Aerobic aquatic degradation study**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [X]** **Other justification [X]**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*26/02/2018*	
Evaluation of applicant's justification	*Agree*	
Conclusion	*Agree*	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.1.2.2.2 Annex Point IIIA, XII.2.1.	Water/sediment degradation study	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The endpoint is not of concern as glyoxal was shown to be readily biodegradable [1]. Moreover a simulation test was conducted, which gave no hint on adsorption or other abiotic elimination processes and confirmed that glyoxal is biodegradable [2]. Low potential for adsorption on soil and sediment was the result of the HPLC screening test for determination of the Koc, which was measured to be 2.1 [3]. Therefore, the performance of further studies on adsorption and desorption in water/sediment systems is not indicated. References [1] [redacted] (1996) Determination of the Biodegradability or Eliminability of [redacted] in the DOC Die-Away Test. [redacted] (unpublished), BPD ID A7.1.1.2.1_01 [2] [redacted] (1996) Determination of the Biodegradability or Eliminability of [redacted] in the Activated Sludge Simulation Test. [redacted] (unpublished), BPD ID A7.1.2.1.1_01 [3] [redacted] (1996) Determination of the sorption coefficient Koc of [redacted] by the HPLC screening method, [redacted] (unpublished), BPD ID A7.1.3_01	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	26/02/2018	
Evaluation of applicant's justification	Agree	
Conclusion	Agree	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.1.3_01 Adsorption / Desorption screening test

Annex Points IIA, VII.7.7. ; IIIA, XII.2.2.

			Official use only
		1 REFERENCE	
1.13	**Reference**	[redacted] (1996) Determination of the sorption coefficient Koc of [redacted] by the HPLC screening method, [redacted] (unpublished), BPD ID A7.1.3_01	
1.14	**Data protection**	Yes	
1.1	Data owner	[redacted]	
1.2	Companies with letter of access	[redacted]	
1.3	Criteria for data protection	Data on new a.s. for first entry to Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
1.15	**Guideline study**	Yes, comparable to OECD TG 121. The study was perfomed following a BASF inhouse SOP which was based on a published method issued by the Fraunhofer Institute for Environmental Chemistry and Ecotoxicology, Schmallenberg, Germany (Kördel W [1993] Validation of the HPLC-Screening Method for the determination of the adsorption coefficient in soil). This method was later integrated in the OECD TG 121 (2001).	
1.16	**GLP**	Yes	
1.17	**Deviations**	No	
		3 MATERIALS AND METHODS	
1.18	**Test material**	[redacted] (1,2 ethanedial)	
3.1	Lot/Batch number	[redacted]	
3.2	Specification	As given in section 2	
3.3	Purity	[redacted]	
3.4	Further relevant properties	Not relevant	
3.5	Method of analysis	Not specified	
1.19	Degradation products		
3.6	Method of analysis for degradation products	Not applicable to this screening method	
1.20	Reference substance	Nitrate was used as reference. The method was calibrated by determination of the k' values of 20 substances with known Koc values. The calibration led to the following equation (log k'/log Koc): y = 0.3216x - 0.4192 (Equation No. 1)	
3.7	Method of analysis for reference substance	HPLC system with UV detector	
1.21	**Soil types**	Not applicable (no soil tested in this method)	
1.22	**Testing procedure**		
3.8	Test system	The log Koc can be determined experimentally by an HPLC screening	X

Section A7.1.3_01 **Adsorption / Desorption screening test**
Annex Points IIA, VII.7.7. ; IIIA, XII.2.2.

		method based on the separation by a cyanopropyl stationary phase under isocratic conditions. Instrument: Liquid chromatograph [redacted] Stationary phase: 125 mm, 4 mm, cyanopropyl, particle diameter 5 µm Flow rate: 1 ml/min Injection volume: 25 µl Detection: UV (simultaneous at 220 nm, 205 nm, 254 nm and 436 nm)	
3.9	Test solution and Test conditions	Concentration of test substance: 51.25 mg in 10 mL water Eluent: 60 % (v/v) 10 mmol/L citrate buffer, pH 7.0; 40 % (v/v) acetonitrile The retention time was measured in triplicate.	
1.23	**Test performance**		
3.10	Preliminary test	According to (a)"OECD 106": No	
3.11	Screening test: Adsorption	According to (a)"OECD 106": No	
3.12	Screening test: Desorption	According to (a)"OECD 106": No	
3.13	HPLC-method	According to (a)"OECD-HPLC-method": Yes (similar) The retention time of the calibration substances determined by means of the HPLC separation is converted into the corresponding k' value: $k' = \frac{t_r - t_0}{t_0}$ (Equation No. 2) where k' = capacity factor t_r = retention time t_0 = dead time (measured with nitrate) The plot of log k' as a function of log Koc is approximately linear and is used for the determination of the Koc for the test substance. The equation for calculating the sorption coefficient for the test substance (or the logarithm of the sorption coefficient) is log k' = a + b log Koc (Equation No. 3) where log k' = common logarithm of the capacity factor k' log Koc = common logarithm of the sorption coefficient Koc a = intersect with the axis b = slope of the straight line	
3.14	Other test	Not relevant	
		4 RESULTS	
1.24	**Preliminary test**	The calibration led to the following equation: y = 0.3216x - 0.4192 (Equation No. 1)	
1.25	**Screening test: Adsorption**	For details of results see Table *A7_1_3-1* For calibration plot see Figure *A7_1_3-1*	X
1.26	**Screening test: Desorption**	Not applicable	
1.27	**Calculations**		

Section A7.1.3_01 **Adsorption / Desorption screening test**
Annex Points IIA, VII.7.7. ; IIIA, XII.2.2.

4.1	Kd	Not applicable	
4.2	Koc	mean Koc = 2.1; log Koc = 0.33	X
1.28	**Degradation product(s)**	No data	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
1.29	**Materials and methods**	The aim of the present study was to estimate the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC) similar the OECD TG 121. Principal method of analysis: HPLC system with UV detector (separation by a cyanopropyl stationary phase under isocratic condition). The test item was dissolved in water. The retention time was measured in triplicate.	
1.30	**Results and discussion**		
5.1	Adsorbed a.s. [%]	Not applicable	
5.2	Percentage of organic carbon	Not applicable	
5.3	Adsorption , K_d	Not applicable	
5.4	Desorption, K_d	Not applicable	
5.5	Kd (adsorption)/Kd (desorption)	Not applicable	
5.6	Koc (adsorption)	The mean Koc is 2.1; the corresponding log Koc is 0.33.	
5.7	Koc (desorption)	Not applicable	
5.8	Degradation products (% of a.s.)	No data	
1.31	**Conclusion**		
5.9	Reliability	[redacted]	X
5.10	Deficiencies	[redacted]	

EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date	26/02/2018
Materials and Methods	3.8: Glyoxal does not belong to any of the chemical classes listed under “applicability of the test” in the OECD Test Guideline 121. Therefore the validity of the Koc value derived by this method seems questionable.

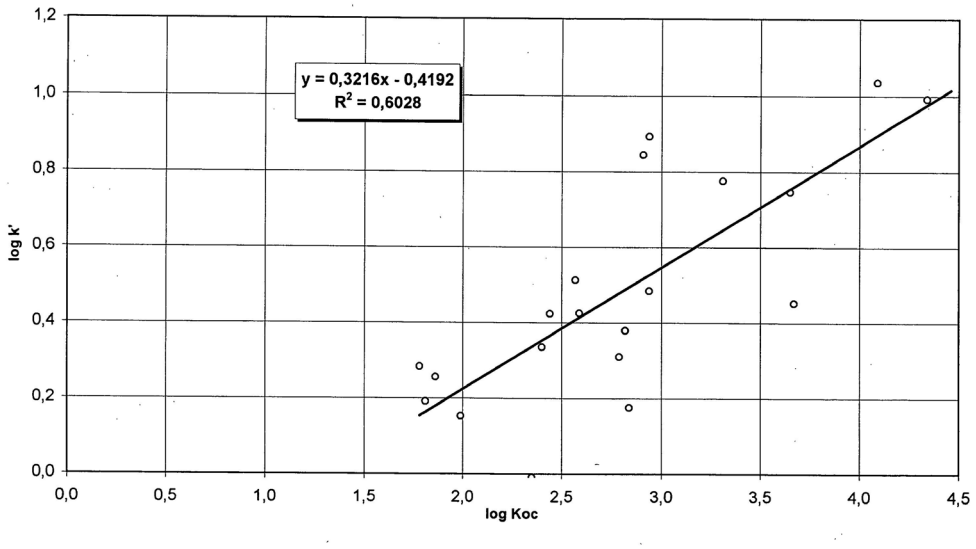
Section A7.1.3_01 **Annex Points IIA, VII.7.7. ; IIIA, XII.2.2.**	**Adsorption / Desorption screening test**
Results and discussion	1.25: In Table A7_1_3-1 it is shown that the Koc values of the reference substances are much higher compared to the calculated Koc-value of 2.1 L/kg for Glyoxal. Figure A7_1_3-1 shows a relatively low R^2 for regression line and calculated Koc values of glyoxal are not in the range of the point cloud. Nevertheless, calculation of the Koc value with the Modelling tool KOCWIN in EPI Suite (Estimation Program Interface) leads to an estimated Koc of 0.3535 L/kg. Compared to Koc of 2.1 L/kg, the adsorption potential of glyoxal approaches zero for both methods. Hence, the Koc of 2.1 L/kg can be seen as acceptable.
Conclusion	The mean Koc is 2.1; the corresponding log Koc is 0.33.
Reliability	[redacted]
Acceptability	
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_1_3-1: Results of HPLC method

Component		t_r [min] *1	t_0 [min] *2	k' *3	log k'	log Koc *4	Koc *4
Monolinuron		2.872	0.983	1.919	0.283	1.78	60.3
Atrazine		2.509	0.984	1.552	0.191	1.81	64.6
Isoproturon		2.775	0.978	1.799	0.255	1.86	72.4
Monuron		2.380	0.981	1.419	0.152	1.99	97.7
Carbendazim		1.946	0.987	0.979	-0.009	2.35	224
Tradimenol		3.110	0.976	2.163	0.335	2.40	251
Fenamiphos		3.593	0.978	2.655	0.424	2.44	275
Triadimefon		4.185	0.974	3.258	0.513	2.57	372
Linuron		3.600	0.979	2.661	0.425	2.59	389
Fensulfothion		2.991	0.987	2.042	0.310	2.79	617
Mercaptodimetur		3.340	0.985	2.393	0.379	2.82	661
Fuberidazol		2.456	0.983	1.496	0.175	2.84	692
Disulfoton		7.850	0.987	6.982	0.844	2.91	813
Methyl azinphos		3.980	0.994	3.048	0.484	2.94	871
Isofenphos		8.669	0.988	7.816	0.893	2.94	871
Fenthion		6.851	0.987	5.970	0.776	3.31	2042
Pyrazophos		6.470	0.984	5.572	0.746	3.65	4467
Anthraquinone		3.770	0.977	2.831	0.452	3.67	4677
Trifluralin		-- *5	0.987	--	--	3.94	8710
Alpha-Endosulfan		11.639	0.984	10.839	1.035	4.09	12303
Quintozen		10.630	0.987	9.817	0.992	4.34	21878
Sulprofos		-- *5	0.990	--	--	4.46	28840
Glyoxal	1	1.446	0.984	0.4695	-0.3284	0.28	1.9
	2	1.465	0.984	0.4888	-0.3109	0.34	2.2
	3	1.473	0.984	0.4970	-0.3037	0.36	2.3

*1 Retention time (t_r) of test substance
*2 Dead time (t_0) of reference substance nitrate
*3 Calculated following equation No. 2
*4 log Koc/Koc for calibration substances were taken from the literature;
log Koc/Koc for glyoxal was calculated following equations No. 1 and 3
*5 Retention time > 12 min (cut-off)

Figure A7_1_3-1: Calibration plot (log k'/logKoc)



Section A7.1.4.1 **Annex Point 7.1**	**Field study on accumulation in the sediment**	
	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 relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*26/02/2018*	
Evaluation of applicant's justification	*Agree*	
Conclusion	*Agree*	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A7_1 _3-1: Classification and physico-chemical properties of soils used as adsorbents

	Soil 1	Soil 2	Soil 3	Soil 4
Classification (USDA)	Not applicable			
Location	Not applicable			
Sand [%]	Not applicable			
Silt [%]	Not applicable			
Clay [%]	Not applicable			
Organic matter [%]	Not applicable			
pH (0.01 M KCl)	Not applicable			
Cation exchange capacity (MEQ/100 g)	Not applicable			

Table A7_1 _3-2: Results of preliminary test:

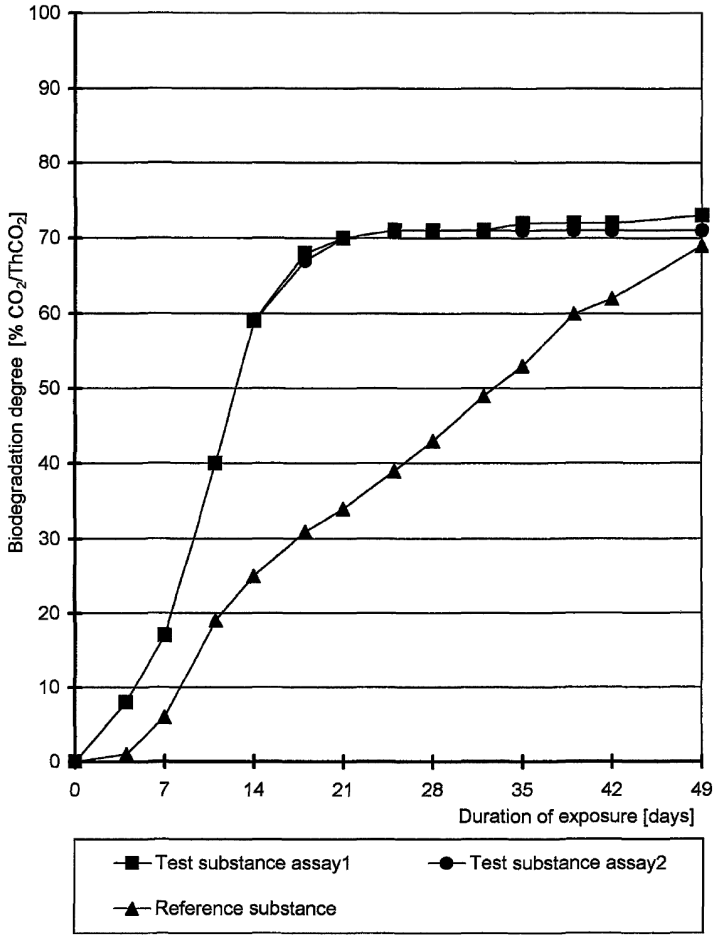
Test substance	[redacted]
Sample purity	[redacted] glyoxal in aqueous solution
Weighed soil	Not applicable
Volume of $CaCl_2$ solution	Not applicable
Nominal concentration of a.s. final solution	Not specified
Analytical concentration final of a.s. solution	Not applicable
Concentration of the test solution (show calculation)	Not applicable
Details of the analytical method used:	High Performance Liquid Chromatograph, Stationary Phase: 125 mm, 4 mm, cyanopropyl, particle diameter 5 µm ([redacted]) Mobile Phase: 60 % vol.: 10 mmol/L citrate buffer, pH = 7; 40 % vol.: acetonitrile Flow rate; 1 mL/min Detection: UV, 220 nm Injection volume: 25 µL
Method	HPLC
Detection limit	No data

Section III A7.1.1.2.1 **Annex Point IIA7.6.1.1**	**Biodegradability (ready)**	
	STATEMENT	Official use only
Comment of the RMS	Information submitted for the PT12 dossier --> Additional required actions: please provide the document III related to the document IVA7.1.1.2.1-04. Validity of submitted information would be checked during the evaluation of the dossier. Depending of new data on vapour pressure, new study performed in closed bottle (guideline OECD 301D) could be required.	
Response of the Notifier	Referrring to the Gerike publication (Gerike P, Gode P (1990) The biodegradability and inhibitory threshold concentration of some disinfectants. Chemosphere 21(6), 799-812, BPD ID A7.1.1.2.1_0471.1.2.1-04), a RSS was written and included in GL.A7_1 in Folder 33. Besides the test substance concentration (2-5 mg/L) and the result (90% degradation related to ThOD in OECD 301D) no further information is provided in the publication of Gerike & Gode (1990), which is a compilation of different methods and results with several test substances. However, results of a DOC Die-away Test (OECD TG 301A; BPD ID A7.1.1.2.1_01) and a MITI (I)-Test following OECD TG 301C were provided (BPD ID A7.1.1.2.1_02 and BPD ID A7.1.1.2.1_03). The latter is also suitable for volatile compounds in case that adequate precaution is taken. Moreover, the measured Henry's Law Constant of ≤ 3.38E-04 Pa*m^3*mol^{-1} at 15-45 °C (BPD ID A3.02.1_01) does not indicate a potential for evaporation.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	

Section III A7.1.1.2.1 **Annex Point IIA7.6.1.1**	**Biodegradability (ready)**
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.2.1_01 **Annex Point IIA7.1**		**Determination of the Biodegradability of Glyoxal in soil under aerobic conditions (Screening Test)**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] (2009) Determination of the Biodegradability of organic compounds in soil under aerobic conditions. [redacted] 2009, BPD ID A7.2.1_02	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, ISO 11266	
2.2	**GLP**	No; however, The test facility is registered for GLP and its ecological part is accredited to Deutsche Akkreditierungsstelle Chemie GmbH (DACH), see number [redacted]	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glyoxal, [redacted] (in aqueous solution)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Substance No.: [redacted]	
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	Homogeneous, liquid, colourless, clear	
3.1.5	Method of analysis	No data	
3.2	**Degradation products**		
3.2.1	Method of analysis for degradation products	The analysis was performed at [redacted]	
3.3	**Reference substance**	Yes, Avicell (substance No [redacted]	
3.3.1	Method of analysis for reference	The analysis was performed at [redacted]	

Section A7.2.1_01 Annex Point IIA7.1		Determination of the Biodegradability of Glyoxal in soil under aerobic conditions (Screening Test)	
	substance		
3.4	**Soil types**	Standard soil type: sandy loam, batch [redacted] December 2008	
3.5	**Testing procedure**		
3.5.1	Test system	The following test assays were prepared: 2 blank control assays (BC) 2 test substance assays (TS) 1 reference substance assay (RS) The biodegradation test was performed in glass columns with a total volume of 500 mL. The columns are equipped with a sintered plate at the base which allows aerating the test batches with carbon dioxide free air. The air was moistened by a humidifier. At the top the columns were connected to two serial scrubbing bottles (total volume 250 mL) filled with 100 mL 0.05 M sodium hydroxide solution for the adsorption of carbon dioxide from biodegradation processes. Usually one time per week the Total Inorganic Carbon (TIC) values of the adsorption solutions of the first trap were determined and used for the calculation of the produced carbon dioxide. After each sampling the second trap was moved forward and the new trap with fresh sodium hydroxide solution was placed into the second position. Each trap was analyzed separately. The TIC-value of the freshly prepared sodium hydroxide solution was determined and considered by the calculation of biogenic produced carbon dioxide amount. The aeration was performed with carbon dioxide free air at a flow of approximately 800 mL per hour. The test batches were prepared as follows: The water content of the soil was determined and adjusted to a water holding capacity (WHC) of 45 % by adding the required volume of demineralized water. For preparation of the test substance assays (TS) about 575 mg test substance, equivalent to 100 mg TOC, were added to about 107 g moistened soil and carefully mixed in a laboratory mixer. The test assay placed into the glass column afterwards. For the preparation of the reference substance assay (RS) the required amount for 100 mg total organic carbon (TOC) of Avicell was added to the moistened soil, mixed and placed into the glass column afterwards. The blank control assays (BC) contained only moistened soil. The test vessels were connected with an aeration unit with a humidifier and aerated by bubble aeration with carbon dioxide free air. The exposure phase was started by connection of the several test vessels with the absorption units. The test was performed at a temperature of 20 ±	

Section A7.2.1_01 Annex Point IIA7.1	Determination of the Biodegradability of Glyoxal in soil under aerobic conditions (Screening Test)	
	2°C.	
3.5.2 Samples analysis	Samples for the TIC analysis were performed as repeat determination, using a TOC analyzer equipped with an auto sampler ([redacted]). The system works with a combustion/non-disperse infrared gas analysis method. For calibration of the TOC-analyzer, standard samples were measured before start of measurements to prove the conformity with the calibration curve. The samples for TIC-analysis (absorption solution) were measured without further treatment.	
	4 RESULTS	
4.1 Test Results		

Section A7.2.1_01 Annex Point IIA7.1	Determination of the Biodegradability of Glyoxal in soil under aerobic conditions (Screening Test)	

Formed Carbon Dioxide, Summarized

[mg CO_2 in the test batches]

Day	RS	IH	TS1	TS2	TS3	TS4
4	3.8	-	29.1	29.7	-	-
7	23.4	-	61.6	63.5	-	-
11	70.4	-	147.8	145.7	-	-
14	91.0	-	217.8	216.1	-	-
18	112.6	-	249.2	247.1	-	-
21	126.6	-	257.2	255.5	-	-
25	144.3	-	260.2	259.3	-	-
28	158.9	-	260.7	260.0	-	-
32	179.5	-	261.9	260.6	-	-
35	196.1	-	262.9	261.0	-	-
39	219.4	-	264.7	262.1	-	-
42	228.3	-	265.3	261.8	-	-
49	252.9	-	268.1	262.6	-	-

mg CO_2 added up = CO_2mv - CO_2mv BC + CO_2pd

CO_2mv = CO_2 amount in the test assay at sampling day

CO_2mv BC = CO_2 amount in the blank control at sampling day

CO2pd = CO_2 amount in the test assay at previous sampling day

Section A7.2.1_01 Annex Point IIA7.1	Determination of the Biodegradability of Glyoxal in soil under aerobic conditions (Screening Test)	

Theoretical Carbon dioxide [mg/test assay] :

RS : 367.8	IH : -	TS1 : 366.9
TS2 : 367.6	TS3 : -	TS4 : -

Biodegradation degree; [CO_2/$ThCO_2$]

Test duration [days]	RS	IH	TS1	TS2	TS3	TS4	TS mv
0	0	-	0	0	-	-	0
4	1	-	8	8	-	-	8
7	6	-	17	17	-	-	17
11	19	-	40	40	-	-	40
14	25	-	59	59	-	-	59
18	31	-	68	67	-	-	68
21	34	-	70	70	-	-	70
25	39	-	71	71	-	-	71
28	43	-	71	71	-	-	71
32	49	-	71	71	-	-	71
35	53	-	72	71	-	-	72
39	60	-	72	71	-	-	72
42	62	-	72	71	-	-	72
49	69	-	73	71	-	-	72

$$[\%\ CO_2/ThCO_2] = \frac{CO_2\ Ta}{ThCO_2Ta} \times 100$$

CO_2 Ta = CO_2 amount at sampling day

$ThCO_2Ta$ = Theoretical CO_2 amount in the test assay

Legend : BC = blank control; RS = reference substance; IH = inhibition control; TS = test substance; mv = mean value

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A7.2.1_01 **Annex Point IIA7.1**	**Determination of the Biodegradability of Glyoxal in soil under aerobic conditions (Screening Test)**	
5.1 Materials and methods	The aim of the present study was to investigate the biodegradability of glyoxal in soil under aerobic conditions. Test substance: Glyoxal, [redacted] in aqueous solution Guideline: ISO 11266 Loamy sand was used as soil. The water content of the soil was adjusted to a water holding capacity (WHC) of 45 %. About 575 mg test substance, equivalent to 100 mg TOC, was added to about 107 g moistened soil and mixed. The biodegradation test was performed in glass columns with a total volume of 500 mL. The produced carbon dioxide was adsorbed by 0.05 M sodium hydroxide solution. Once per week the Total Inorganic Carbon (TIC) of the adsorption solutions was determined and used for the calculation of the produced carbon dioxide. Avicell was used as reference substance. The test vessels (duplicates) were connected with an aeration unit with a humidifier and aerated by bubble aeration with carbon dioxide free air. The exposure phase was started by connection of the several test vessels with the absorption units. The test was performed over a test period of 49 days at 20 ± 2°C.	
5.2 Results and discussion	Biodegradation degree ($C0_2$/$ThC0_2$) after 49 days: 72 % (mean value of two test assays). The reference substance was degraded by 69%.	
5.3 Conclusion	Glyoxal was shown to be susceptible to biodegradation in soil under aerobic conditions.	
5.3.1 Reliability	[redacted]	X
5.3.2 Deficiencies	[redacted]	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	26/02/2018	
Materials and Methods	Some information like soil pH, soil organic matter content are lacking. Soil properties are not sufficiently described.	
Results and discussion	No DT_{50} or DT_{90} for dissipation have been estimated. Nevertheless, this study is used only as supportive data as it was performed with only one soil system.	
Conclusion	This study gave indication on the biodegradability of Glyoxal in soil under aerobic conditions	
Reliability	[redacted]	
Acceptability	[redacted]	
Remarks		
	Comments from ...	

Section A7.2.1_01 **Annex Point IIA7.1**	**Determination of the Biodegradability of Glyoxal in soil under aerobic conditions (Screening Test)**	
Date	*Give date of 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*	
Remarks		

Section A7.2.2.1 **Annex Point 7.1**	**The rate and route of degradation including 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 [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	[redacted]	X
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	26/02/2018	
Evaluation of applicant's justification	[redacted]	
Conclusion		
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	

Section A7.2.2.1 **Annex Point 7.1**	**The rate and route of degradation including identification of the processes involved and identification of any metabolites and degradation products in at least three soil types under appropriate conditions**
Conclusion **Remarks**	*Discuss if deviating from view of rapporteur member state*

Section A7.2.2.2 **Annex Point XII.1.1**	**Field soil dissipation and accumulation**	
	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 relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	26/02/2018	
Evaluation of applicant's justification	*Agree*	
Conclusion	*Agree*	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.2.2.3 **Annex Point 7.1**	**Extent and nature of bound residues**	
	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]	X
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	26/02/2018	
Evaluation of applicant's justification **Conclusion** **Remarks**	[redacted]	
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	

Section A7.2.2.3 **Annex Point 7.1**	**Extent and nature of bound residues**
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.2.2.4 **Annex Point 7.1**	**Other soil degradation studies**	
	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]	X
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*26/02/2018*	
Evaluation of applicant's justification	[redacted]	
Conclusion		
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	

Section A7.2.2.4 **Annex Point 7.1**	**Other soil degradation studies**
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.2.3.1 **Annex Point 7.1**	**Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106 and, where relevant, adsorption and Desorption of metabolites and degradation products**	
	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 relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*26/02/2018*	
Evaluation of applicant's justification	Agree.	
Conclusion	Agree.	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.2.3.2 Annex Point IIA7.1	Mobility in at least three soil types and where relevant mobility of metabolites and degradation products	
	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 relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	26/02/2018	
Evaluation of applicant's justification	Agree.	
Conclusion	Agree. No further data required as glyoxal is readily biodegradable.	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.3.1_01&02 **Phototransformation in air (estimation method), including identification of breakdown products**
Annex Point IIIA VII.5

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2008) Glyoxal - Calculation of the half-life of glyoxal in air from measured OH radical reaction rate constant. [redacted] 2008, (unpublished), BPD ID A7.3.1_01	
1.2	**Reference**	Plum CN, Sanhueza E, Atkinson R, Carter WPL, Pitts Jr. JN (1983) OH radical rate constants and photolysis rates of alpha-dicarbonyls. Environ Sci Technol 17, 479-484, BPD ID A7.3.1_02	
1.3	**Data protection**	Yes	
1.3.1	Data owner	[redacted]	
1.3.2	Companies with letter of access		
1.3.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glyoxal, CAS 107-22-2	
3.1.1	Lot/Batch number	Not relevant	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	Not relevant	
3.2	**Reference substances**	None	
3.3	**Test solution**	None	
3.4	**Testing procedure**	Not relevant	
3.5	**Calculation**	Model: Calculation of half-life ($t_{1/2}$) based on the (measured) OH radical reaction rate constant and the following pseudo first-order equation: $t_{1/2} = \frac{\ln(2)}{k * [OH\bullet]}$ where k = reaction rate constant [OH•] = OH radical concentration Data used for calculation: Reaction rate constant = **1.15E-11** +/- 0.04 cm³/(molecule*s) at 25 °C Reference: *Plum et al. (1983), Environ Sci Technol 17, 479-484; BPD ID A7.03.1_02* Assumptions (based on SRC AOPWIN v1.92 model): a) 12-hour day, OH radical concentration = 1500000 molecules/cm³ b) 24-hour day, OH radical concentration = 500000 molecules/cm³	
		4 RESULTS	

Section A7.3.1_01&02 Annex Point IIIA VII.5		Phototransformation in air (estimation method), including identification of breakdown products
4.1	**Photolysis data**	Half-lives: a) $t_{1/2}$ = 11.2 hours (0.93 days) b) $t_{1/2}$ = 33.5 hours (1.4 days)
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Based on a measured OH radical reaction rate constant of 1.15E-11 cm³/(molecule*s) at 25 °C and assuming specific OH radical concentrations in air (corresponding to the SRC AOPWIN model), the half-life of glyoxal in air was calculated.
5.2	**Results and discussion**	A half-life of 1.4 days was estimated for a 24-hour day with an OH radical concentration of 500000 molecules/cm³.
5.3	**Conclusion**	The substance is subjected to rapid photodegradation in air with an estimated half-life of about 1 day.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date	09/02/2018
Materials and Methods	Agree
Results and discussion	Agree
Conclusion	The substance is subjected to rapid photodegradation in air with an estimated half-life of 1.4 days.
Reliability	Agree
Acceptability	acceptable
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Section A7.3.2 **Annex Point IIIA, XII.3**	**Fate and behaviour in air, further studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*09/02/2018*	
Evaluation of applicant's justification	Agree	
Conclusion	Agree	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.4.1.1_01 Acute toxicity to fish
Annex Point IIA7.1 **Golden Orfe (*Leuciscus idus*)**

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1989) Report on the study of the acute toxicity to golden orfe (*Leuciscus idus* L., golden variety). [redacted] 1985 (unpublished), BPD ID A7.4.1.1_01
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, according to the guideline of German Industrial Standard DIN 38412 "Testverfahren mit Wasserorganismen (Gruppe L). Allgemeine Hinweise zur Planung, Durchführung und Auswertung biologischer Testverfahren (L1)" and "Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische - Fischtests (L15)", June 1982, using a static procedure.
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Yes, besides the control only three test concentrations were set up.
		3 MATERIALS AND METHODS
3.1	**Test material**	Glyoxal [redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	[redacted] active ingredient
3.1.5	Further relevant properties	No data
3.1.6	Method of analysis	No data
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant
3.3	**Reference substance**	Yes. Chloroacetamide; positive control of animals: 48-h LC_{50}: ca. 25 mg/l; this lethal concentration corresponds to the normal sensitivity.
3.3.1	Method of analysis for reference substance	No data
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_1-2
3.4.2	Test organisms	See table A7_4_1_1-3
3.4.3	Test system	See table A7_4_1_1-4
3.4.4	Test conditions	See table A7_4_1_1-5
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Mortality, clinical symptoms of toxicity
3.4.7	Sampling	The biological parameters (mortality, symptoms) were recorded after 1, 4, 24, 48, 72 and 96 hours. The pH, temperature and dissolved oxygen measurements were performed at test initiation and after 24, 48, 72 and 96 hours.
3.4.8	Monitoring of TS concentration	Not performed
3.4.9	Statistics	Probit analysis could not be conducted due to a missing concentration-response relationship.

Section A7.4.1.1_01 **Acute toxicity to fish**
Annex Point IIA7.1 **Golden Orfe (*Leuciscus idus*)**

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 316, 464 and 681 mg test material/L
4.2.2	Actual concentrations of test substance	No analytical monitoring was performed.
4.2.3	Effect data (Mortality)	See table A7_4_1_1-6 and table A7_4_1_1-7
4.2.4	Concentration / response curve	None
4.2.5	Other effects	Following symptoms were reported:

Test conc. (mg test mat./L)	1 h	4 h	24 h	48 h	72 h	96 h
0 (control)	-	-	-	-	-	-
316	-	-	-	-	-	-
464	-	-	-	-	apathy	apathy
681	-	-	-	apathy	apathy, tumbling	apathy

4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were reported for the control animals.
4.3.2	Nature of adverse effects	Not relevant
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	Not specified
4.4.2	Results	Chloroacetamide; positive control of animals: 48-h LC_{50}: ca. 25 mg/L This lethal concentration corresponds to the normal sensitivity.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The acute toxicity of glyoxal ([redacted]) to the golden orfe (*Leuciscus idus* L., golden variety) was studied according to the German Industrial Standard DIN 38412, Part 15.
Fish were purchased from a commercial supplier and breeder. They were housed over a period of about 4 months and adapted to the test conditions for 3 days. At test initiation, they had a mean weight of 2.6 g (1.9 - 3.1 g) and a mean length of 6.8 cm (6.2 - 7.2 cm). The resulting corpulence factor of the batch was 0.83.
The acute toxicity of the test material was determined in a static test system at following concentrations: 0, 316, 464 and 681 mg test mat./L. Ten fish were used per concentration. The test water was reconstituted water according to the German Industrial Standard DIN 38412, Part 11

X

Section A7.4.1.1_01 Annex Point IIA7.1		Acute toxicity to fish Golden Orfe (*Leuciscus idus*)	
		(Draft Sep. 1981). It was prepared from fully demineralized tap water with a conductivity of 10 µS. The fish were checked for mortality and symptoms of toxicity after 1, 4, 24, 48, 72 and 96 hours. The temperature, pH and dissolved oxygen measurements were performed at test initiation and after 24, 48, 72 and 96 hours.	
5.2	**Results and discussion**	**Mortality:** No mortality was reported for the control group. In the treated groups, no mortality occurred at the nominal test material concentrations 316 and 464 mg test mat./L. At 681 mg test mat./L, the mortality was 20 % after 72 h and 80 % after 96 h, respectively. **Symptoms of toxicity:** After 72 h of exposure in the 464 mg test mat./L test concentration and after 48 h in the 681 mg test mat./L concentration, fish were observed to be apathetic until test end. At 681 mg test mat./L, also tumbling was reported after 72 h. The NOEC was 316 mg test mat./L. **Temperature, dissolved oxygen and pH:** The temperature of the test solutions was 21 °C and remained constant in all test vessels and over the whole testing period. The concentration of dissolved oxygen ranged between 6.2 and 8.8 mg/L (> 60 % of maximum saturation). The pH value was constantly between 7.2 and 7.4.	
5.2.1	LC_0	464 mg test mat./L (96 h)	
5.2.2	LC_{50}	464 – 681 mg test mat./L (96 h)	X
5.2.3	LC_{100}	> 681mg test mat./L (96 h)	
5.3	**Conclusion**	The testing of the acute toxicity of glyoxal ([redacted]) to the golden orfe resulted in a 96-h LC_{50} of 464 – 681 mg test mat./L. The LC0 was 464 mg test mat./L. Symptoms of toxicity occurred in the test concentrations of ≥ 464 mg test mat./L, resulting in a NOEC of 316 mg test mat./L. Besides the missing analytical verification of nominal test concentrations, the test is valid according to OECD TG 203 (1992).	X
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	[redacted]	

Section A7.4.1.1_01 **Acute toxicity to fish**
Annex Point IIA7.1 **Golden Orfe (*Leuciscus idus*)**

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	1/03/2018
Materials and Methods	5.1: The loading is of 2.6 g/L whereas the maximum recommended for static test is 1g/L. Besides the control only three test concentrations were set up instead of a minimum of 5 concentrations. No analytical verification of nominal test concentrations was done.
Results and discussion	5.2: As probit analysis could not be conducted due to a missing concentration-response relationship, an approximation for the LC_{50} by considering the geometric mean between the highest concentration causing no mortality and the lowest concentration causing 100% mortality, i.e. 464 – 681 mg test mat./L (96 h) respectively. Then, the LC_{50} = 225 mg a.i./L.
Conclusion	The LC_{50} is approximatively equal to 225 mg a.i./L. Note the presence of several defisciencies in this no GLP study (3 tested concentrations only, load of fish more than two fold higher than maximum accepted). Since the missing analytical verification of nominal test concentrations of glyoxal, it is not possible to know if the concentration of test substance is ≥80% of initial concentration during test and this validity criterion can not be checked.
Reliability	[redacted]
Acceptability	
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Reconstituted water prepared from fully demineralized water (conductivity: 10 µS) according to German Industrial Standard DIN 38412, Part 11 (draft Sep. 1981).
Alkalinity	No data
Hardness	Total: 2.5 mmol/L Carbonate: 0.8 mmol/L
pH	About 7.8
Oxygen content	No data (continuously aerated with oil-free air)
Conductance	*No data*
Holding water different from dilution water	Yes, holding water was tap water cleaned by active carbon and aerated with oil-free air. This water had a carbonate hardness of about 2.6 mmol/L.. The pH was about 7.5. The oxygen content was above 60 % of maximum saturation. The temperature in the holding tank was 15 to 20 °C.

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Golden orfe (Leuciscus idus L., golden variety)
Source	[redacted]
Wild caught	No
Age/size	Mean weight of 2.6 g (1.9 – 3.1 g) Mean total length of 6.8 cm (6.2 - 7.2 cm)
Kind of food	“[redacted]” growing feed 1 ([redacted])
Amount of food	Ad libitum
Feeding frequency	No data
Pretreatment	Fish were adapted to test water and test temperature for 3 days. Food was withdrawn 1 day before the exposure.
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	No
Volume of test vessels	30 x 22 x 24 cm all-glass aquaria
Volume/animal	1 L/fish (each vessel contained 10 L of test solution)
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

<table>
<tr><th>Criteria</th><th>Details</th></tr>
<tr><td>Test temperature</td><td>Maintained at 21 °C for all test solutions</td></tr>
<tr><td>Dissolved oxygen</td><td>
<table>
<tr><td rowspan="2">Test conc. (mg test mat./L)</td><td colspan="5">Oxygen content (mg/L)</td></tr>
<tr><td>Initial</td><td>24 h</td><td>48 h</td><td>72 h</td><td>96 h</td></tr>
<tr><td>0 (control)</td><td>7.6</td><td>7.2</td><td>8.2</td><td>7.7</td><td>7.7</td></tr>
<tr><td>316</td><td>7.3</td><td>6.7</td><td>7.7</td><td>6.8</td><td>6.2</td></tr>
<tr><td>464</td><td>7.6</td><td>7.8</td><td>8.4</td><td>7.1</td><td>6.4</td></tr>
<tr><td>681</td><td>7.9</td><td>8.2</td><td>8.8</td><td>7.5</td><td>8.2</td></tr>
</table>
</td></tr>
<tr><td>pH</td><td>
<table>
<tr><td rowspan="2">Test conc. (mg test mat./L)</td><td colspan="5">pH</td></tr>
<tr><td>Initial</td><td>24 h</td><td>48 h</td><td>72 h</td><td>96 h</td></tr>
<tr><td>0 (control)</td><td>7.4</td><td>7.3</td><td>7.3</td><td>7.3</td><td>7.3</td></tr>
<tr><td>316</td><td>7.4</td><td>7.2</td><td>7.3</td><td>7.2</td><td>7.2</td></tr>
<tr><td>464</td><td>7.4</td><td>7.3</td><td>7.3</td><td>7.3</td><td>7.2</td></tr>
<tr><td>681</td><td>7.4</td><td>7.4</td><td>7.4</td><td>7.3</td><td>7.4</td></tr>
</table>
</td></tr>
<tr><td>Adjustment of pH</td><td>No</td></tr>
<tr><td>Aeration of dilution water</td><td>No</td></tr>
<tr><td>Intensity of irradiation</td><td>Not specified</td></tr>
<tr><td>Photoperiod</td><td>16 hours light / 8 hours dark</td></tr>
</table>

Table A7_4_1_1-6: Mortality data

Testsubstance conc. (nominal) [mg test mat./L]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0 (control)	0	0	0	0	0	0	0	0
316	0	0	0	0	0	0	0	0
464	0	0	0	0	0	0	0	0
681	0	0	2	8	0	0	20	80
Temperature [°C]	21 °C	21 °C	21 °C	21 °C				
pH	7.2 – 7.4	7.3 – 7.4	7.2 – 7.3	7.2 – 7.4				
Oxygen [mg/L]	6.7 – 8.2	7.7 – 8.8	6.8 – 7.7	6.2 – 8.2				

Table A7_4_1_1-7: Effect data

	48 h [mg test mat./L][1]	95 % c.l.	96 h [mg test mat./L][1]	95 % c.l.
LC_0	681 (n)	-	464 (n)	-
LC_{50}	> 681 (n)	-	464 – 681 (n)	-
LC_{100}	> 681 (n)	-	> 681 (n)	-

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

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	Fulfilled	Not fullfilled
Mortality of control animals <10%	Yes	
Concentration of dissolved oxygen in all test vessels > 60% saturation	Yes	
Concentration of test substance ≥80% of initial concentration during test	No analytical monitoring performed	**X**

Criteria for poorly soluble test substances	Not relevant	

Section A7.4.1.1_02 Acute toxicity to fish
Annex Point IIA7.1 **Carp (*Cyprinus carpio*)**

Official use only

		1 REFERENCE
1.1	**Reference**	Anonymous (1984) Static nonreplacement acute toxicity test of glyoxal and carp. Medical College of Wisconsin's Aquatic Biomedical Laboratory, Wisconsin, USA, Jan 1984. TSCATS OTS0534429, New Doc ID 86-920000342. Submitted to US EPA by American Cyanamid Company (published), BPD ID A7.4.1.1_02
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Companies with letter of access	No as data published
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No, but method comparable to OECD TG 203
2.2	**GLP**	No
2.3	**Deviations**	Yes, besides the control only three test concentrations were set up; no analytical monitoring; test duration: 216 h (9 days).
		3 MATERIALS AND METHODS
3.1	**Test material**	Glyoxal (Aldrich Chemical Company)
3.1.1	Lot/Batch number	2606JK
3.1.2	Specification	As given in section 2
3.1.3	Purity	Not specified
3.1.4	Composition of Product	Glyoxal (ethanedial), 40 % (w/w) aqueous solution
3.1.5	Further relevant properties	No data
3.1.6	Method of analysis	No data
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_1-2
3.4.2	Test organisms	See table A7_4_1_1-3
3.4.3	Test system	See table A7_4_1_1-4
3.4.4	Test conditions	See table A7_4_1_1-5
3.4.5	Duration of the test	216 hours
3.4.6	Test parameter	Mortality, clinical symptoms of toxicity
3.4.7	Sampling	The biological parameters (mortality, symptoms) were recorded every 24 hours until the end of the test after 216 h. Temperature and pH were measured at the same time intervals. Dissolved oxygen measurements were performed at 24, 48, 72 and 96 hours.
3.4.8	Monitoring of TS concentration	Not performed
3.4.9	Statistics	No satistics performed (missing concentration-response relationship)
		4 RESULTS

Section A7.4.1.1_02 **Acute toxicity to fish**
Annex Point IIA7.1 **Carp (*Cyprinus carpio*)**

4.1	**Limit Test**	Not performed	
4.1.1	Concentration	Not relevant	
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant	
4.1.3	Nature of adverse effects	Not relevant	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0, 50, 100 and 200 mg a.i./L (nominal test concentrations, corrected for purity)	
4.2.2	Actual concentrations of test substance	No analytical monitoring was performed.	
4.2.3	Effect data (Mortality)	See table A7_4_1_1-6 and table A7_4_1_1-7	
4.2.4	Concentration / response curve	None	
4.2.5	Other effects	Behavioural or physiological symptoms of toxicity were not observed.	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were reported for the control animals.	
4.3.2	Nature of adverse effects	Not relevant	
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 acute toxicity of glyoxal to common carp (*Cyprinus carpio*) was studied following a method similar to OECD TG 203, but with some deviations (no analytical monitoring, low number of test concentrations). Fish were housed over a period of more than six months. At test initiation, they had a mean weight of 4.54 – 4.79 g (range of 4 groups, 10 fish each). The acute toxicity of the test material was determined in a static test system over a period of 216 h (9 days) at the following nominal concentrations: 0, 50, 100 and 200 mg a.i./L. Ten fish were used per concentration. The test and dilution water was dechlorinated Milwaukee tap water. It had a conductivity of 350 µmhos/cm, a pH of 7.55, a dissolved oxygen content of 10.9 mg/L and a temperature of 12 °C. The fish were checked for mortality and symptoms of toxicity every 24 h until the end of the test after 216 h (9 days). The same intervals applied to the measurements of pH and temperature. Dissolved oxygen measurements were performed at 24, 48, 72 and 96 hours.	X
5.2	**Results and discussion**	**Mortality:** No mortality was reported for the control and treated groups over the whole test period of 216 h. **Symptoms of toxicity:** No symptoms of toxicity were observed. **Temperature, dissolved oxygen and pH:** The temperature of the test solutions ranged from 9.8 to 11.0 °C. The pH value was between 7.60	

Section A7.4.1.1_02 Annex Point IIA7.1		Acute toxicity to fish Carp (*Cyprinus carpio*)	
		and 7.68. The concentration of dissolved oxygen was > 80 % of the maximum saturation during the whole test period.	
5.2.1	LC_0	200 mg a.i./L (96 h, nominal)	
5.2.2	LC_{50}	> 200 mg a.i./L (96 h, nominal)	
5.2.3	LC_{100}	> 200 mg a.i./L (96 h, nominal)	
5.3	**Conclusion**	The testing of the acute toxicity of glyoxal to carp resulted in a 96-h LC_{50} of > 200 mg a.i./L, based on nominal concentrations. The LC0 was 200 mg a.i./L. Symptoms of toxicity were not observed, resulting in a 96-h NOEC of 200 mg a.i./L. Besides the missing analytical verification of nominal test concentrations, the test is valid according to OECD TG 203 (1992).	X
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	Yes, no analytical monitoring was performed; the number of test concentrations was low.	

EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	16/03/2018
Materials and Methods	5.1: "but with some deviations (no analytical monitoring, low number of test concentrations, load of fish more than two fold higher than maximum accepted, temperatures more than 10 degrees lower than recommended)"
Results and discussion	Agree
Conclusion	Note the presence of several defisciencies in this no GLP study (3 tested concentrations only, load of fish more than two fold higher than maximum accepted, low temperature, no indication of fish adaptation to the test conditions). Since the missing analytical verification of nominal test concentrations of glyoxal, it is not possible to know if the concentration of test substance is ≥80% of initial concentration during test and this validity criterion can not be checked.
Reliability	[redacted]
Acceptability	
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Chemically dechlorinated Milwaukee tap water; supersaturated gasses were removed
Alkalinity	No data
Hardness	No data
pH	7.55
Oxygen content	10.9 mg/L at 12 °C
Conductance	350 µmhos/cm
Holding water different from dilution water	No data

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Carp (Cyprinus carpio)
Source	No data
Wild caught	No data
Age/size	Mean weight: Nominal test conc. (mg/L) / Mean ± SD / n 0 / 4.68 ± 0.84 / 10 50 / 4.79 ± 0.49 / 10 100 / 4.54 ± 0.65 / 10 200 / 4.57 ± 0.63 / 10 Mean total length: no data
Kind of food	No data
Amount of food	No data
Feeding frequency	No data
Pretreatment	Fish were kept inhouse for > six months
Feeding of animals during test	No feeding reported

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	No
Volume of test vessels	Twenty gallon (ca. 90 L) all glass aquaria, covered
Volume/animal	2 L/fish (each vessel contained 20 L of test solution)
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	Nominal test conc. (mg/L) / Temperature (°C): 24 h / 48 h / 72 h / 96 h 0 (control): 10.0 / 10.0 / 10.8 / 11.0 50: 10.0 / 9.8 / 10.5 / 11.0 100: 10.1 / 9.8 / 10.2 / 11.0 200: 10.0 / 9.8 / 10.5 / 10.8
Dissolved oxygen	Nominal test conc. (mg/L) / Oxygen content (% max. saturation): 24 h / 48 h / 72 h / 96 h 0 (control): 96.6 / 96.6 / 93.9 / 83.5 50: 86.6 / 95.7 / 113.0 / 104.0 100: 107.7 / 96.6 / 96.3 / 103.8 200: 99.0 / 98.4 / 93.9 / 99.6
pH	Nominal test conc. (mg/L) / pH: 24 h / 48 h / 72 h / 96 h 0 (control): 7.64 / 7.63 / 7.62 / 7.69 50: 7.60 / 7.63 / 7.61 / 7.66 100: 7.61 / 7.64 / 7.61 / 7.63 200: 7.64 / 7.68 / 7.65 / 7.68
Adjustment of pH	No
Aeration of dilution water	Yes
Intensity of irradiation	Not specified
Photoperiod	Not specified

Table A7_4_1_1-6: Mortality data

Testsubstance conc. (nominal) [mg a.i./L]	Mortality (n)								
	24 h	48 h	72 h	96 h	120 h	144 h	168 h	192 h	216 h
0 (control)	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0
200	0	0	0	0	0	0	0	0	0
Temperature [°C]	10.0-10.1	9.8-10.0	10.2-10.8	10.8-11.0	12.0	12.0	12.0	12.1	12.3-12.7
pH	7.60-7.64	7.63-7.68	7.61-7.65	7.63-7.69	7.73-7.79	7.61-7.73	7.60-7.72	7.52-7.62	7.51-7.58
Oxygen [% max. sat.]	86.6-107.7	95.7-98.4	93.9-113.0	83.5-104.0	-	-	-	-	-

Table A7_4_1_1-7: Effect data

	48 h [mg a.i./L][1]	95 % c.l.	96 h [mg a.i./L][1]	95 % c.l.
LC_0	200 (n)	-	200 (n)	-
LC_{50}	> 200 (n)	-	> 200 (n)	-
LC_{100}	> 200 (n)	-	> 200 (n)	-

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

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	**X**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**X**	
Concentration of test substance ≥80% of initial concentration during test	**No analytical monitoring performed**	

Criteria for poorly soluble test substances	**Not relevant**	

Section A7.4.1.1_03 Acute toxicity to fish
Annex Point IIA7.1 **Fathead minnow (*Pimephales promelas*)**

Official use only

1 REFERENCE

1.1 Reference	Conway RA, Waggy GT, Spiegel MH, Berglund RL (1983) Environmental Fate and Effects of Ethylene Oxide. Research and Development Department, Solvents and Coating Materials Division, Union Carbide Corporation, South Charleston, West Virginia 25303. Environ. Sci. Technol. 1983, 17, 107-112.BPD ID A7.4.1.1_03_fish_fathead".
1.2 Data protection	No
1.2.1 Data owner	Not applicable
1.2.2 Companies with letter of access	No as data published
1.2.3 Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study	No, but Fish toxicity data were collected by using procedures as published by EPA"Committee on Methods for Toxicity Tests with Aquatic Organisms, "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians", EPA-660/3-75-009, Apr 1975.
2.2 GLP	No GLP was not compulsory at the time the study was performed.
2.3 Deviations	Some test modifications were required to meet sample size limitations and dissolved oxygen requirements details not given.

3 MATERIALS AND METHODS

3.1 Test material	Glyoxal
3.1.1 Lot/Batch number	Not specified
3.1.2 Specification	As given in section 2
3.1.3 Purity	Not specified
3.1.4 Composition of Product	Not specified
3.1.5 Further relevant properties	Not specified
3.1.6 Method of analysis	Not specified
3.2 Preparation of TS solution for poorly soluble or volatile test substances	Not relevant
3.3 Reference substance	Sodium lauryl sulfate
3.3.1 Method of analysis for reference substance	Not specified
3.4 Testing procedure	
3.4.1 Dilution water	Dechlorinated (carbon-treated) Charleston tap water, see table A7_4_1_1-2.
3.4.2 Test organisms	See table A7_4_1_1-3
3.4.3 Test system	See table A7_4_1_1-4
3.4.4 Test conditions	See table A7_4_1_1-5
3.4.5 Duration of the test	96 hours
3.4.6 Test parameter	Mortality

Section A7.4.1.1_03 **Acute toxicity to fish**
Annex Point IIA7.1 **Fathead minnow (*Pimephales promelas*)**

3.4.7	Sampling	The biological parameters (mortality) were recorded every 24 hours until the end of the test after 96 h.
3.4.8	Monitoring of TS concentration	Not performed
3.4.9	Statistics	LC_{50} endpoints determined at 24, 48 and 96 h, method not reported
		4 RESULTS
4.1 Limit Test		Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2 Results test substance		
4.2.1	Initial concentrations of test substance	Not stated
4.2.2	Actual concentrations of test substance	No analytical monitoring was performed.
4.2.3	Effect data (Mortality)	See table A7_4_1_1-6
4.2.4	Concentration / response curve	None
4.2.5	Other effects	Behavioral or physiological symptoms of toxicity were not reported.
4.3 Results of controls		
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were reported for the control animals
4.3.2	Nature of adverse effects	No information provided
4.4 Test with reference substance		Not performed
4.4.1	Concentrations	Not reported
4.4.2	Results	LC_{50} of 6.6 (5.8 – 7.5) and 6.9 (5.3 – 90) mg Na lauryl sulfate, but it is not clear which (if any) of these is most relevant to the test done with glyoxal
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods		The acute toxicity of glyoxal to the fathead minnow (*Pimephales promelas)* was studied following the "Committee on Methods for Toxicity Tests with Aquatic Organisms, "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians", EPA-660/3-75-009, Apr 1975, but with some deviations (to meet sample size limitations and dissolved oxygen requirements details not given). The acute toxicity of the test material was determined in a static test system over a period of 96 hours. Ten fish were used per concentration. The test and dilution water was Dechlorinated (carbon-treated) Charleston tap water. The fish were checked for mortality after 24, 48

Section A7.4.1.1_03 Annex Point IIA7.1	Acute toxicity to fish Fathead minnow (*Pimephales promelas*)	
	and 96 hour.	
5.2 Results and discussion		
5.2.1 96 hr LC_0	215 mg/L	
5.2.2 48 hr LC_{50}	230 mg/L	
5.2.3 24 hr LC_{100}	550 mg/L	
5.3 Conclusion	The testing of the acute toxicity of glyoxal to the fathead minnow resulted in a 96-h LC_{50} of 215 mg/L.	X
5.3.1 Reliability	[redacted]	
5.3.2 Deficiencies	[redacted]	

EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	16/03/2018
Materials and Methods	The testing procedure is not available
Results and discussion	Results are not detailed
Conclusion	The test material is not described and the correction for purity is not indicated, then 96-h LC_{50} should be expressed as 215 mg/L of test material and not active ingredient.
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Dechlorinated (carbon-treated) Charleston tap water
Alkalinity	No data
Hardness	No data
pH	No data
Oxygen content	No data
Conductance	No data
Holding water different from dilution water	No data

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Fathead minnow
Source	No data
Wild caught	No data
Age/size	No data
Kind of food	No data
Amount of food	No data
Feeding frequency	No data
Pretreatment	No data
Feeding of animals during test	No feeding reported

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	No
Volume of test vessels	10-15L
Volume/animal	10 fish/test concentation
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	Not specified
Dissolved oxygen	Not specified
pH	Not specified
Adjustment of pH	Not specified
Aeration of dilution water	Minimal aeration or oxygen blankets
Intensity of irradiation	Not specified
Photoperiod	Not specified

Table A7_4_1_1-6: Effect data

	Time		
	24 h	**48 h**	**96 h**
LC50	550 mg/L	230 mg/L	215 mg/L

Section A 7.4.1.1_04 Acute toxicity to fish
Annex Point IIA7.1 **Bluegill sunfish (*Lepomis Macrochirus*)**

Official use only

1 REFERENCE

1.1 Reference		[redacted] (1984a) Acute toxicity of [redacted] to Bluegill (*Lepomis macrochirus*). [redacted] BPD ID A7.4.1.1_04.
1.2 Data protection		Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study	In-house protocol based on 'Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians' (US EPA, 1975). Comparable to OECD 203.
2.2 GLP	No, GLP was not compulsory at the time the study was performed.
2.3 Deviations	None reported

3 MATERIALS AND METHODS

3.1 Test material		[redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Not stated
3.1.3	Purity	Not specified
3.1.4	Composition of Product	Not specified
3.1.5	Further relevant properties	An amber coloured liquid
3.1.6	Method of analysis	No data
3.2 Preparation of TS solution for poorly soluble or volatile test substances		Not relevant
3.3 Reference substance		No reference substance used
3.3.1	Method of analysis for reference substance	Not applicable
3.4 Testing procedure		
3.4.1	Dilution water	See table A7_4_1_1-2
3.4.2	Test organisms	See table A7_4_1_1-3
3.4.3	Test system	See table A7_4_1_1-4
3.4.4	Test conditions	See table A7_4_1_1-5
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Mortality and sub-lethal effects
3.4.7	Sampling	No samples were taken
3.4.8	Monitoring of TS concentration	Not performed
3.4.9	Statistics	A computer program ([redacted] 1982, personal communication) was used to calculate LC50 values, three statistical methods in the following

		order of preference were available, in the computer program; moving average angle analysis, probit analysis, binomial probability. The method of selection was determined by the above order of preference and by the characteristics of the data base (e.g. presence or absence of several test concentrations causing mortality of a partial number of animals in the respective test populations).
		4 RESULTS
4.1 Limit Test		Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2 Results test substance		
4.2.1	Initial concentrations of test substance	130, 220, 360, 600, and 1000 mg test substance/L (nominal test concentrations.)
4.2.2	Actual concentrations of test substance	No analytical monitoring was performed. Turbidity (possibly indicating microbial growth) was noted at 360 mg/L from 72 h onwards and from 48 h at 600 and 1000 mg/L. Depletion of DO in response to dose was noted where measured in all treatments ≥130 mg/L from 48 h onwards. Taken together, these observations suggest that biological degradation occurred and that exposure concentrations were not maintained at nominals or initials during the 96 h test.
4.2.3	Effect data (Mortality)	See table A7_4_1_1-6 and table A7_4_1_1-7
4.2.4	Concentration / response curve	None
4.2.5	Other effects	None reported
4.3 Results of controls		
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were reported for the control animals.
4.3.2	Nature of adverse effects	Not relevant
4.4 Test with reference substance		Not performed
4.4.1	Concentrations	Not relevent
4.4.2	Results	Not relevant
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods		The acute toxicity of [redacted] to *Lepomis macrochirus* was determined

Section A 7.4.1.1_04 **Acute toxicity to fish**
Annex Point IIA7.1 **Bluegill sunfish (*Lepomis Macrochirus*)**

under static conditions in a 96-h test according to methodology of the US EPA. Fish (10 in single vessels per treatment) were exposed to nominal concentrations of 0 (control), 130, 220, 360, 600 and 1000 mg [redacted] in a soft dilution water reconstituted from deionised water. Observations of mortality and abnormal appearance and behaviour were made at daily intervals, together with checks on the condition of the test media. Measurements of water quality were made in the control and the lowest, middle and highest exposure treatments at test initiation and termination, and at intervals during the test.

5.2 Results and discussion

Mortality: There was no mortality or any overt behavior or gross physical indications of toxicity among the fish exposed to test concentrations of [redacted] ranging from 130 to 1000 mg/L.
Temperature, dissolved oxygen and pH: The temperature of the test solutions in the control was 22°C and remained constant in all test vessels and over the whole testing period. The concentration of dissolved oxygen ranged between 1.4 and 8.9 mg/L. The pH value ranged betweeen 6.8 and 7.6.

5.2.1 LC_0 >1000 mg/L (96 h)

5.2.2 LC_{50} >1000 mg/L (96 h)

5.2.3 LC_{100} > 1000 mg/L (96 h)

5.3 Conclusion

The testing of the acute toxicity of [redacted] to *Lepomis macrochirus* under static conditions resulted in a 96-h LC_{50} of >1000 mg/L. No mortalities occurred.

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Section A 7.4.1.1_04 **Acute toxicity to fish**
Annex Point IIA7.1 **Bluegill sunfish (*Lepomis Macrochirus*)**

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	19/03/2018
Materials and Methods	Agree
Results and discussion	Agree
Conclusion	Agree
Reliability	[redacted]
Acceptability	
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	The dilution water used was soft water reconstituted from deionised water according to recommended Procedures (U.S EPA, 1975).
Alkalinity	$CaCO_3$ of 32 mg/L
Hardness	$CaCO_3$ of 44 mg/L
pH	7.5
Oxygen content	No data
Conductance	130 µmhos/cm
Holding water different from dilution water	Yes, holding water was well water and was characterized as having total hardness and alkalinity ranges as calcium carbonate ($CaCO_3$) of 28-36 mg/l and 24-26 mg/l, respectively, and a specific conductance range of 100-110 micrometers per centimetre (µmhos/cm).

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Bluegill (*Lepomis macrochirus)*
Source	Fish were obtained from a commercial fish supplier [redacted]
Wild caught	No
Age/size	The mean (range, n=30) wet weight and total length of the test fish population was 0 .64(0.29 – 1.0) grams and 37(28-43) millimeters.
Kind of food	All fish were fed a dry commercial pelleted food, ad libitum, daily except during the 48 hours prior to testing.
Amount of food	Ad libitum
Feeding frequency	No data
Pretreatment	Fish were adapted to test water and test temperature for 14 days. Food was withdrawn 2 days before the exposure.
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	No
Volume of test vessels	19.6 L glass jars containing 15 L test medium
Volume/animal	1.5 L
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	Maintained at 22 °C for all test solutions
Dissolved oxygen	Test conc. (mg/L) / Oxygen content (mg/L): Initial / 24 h / 48 h / 72 h / 96 h 0 (control): 8.9 / 7.6 / 6.3 / - / 6.3 130: 8.9 / 7.6 / 5.1 / - / 3.9 360: 8.7 / 7.5 / 4.3 / - / 3.6 1000: 8.8 / 7.3 / 1.8 / - / 1.4
pH	Test conc. (mg/L) / pH: Initial / 24 h / 48 h / 72 h / 96 h 0 (control): 7.6 / 7.4 / 7.3 / - / 7.1 130: 7.4 / 7.2 / 7.0 / - / 6.9 360: 7.2 / 7.2 / 7.0 / - / 6.8 1000: 6.9 / 7.1 / 6.9 / - / 6.7
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	Not specified
Photoperiod	16 hours light / 8 hours dark

Table A7_4_1_1-6: Mortality data

Test substance conc. (nominal) [mg test mat./L]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0 (control)	0	0	0	0	0	0	0	0
130	0	0	0	0	0	0	0	0
220	0	0	0	0	0	0	0	0
360	0	0	0	0	0	0	0	0
600	0	0	0	0	0	0	0	0
1000	0	0	0	0	0	0	0	0

Table A7_4_1_1-7: Effect data

	24 h [mg /L][1]	48 h [mg /L][1]	72 h [mg /L][1]	96 h [mg /L][1]
LC_0	>1000 (n)	>1000 (n)	>1000 (n)	>1000 (n)
LC_{50}	>1000 (n)	>1000 (n)	>1000 (n)	>1000 (n)
LC_{100}	>1000 (n)	>1000 (n)	>1000 (n)	>1000 (n)

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

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled

Mortality of control animals <10%	yes	
Concentration of dissolved oxygen in all test vessels > 60% saturation	No	DO was below 60% ASV in all treatments ≥130 mg/L from 48 h onwards and 16% ASV was recorded after 96 h in the 1000 mg/L treatment. There were no mortalities or any other effects in spite of the low DO concentrations.
Concentration of test substance ≥80% of initial concentration during test	No analytical monitoring performed	**X**

Criteria for poorly soluble test substances	Not relevant	

Section A7.4.1.1_05 **Acute toxicity to fish**
Annex Point IIA7.1 **Rainbow trout (*Salmo gairdneri*)**

Official use only

	1 REFERENCE	
1.1 Reference	[redacted] (1984a) Acute toxicity of [redacted] to rainbow trout (*salmo gairdneri*). [redacted] 1984. BPD ID A7.4.1.1_05.	
1.2 Data protection	Yes	
1.2.1 Data owner	[redacted]	
1.2.2 Companies with letter of access	[redacted]	
1.2.3 Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	In-house protocol based on 'Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians' (US EPA, 1975). Comparable to OECD 203.	
2.2 GLP	No, GLP was not compulsory at the time the study was performed.	
2.3 Deviations	None reported	
	3 MATERIALS AND METHODS	
3.1 Test material	[redacted]	
3.1.1 Lot/Batch number	[redacted]	
3.1.2 Specification	Not stated	
3.1.3 Purity	Not specified	
3.1.4 Composition of Product	Not specified	
3.1.5 Further relevant properties	An amber coloured liquid	
3.1.6 Method of analysis	No data	
3.2 Preparation of TS solution for poorly soluble or volatile test substances	Not relevant	
3.3 Reference substance	No reference substance used	
3.3.1 Method of analysis for reference substance	Not applicable	
3.4 Testing procedure		
3.4.1 Dilution water	See table A7_4_1_1-2	
3.4.2 Test organisms	See table A7_4_1_1-3	
3.4.3 Test system	See table A7_4_1_1-4	
3.4.4 Test conditions	See table A7_4_1_1-5	
3.4.5 Duration of the test	96 hours	
3.4.6 Test parameter	Mortality and sub-lethal effects.	
3.4.7 Sampling	No samples were taken	
3.4.8 Monitoring of TS concentration	Not performed	
3.4.9 Statistics	A computer program ([redacted], 1982, personal communication) was used to calculate LC50 values, three statistical methods in the following	

order of preference were available in the computer program; moving average angle analysis, probit analysis, binomial probability. The method of selection was determined by the above order of preference and by the characteristics of the data base (e.g. presence or absence of several test concentrations causing mortality of a partial number of animals in the respective test populations).

4 RESULTS

4.1 Limit Test		Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2 Results test substance		
4.2.1	Initial concentrations of test substance	130, 220, 360, 600, and 1000 mg test substance/L (nominal test concentrations.)
4.2.2	Actual concentrations of test substance	No analytical monitoring was performed.
4.2.3	Effect data (Mortality)	See table A7_4_1_1-6 and table A7_4_1_1-7
4.2.4	Concentration / response curve	None
4.2.5	Other effects	None reported
4.3 Results of controls		
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects were reported for the control animals.
4.3.2	Nature of adverse effects	Not relevant
4.4 Test with reference substance		Not performed
4.4.1	Concentrations	Not relevent
4.4.2	Results	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods The acute toxicity of [redacted] to *Oncorhynchus mykiss* (formerly known as *Salmo gairdneri*) was determined under static conditions in a 96-h test according to methodology of the US EPA. Fish (10 in single vessels per treatment) were exposed to nominal concentrations of 0 (control), 130, 220, 360, 600 and 1000 mg [redacted] in a soft dilution water reconstituted from deionised water. Observations of mortality and abnormal appearance and behaviour were made at daily intervals,

Section A7.4.1.1_05 **Acute toxicity to fish**
Annex Point IIA7.1 **Rainbow trout (*Salmo gairdneri*)**

		together with checks on the condition of the test media. Measurements of water quality were made in the control and the lowest, middle and highest exposure treatments at test initiation and termination, and at intervals during the test.
5.2 Results and discussion		**Mortality:** There was no mortality or any overt behavior or gross physical indications of toxicity among the fish exposed to test concentrations of [redacted] ranging from 130 to 1000 mg/l. **Temperature, dissolved oxygen and pH:** The temperature of the test solutions in the control was 12°C and remained constant in all test vessels and over the whole testing period. The concentration of dissolved oxygen ranged between 6.5 and 10.4 mg/L. The pH value ranged betweeen 6.7 and 7.5.
5.2.1	LC_0	>1000 mg/L (96 h)
5.2.2	LC_{50}	>1000 mg/L (96 h)
5.2.3	LC_{100}	> 1000 mg/L (96 h)
5.3 Conclusion		The testing of the acute toxicity of [redacted] to *Salmo gairdneri* under static conditions resulted in a 96-h LC_{50} of >1000 mg/L. No mortalities occurred.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	19/03/2018
Materials and Methods	Agree
Results and discussion	Agree
Conclusion	Agree
Reliability	[redacted]
Acceptability	
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	The dilution water used was soft water reconstituted from deionised water according to recommended Procedures (U.S EPA, 1975)
Alkalinity	As $CaCO_3$ 32 mg/l
Hardness	As $CaCO_3$ 44 mg/l
pH	7.5
Oxygen content	No data
Conductance	130 µmhos/cm
Holding water different from dilution water	Yes, holding water was well water and was characterized as having total hardness and alkalinity ranges as calcium carbonate ($CaCO_3$) of 28-36 mg/l and 24-26 mg/l, respectively, and a specific conductance range of 100-110 micrometers per centimetre (µmhos/cm)

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Rainbow trout (*Salmo gairdneri)*
Source	Fish were obtained from a commercial fish supplier [redacted]
Wild caught	No
Age/size	The mean (range, n=30) wet weight and total length of the test fish population was 1.1 (0.65 – 1.4) grams and 47(40-54) millimetres
Kind of food	All fish were fed a dry commercial pelleted food, ad libitum, daily except during the 48 hours prior to testing
Amount of food	Ad libitum
Feeding frequency	No data
Pretreatment	Fish were adapted to test water and test temperature for 14 days. Food was withdrawn 2 days before the exposure
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	No
Volume of test vessels	19.6 L containing 15 L test medium
Volume/animal	1.5 L
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	Maintained at 12 °C for all test solutions
Dissolved oxygen	
pH	
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	Not specified
Photoperiod	16 hours light / 8 hours dark

Test conc. (mg/L)	Oxygen content (mg/L)				
	Initial	24 h	48 h	72 h	96 h
0 (control)	10.2	9.5	8.5	-	8.0
130	10.2	9.6	8.0	-	7.0
360	10.2	9.2	7.7	-	7.2
1000	10.4	9.7	7.5	-	6.5

Test conc. (mg/L)	pH				
	Initial	24 h	48 h	72 h	96 h
0 (control)	7.5	7.4	7.1	-	7.0
130	7.4	7.2	7.1	-	7.0
360	7.3	7.1	7.1	-	6.9
1000	7.0	7.0	7.0	-	6.8

Table A7_4_1_1-6: Mortality data

Test substance conc. (nominal) [mg test mat./L]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0 (control)	0	0	0	0	0	0	0	0
130	0	0	0	0	0	0	0	0
220	0	0	0	0	0	0	0	0
360	0	0	0	0	0	0	0	0
600	0	0	0	0	0	0	0	0
1000	0	0	0	0	0	0	0	0

Table A7_4_1_1-7: Effect data

	24 h [mg /L][1]	48 h [mg /L][1]	72 h [mg /L][1]	96 h [mg /L][1]
LC_0	>1000 (n)	>1000 (n)	>1000 (n)	>1000 (n)
LC_{50}	>1000 (n)	>1000 (n)	>1000 (n)	>1000 (n)
LC_{100}	>1000 (n)	>1000 (n)	>1000 (n)	>1000 (n)

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

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	yes	
Concentration of dissolved oxygen in all test vessels > 60% saturation	Yes	
Concentration of test substance ≥80% of initial concentration during test	No analytical monitoring performed	**X**

Criteria for poorly soluble test substances	Not relevant	

Section A7.4.1.2_01 Acute toxicity to invertebrates
Annex Point IIA7.2 ***Daphnia magna***

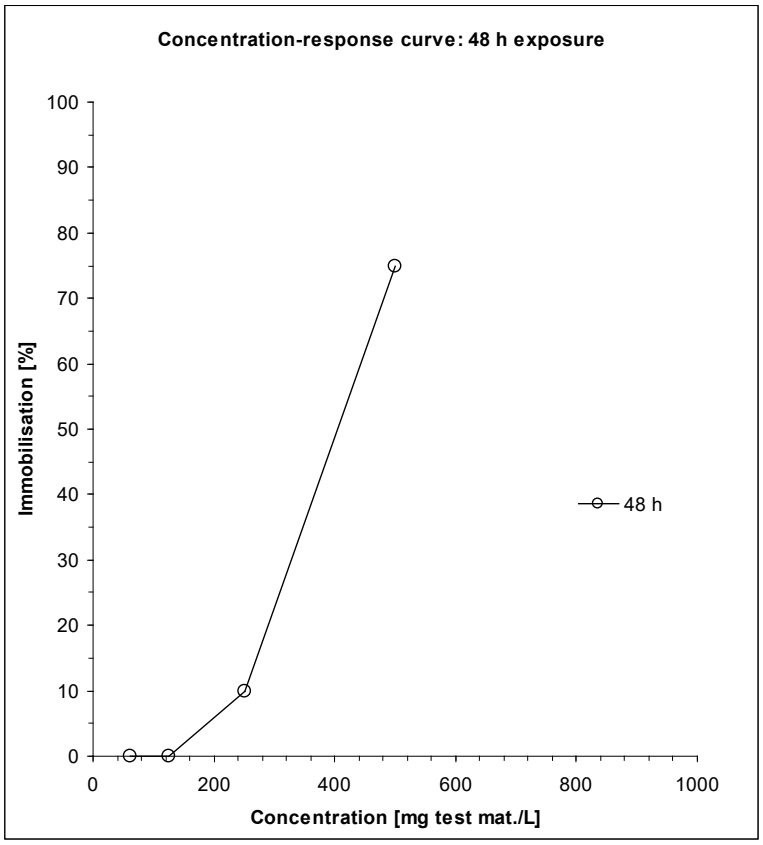
			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1988) Determination of the acute toxicity of Glyoxal pure solution [redacted] to the waterflea *Daphnia magna* Straus. [redacted] 1988, (in German, unpublished), BPD ID A7.4.1.2_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, according to method C.2 of Annex V of Directive 79/831/EEC	
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Yes, no analytical monitoring of test concentrations was performed.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glyoxal, [redacted] aqueous solution	
3.1.1	Lot/Batch number	Not specified	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted] (aqueous solution)	
3.1.4	Composition of Product	[redacted]	
3.1.5	Further relevant properties	Solubility in water > 500 mg/L at ca. 20 °C	
3.1.6	Method of analysis	No data	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not relevant	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_2-2	
3.4.2	Test organisms	See table A7_4_1_2-3	
3.4.3	Test system	See table A7_4_1_2-4	

Section A7.4.1.2_01 **Acute toxicity to invertebrates**
Annex Point IIA7.2 ***Daphnia magna***

3.4.4	Test conditions	See table A7_4_1_2-5
3.4.5	Duration of the test	48 hours
3.4.6	Test parameter	Immobilisation
3.4.7	**Sampling**	The test parameter was checked after 3, 6, 24 and 48 hours. The oxygen and pH measurements were performed at test initiation and after 48 hours.
3.4.8	**Monitoring of TS concentration**	No
3.4.9	**Statistics**	Not specified in the report; probably determination of EC_{50} values based on the Spearman-Kaerber method (Sachs, Lothar: Angewandete Statistik, Springer Verlag, Berlin, Heidelberg, New York, 4. Auflage, 1974)
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 62.5, 125, 250 and 500 mg test mat./L
4.2.2	Actual concentrations of test substance	No analytical monitoring was performed.
4.2.3	Effect data (Immobilisation)	See table A7_4_1_2-6 and table A7_4_1_2-7

Section A7.4.1.2_01 **Acute toxicity to invertebrates**
Annex Point IIA7.2 ***Daphnia magna***

4.2.4 Concentration / response curve



4.2.5 Other effects: None

4.3 Results of controls: See table A7_4_1_2-6
All animals of the control group remained mobile.

4.4 Test with reference substance: Not performed

4.4.1 Concentrations: Not relevant

4.4.2 Results: Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The acute toxicity of glyoxal [redacted] to aquatic invertebrates (*Daphnia magna* Straus) was studied according to method C.2 of Annex V of Directive 79/831/EEC (no GLP).
The immobilisation potential was assessed over a 48-hour exposure period under static conditions. The following nominal concentrations were tested: 0, 62.5, 125, 250 and 500 mg test mat./L. These concentrations were prepared starting from a 500 mg/L stock solution of the test substance. The dilution/test water was chlorine free, filtered tap water, which was supplemented (1) with sulphuric acid for the reduction of the buffering capacity of the carbonic acid system, and (2) with deionized water for the reduction of the total hardness. Reagent tubes with flat bottom were used as test vessels and were filled with 10 ml of test solution with the correspoding test concentration. Four vessels were used per test concentration, and five *Daphnia* (age: 2-24 h) were placed in each vessel. Hence, a total number of 20 *Daphnia* was used per test

Section A7.4.1.2_01 **Acute toxicity to invertebrates**
Annex Point IIA7.2 ***Daphnia magna***

concentration. The test was performed at a temperature of 19-21 °C. The *Daphnia* were examined for their mobility at the following time points: 0, 3, 6, 24 and 48 hours. Oxygen and pH measurements were performed at test initiation and after 48 hours.

5.2 Results and discussion

Immobilisation of *Daphnia*, dissolved oxygen and pH:

Nominal concen-tration	*Immobilisation*				*Dissolved oxygen*		*pH*	
[mg test mat./L]	*[%]*				*[mg/L]*			
	3 h	*6 h*	*24 h*	*48 h*	*0 h*	*48 h*	*0 h*	*48 h*
0	*0*	*0*	*0*	*0*	*8.83*	*8.60*	*7.70*	*8.00*
62.5	*0*	*0*	*0*	*0*	*8.82*	*8.50*	*7.60*	*7.98*
125	*0*	*0*	*0*	*0*	*8.72*	*8.44*	*7.61*	*7.96*
250	*0*	*0*	*0*	*10*	*8.58*	*8.21*	*7.58*	*7.88*
500	*0*	*0*	*5*	*75*	*8.53*	*7.54*	*7.56*	*7.73*

Temperature: 20 ± 1 °C

5.2.1 EC_0 125 mg test mat./L (nominal)

5.2.2 EC_{50} 404 mg test mat./L (nominal)

5.2.3 EC_{100} > 500 mg test mat./L (nominal)

5.3 Conclusion

The testing of the acute toxicity of glyoxal [redacted] to the freshwater crustacean *Daphnia magna* resulted in an EC_{50} value (48 h) of 404 mg/L, referring to the test material as such.
Besides the missing analytical monitoring, the test is valid according to OECD TG 202 (no data on control animals staying at the surface).

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Section A7.4.1.2_01 **Acute toxicity to invertebrates**
Annex Point IIA7.2 ***Daphnia magna***

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	19/03/2018
Materials and Methods	Agree.
Results and discussion	Agree
Conclusion	Agree
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Tap water purified by charcoal to remove chlorine, and filtered through a 6 µm filter. Sulfuric acid was added to reduce the buffering capacity of the carbonic acid system. Deionized water was added to reduce the total hardness of the water.
Acid capacity (Ks) up to pH 4.3	0.80 ± 0.1 mmol/L
Hardness	2.70 ± 0.50 mmol/L
pH	7.7 – 8.3
Ca / Mg ratio	4:1
Na / K ratio	10:1
Oxygen content	Aeration of the test water with oil free air until saturation, followed by stabilization over 24 hours preceding test initiation
Conductance	550 - 650 µSiemens/cm
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	[redacted]
Age	2 to 24 hours old
Breeding method	Not specified
Kind of food	Brewer´s yeast after each water change, washed green algae once a day
Amount of food	Not specified
Feeding frequency	See above
Pretreatment	No particularities
Feeding of animals during test	No data

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	None
Volume of test vessels	10 mL
Volume/animal	2 mL/animal
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	19 to 21 °C
Dissolved oxygen	Concentration (mg test mat./L) / Dissolved oxygen (mg/L) after 0 h / 48 h 0 / 8.83 / 8.60 62.5 / 8.82 / 8.50 125 / 8.72 / 8.44 250 / 8.58 / 8.21 500 / 8.53 / 7.54
pH	Concentration (mg test mat./L) / pH after 0 h / 48 h 0 / 7.70 / 8.00 62.5 / 7.60 / 7.98 125 / 7.61 / 7.96 250 / 7.58 / 7.88 500 / 7.56 / 7.73
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Light intensity ca. 5 µEinstein/(m*m*s) in the wavelength range of 400 to 750 nm
Photoperiod	16:8 hours day:night

Table A7_4_1_2-6: Immobilisation data

	Immobilisation				Phys.-chem. parameter		
Nominal concentration [mg test mat./L]	**Number**		**Percentage**		**O_2 content [mg/L]**	**pH**	**Tempera-ture [°C]**
	24 h	**48 h**	**24 h**	**48 h**	**48 h**	**48 h**	**48 h**
0	0	0	0	0	8.60	8.00	20 ± 1
62.5	0	0	0	0	8.50	7.98	20 ± 1
125	0	0	0	0	8.44	7.96	20 ± 1
250	0	2	0	10	8.21	7.88	20 ± 1
500	1	15	5	75	7.54	7.73	20 ± 1

Table A7_4_1_2-7: Effect data

	EC_{50}[1]	**95 % c.l.**	**EC_0[1]**	**EC_{100}[1]**
24 h [mg test mat./L]	> 500 (n)	-	250 (n)	> 500 (n)
48 h [mg test mat./L]	404 (n)	-	125 (n)	> 500 (n)

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

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	Fulfilled	**Not fullfilled**
Immobilisation of control animals <10%	**X**	
Control animals not staying at the surface	**Not reported**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**X**	
Concentration of test substance ≥80% of initial concentration during test	**No analytical monitoring performed**	

Criteria for poorly soluble test substances	**Not relevant**	

Section A7.4.1.2_02 Acute toxicity to invertebrates
Annex Point IIA7.2 **Marine species Mysid Shrimp (*Americamysis bahia*)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2009) Glyoxal [redacted] - A 96-hour static-renewal acute toxicity test with the salt water Mysid (*Americamysis bahia*). [redacted] 2009 (unpublished), BPD ID A7.4.1.2_02.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s.] for first entry to Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, US EPA OPPTS 850.1035 (1996)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, the photoperiod was 16 hours light to 8 hours darkness instead of 14 to 10 hours as indicated in the guideline. This is, however, not regarded to have a significant impact on the results of the study.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glyoxal [redacted] (CAS no 107-22-2), [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	[redacted] active ingredient (ethanedial in water)	
3.1.5	Further relevant properties	Colourless and clear liquid, stored unter nitrogen at ambient temperature	
3.1.6	Method of analysis	No data	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant	
3.3	**Reference substance**	No	

Section A7.4.1.2_02 **Acute toxicity to invertebrates**
Annex Point IIA7.2 **Marine species Mysid Shrimp (*Americamysis bahia*)**

3.3.1	Method of analysis for reference substance	Not relevant
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_2-2
3.4.2	Test organisms	See table A7_4_1_2-3
3.4.3	Test system	See table A7_4_1_2-4
3.4.4	Test conditions	See table A7_4_1_2-5
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Mortality and signs of toxicity (i.e. lethargy and surfacing) as well as physical characteristics of the test solutions (pH, dissolved oxygen, salinity, light intensity and temperature)
3.4.7	Sampling	The mortality and biological observations were checked at 5.5, 24, 48, 72 and 96 hours. The parameters of the test solutions were measured at test initation after 48 hours (prior and post the renewal of the test solutions) and at the test ending.
3.4.8	Monitoring of TS concentration	Samples were collected from the batch solution of each concentration at the beginning of the test. After 48 hours samples of each concentration were collected before and after the renewal of the test solutions and after 96 hours. The analytical method consisted of diluting the samples in saltwater, derivatizing and analyzing by high performance liquid chromatography (HPLC) using a UV-detector.
3.4.9	Statistics	The 24-hour LC_{50} value was calculated using probit analysis, while the 48, 72 and 96- hour LC_{50} were calculated using binomial probability. The no-mortality concentration and the NOEC were determined by visual interpretation of the mortality and observation data.
		4 RESULTS
4.1	**Limit Test**	The nominal test concentrations were based upon results of exploratory range finding toxicity data.
4.1.1	Concentration	Not reported
4.1.2	Number/ percentage of animals showing adverse effects	Not reported
4.1.3	Nature of adverse effects	Not reported

Section A7.4.1.2_02 **Acute toxicity to invertebrates**
Annex Point IIA7.2 **Marine species Mysid Shrimp (*Americamysis bahia*)**

4.2 Results test substance

4.2.1 Initial concentrations of test substance: 0, 20, 50, 100, 250 and 500 mg test material/L

4.2.2 Actual concentrations of test substance: 0, 19, 43, 89, 227 and 462 mg test material/L

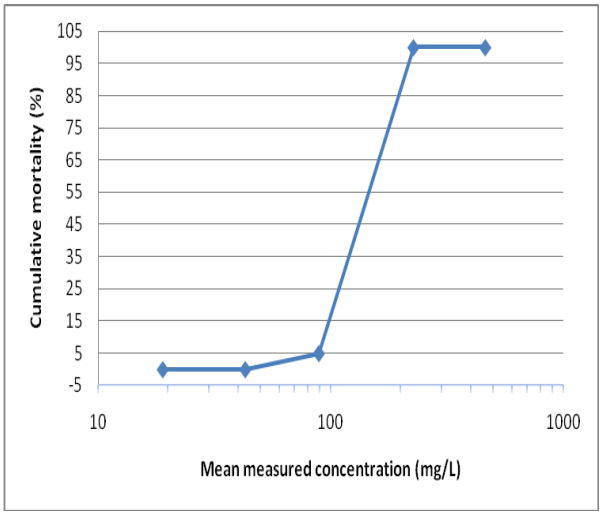
Results of the analytical monitoring over the test period:

Nominal concentration (mg test material /L)	Mean measured concentration (mg test material/L)	Mean percent of nominal concentration (%)
Control	<10	-
20	19	95
50	43	86
100	89	89
250	227	91
500	462	92

4.2.3 Effect data: See table A7_4_1_2-6 and table A7_4_1_2-7

4.2.4 Concentration / response curve

Concentration response curve after 96-hours:



4.2.5 Other effects: For sublethal effects, see table A7_4_1_2-6

4.3 Results of controls: See table A7_4_1_2-6

Section A7.4.1.2_02 **Acute toxicity to invertebrates**
Annex Point IIA7.2 **Marine species Mysid Shrimp (*Americamysis bahia*)**

4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the acute toxicity of Glyoxal [redacted] to marine invertebrates (Mysids, *Americamysis bahia*).

Test material: [redacted]

The test was carried out according to US EPA OPPTS 850.1035 (1996) under GLP conditions.

For the test juvenile mysids (<24 hours old) from a stock culture maintaimed [redacted] The stock culture was maintained in natural seawater, collected at [redacted], filtered (0.45 µm) and diluted with well water to a salinity of approximately 20‰. The adult mysids were held under the same conditions as for the test (water temperature: 25.5-26.9°C, pH: 7.6-7.9, dissolved oxygen: 5.9-7.2 mg/L, salinity: 20-25‰). The mysids in the culture were fed with live brine shrimp (*Artemia nauplii*) daily and occasionally with [redacted]. During the test the juvenile mysids were fed daily with brine shrimb.

Based on results of exploratory range finding toxicity data, the following nominal concentrations were tested: 0, 20, 50, 100, 250 and 500 mg test material/L.

The test concentrations were subjected to an analytical monitoring based on HPLC-UV.

The test system consisted of 2 L glass beakers filled with approximately 1.5 L of the test solution. Each test chamber contained ten mysids.

After 48 hours each test solution was renewed with a newly prepared solution.

The test temperature was maintained at 25±2°C and the animals were subjected to a 16:8 hours light/dark photoperiod. Dissolved oxygen, pH, salinity and temperature measurements were performed at 0, 48 (prior and post renewal) and 96 hours. The mysids were observed for mortality and sublethal effects indicative of toxicity at 5.5, 24, 48, 72 and 96 hours.

The LC_{50} values were calculated using probit analysis and binomial probality. The no-mortality and the NOEC were determined by visual interpretation of the effect data.

Section A7.4.1.2_02 **Acute toxicity to invertebrates**
Annex Point IIA7.2 **Marine species Mysid Shrimp (*Americamysis bahia*)**

5.2 Results and discussion

Analytical monitoring:

The mean measured concentrations of Glyoxal [redacted] were 19, 43, 89, 227 and 462 mg test material/L. The measured concentrations ranged from 86-95% of nominal. The results of the study were based on the mean measured concentrations.

Mortality and signs of toxicity:

In the control group one mysid was missing and assumed dead, therefore the cumulative mortality was 5% and the test is valid. The surviving mysids appeared normal throughout the test.

In the 19 and 43 mg test material/L no mortality or signs of toxicity were noted throughout the test.

The mortality in the 89, 227 and 462 mg test material/L were 5, 100 and 100% at the test end. Signs of toxicity were lethargy and surfacing.

Test parameters:

The water temperature was within the range of 25±2°C. Dissolved oxygen concentrations were >5.5 mg/L (>75% of saturation). The pH ranged from 7.6-8.2 and the salinity was 20‰. The light intensity measured in one representative test chamber at the water surface was 610 lux.

5.2.1 NOEC

The no-mortality was 43 mg test material/L, corresponding to 17.1 mg Glyoxal/L.

	mg test material/L	**mg Glyoxal/L**
NOEC	43	17.1

5.2.2 LC_{50}

Time	**LC_{50} (mg test material/L)**	**LC_{50} (mg Glyoxal/L)**
48 hours (95% confidence limits)	157 (89-227)	62.3 (35.3-90.1)
96 hours (95% confidence limits)	134 (89-227)	53.2 (35.3-90.1)

5.2.3 LC_{100}

Not reported

5.3 Conclusion

The testing of the acute toxicity of Glyoxal [redacted] to the marine Mysid *Americamysis bahia* using a static-renewal system resulted in a LC_{50} value (96 h) of 53.2 mg a.s./L, referring to the active substance Glyoxal; the NOEC was 17.1 mg a.s./L.

The validity criteria for the mysid acute toxicity test according to US EPA OPPTS 850.1035 (1996) were fulfilled.

Section A7.4.1.2_02 **Acute toxicity to invertebrates**
Annex Point IIA7.2 **Marine species Mysid Shrimp (*Americamysis bahia*)**

5.3.1 Reliability [redacted]
5.3.2 Deficiencies [redacted]

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	19/03/2018
Materials and Methods	Agree.
Results and discussion	Agree
Conclusion	Agree
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Natural seawater collected at [redacted], passed through a sand filter (ca. 25 µm), diluted with fresh water to a salinity of 20‰, aerated, filtered through 0.45 µm (UV sterilized for stock solution preparation)
Acid capacity (Ks) up to pH 4.3	Not specified
Hardness	Not specified
pH	7.6 – 8.2
Ca / Mg ratio	ca. 1:3
Na / K ratio	Not specified
Oxygen content	fresh media: 7.1-7.4 mg/L aged media: 5.5-6.6 mg/L (4.4 mg/L represents 60% saturation at 25 °C in saltwater with a salinity of 20‰)
Conductance	Not specified
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Americamysis bahia (previous name: Mysidopsis bahia)*
Source	in-house culture
Age	< 24 hours old
Breeding method	in natural seawater collected at [redacted] (salinity 20-25‰);
Kind of food	fed daily live brine shrimp nauplii (Artemia sp.) occasionanlly enriched with [redacted] to prevent cannibalism
Amount of food	Not specified
Feeding frequency	See above
Pretreatment	No particularities
Feeding of animals during test	live brine shrimp nauplii (Artemia sp.)

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	After 48 h
Volume of test vessels	2 L
Volume/animal	150 mL/animal
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	25 ± 2 °C
Dissolved oxygen	fresh media: 7.1-7.4 mg/L aged media: 5.5-6.6 mg/L (4.4 mg/L represents 60% saturation at 25 °C in saltwater with a salinity of 20‰)
pH	7.6-8.2
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	ca. 600 lux
Photoperiod	16 h light: 8 h dark (30-min transition period)

Table A7_4_1_2-6: Immobilisation data

	Immobilisation				Phys.-chem. Parameter		
Nominal concentration [mg test mat./L]	**Number**		**Percentage**		**O_2 content [mg/L]**	**pH**	**Tempera-ture [°C]**
	24 h	**96 h**	**24 h**	**96 h**	**96 h**	**96 h**	**96 h**
0	0	1	-	5	6.7	8.1	26.5
19	0	0	-	0	6.5	8.1	26.0
43	0	0	-	0	6.0	8.0	26.2

89	0	1	-	5	5.7	7.9	25.3
227	3	10	-	100	6.5	7.9	25.3
462	7	10	-	100	-	-	-

Table A7_4_1_2-7: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg test mat./L]	34 (m)	270 - 457	-	-
96 h [mg test mat./L]	134 (m)	89 - 227	-	-

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

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

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

Criteria for poorly soluble test substances	**Not relevant**	

Section A7.4.1.2_03 Acute toxicity to invertebrates
Annex Point IIA7.2 ***Daphnia magna***

Official use only

1 REFERENCE

1.1 Reference [redacted] (1984b) Acute toxicity of CT-194 to daphnids (*Daphnia magna*). S[redacted]. BPD ID A7.4.1.2_03.

1.2 Data protection Yes

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study In-house protocol based on 'Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians' (US EPA, 1975). Comparable to OECD 203.

2.2 GLP No, GLP was not compulsory at the time the study was performed.

2.3 Deviations Yes, no analytical monitoring of test concentrations was performed

3 MATERIALS AND METHODS

3.1 Test material [redacted]

3.1.1 Lot/Batch number [redacted]

3.1.2 Specification Not stated

Section A7.4.1.2_03 Acute toxicity to invertebrates
Annex Point IIA7.2 ***Daphnia magna***

3.1.3	Purity	Not specified
3.1.4	Composition of Product	Not specified
3.1.5	Further relevant properties	Clear, light yellow liquid
3.1.6	Method of analysis	No data
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant
3.3	**Reference substance**	No reference substance used
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_2-2
3.4.2	Test organisms	See table A7_4_1_2-3
3.4.3	Test system	See table A7_4_1_2-4
3.4.4	Test conditions	See table A7_4_1_2-5
3.4.5	Duration of the test	48 hours
3.4.6	Test parameter	Mortality and observations of physical characteristics of each replicate test solution.
3.4.7	**Sampling**	No samples were taken
3.4.8	**Monitoring of TS concentration**	No
3.4.9	**Statistics**	The 24-h LC50 and its 95% confidence limits were determined by nonlinear interpolation and the binomial probability method. Moving angle average angle analysis was used to calculate the 48-h LC50 and corresponding 95% CL.
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 78, 130, 220, 360, 600 and 1000 mg CT-194/L
4.2.2	Actual concentrations	No analytical monitoring was performed.

Section A7.4.1.2_03 **Acute toxicity to invertebrates**
Annex Point IIA7.2 ***Daphnia magna***

	of test substance	
4.2.3	Effect data (mortality	See table A7_4_1_2-6 and table A7_4_1_2-7
4.2.4	Concentration / response curve	None
4.2.5	Other effects	None
4.3	**Results of controls**	No adverse effects were reported for the control animals
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The acute toxicity of [redacted] to *Daphnia magna* was determined under static conditions in a 48-h test according to methodology of the US EPA. Daphnids (5 in each of three vessels per treatment) were exposed to nominal concentrations of 0 (control), 78, 130, 220, 360, 600 and 1000 mg [redacted] in a hard dilution water prepared by fortification of well water. Observations of mortality and abnormal appearance and behaviour were made at daily intervals, together with checks on the condition of the test media. Measurements of water quality were made in the control and the lowest, middle and highest exposure treatments at test initiation and termination.

5.2 Results and discussion

With the exception of the control group, sublethal effects were observed in all treatments containing surviving daphnids after 24 and 48 h: between half and all survivors exhibited lethargy and were at the bottom of the test vessels. Some daphnids at concentrations ≥130 mg [redacted] were trapped at the surface of the test media. Some daphnids of the 78 and 130 mg/L treatments were trapped on shed exoskeletons at 48 h.

Mean Mortality %		
Nominal concentration mg/l	**24 h**	**48 h**
0	0	0
78	0	13
130	0	47
220	0	27
360	0	60
600	0	93

Section A7.4.1.2_03 **Acute toxicity to invertebrates**
Annex Point IIA7.2 ***Daphnia magna***

1000	100	100

Temperature, dissolved oxygen and pH: The temperature of the test solutions in the control was 21°C and remained constant over the whole testing period. The concentration of dissolved oxygen ranged between 7.9 and 8.8 mg/L. The pH value ranged betweeen 7.6 and 8.0.

5.2.1 NOEC <78 mg/L (nominal at 48 hours)

5.2.2 LC_{50} 220 mg/L (nominal at 48 hours)

5.2.3 LC_{100} Not specified

5.3 Conclusion The testing of the acute toxicity of [redacted] to the freshwater crustacean *Daphnia magna* under static conditions resulted in an LC_{50} value (48 h) of 220 mg/L. No control mortalities occurred.

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Section A7.4.1.2_03 **Acute toxicity to invertebrates**
Annex Point IIA7.2 ***Daphnia magna***

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	19/03/2018
Materials and Methods	Agree
Results and discussion	Agree
Conclusion	Agree
Reliability	[redacted]
Acceptability	
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	**Details**
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Fortified well water based on the formula for hard water (U.S. EPA, 1975) and filtering it through a carbon filter and an Amberlite XAD-7 resin column to remove any potential organic contaminants
Alkalinity	As $CaCO_3$ 180 mg/l
Hardness	As $CaCO_3$ 120 mg/l
pH	8.3
Oxygen content	>60% saturation maintained
Conductance	500 µmhos/cm
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	The daphnids used were obtained from laboratory cultures maintained [redacted]
Age	< 24 hours old
Breeding method	Not specified
Kind of food	Green algae (*Ankistrodesmus* sp. or *Selenastrum* sp.) and yeast.
Amount of food	Not specified
Feeding frequency	Once daily
Pretreatment	Other than culture conditions, not specified
Feeding of animals during test	None

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	None
Volume of test vessels	250 mL
Volume/animal	200 mL/5 animals
Number of animals/vessel	5
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	Maintained at 21 °C for all test solutions
Dissolved oxygen	Concentration (mg test mat./L) / Dissolved oxygen (mg/L) 0 h / 48 h 0 / 8.7 / 8.2 78 / 8.6 / 7.9 360 / 8.7 / 8.6 100 / 8.7 / 8.8
pH	Concentration (mg test mat./L) / pH 0 h / 48 h 0 / 8.0 / 8.0 78 / 8.0 / 7.9 360 / 7.9 / 8.0 100 / 7.6 / 7.9
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Not specified for test chambers, culture area received a light intensity of 5-10 hectolux.
Photoperiod	16 hours light / 8 hours dark

Table A7_4_1_2-6: Cumulative mortality data

	Mortality	
Nominal concentration [mg/L]	**Mean Percentage**	
	24 h	**48 h**
0	0	0
78	0	13
130	0	47
220	0	27
360	0	60
600	0	93
1000	100	100

Table A7_4_1_2-7: Effect data

	LC_{50}	**95 % C.I.**	**NOEC**
24 h [mg/L]	700 (n)	600-1000	-
48 h [mg/L]	220 (n)	160-290	<78

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	**Not fullfilled**
Immobilisation of control animals <10%	**yes**	
Control animals not staying at the surface	**yes**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**No analytical monitoring performed**	
Criteria for poorly soluble test substances	**Not relevant**	

Section A7.4.1.2_04 Acute toxicity to invertebrates
Annex Point IIA7.2 **Marine species Mysid Shrimp (*Mysidopsis bahia*)**

Official use only

1 REFERENCE

1.1 Reference		[redacted] (1984) Acute toxicity of [redacted] to mysid (*Mysidopsis bahia*). [redacted] 1984. BPD ID A7.4.1.2_04
1.2 Data protection		Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study	None cited, but compatible with US EPA requirements
2.2 GLP	No, GLP was not compulsory at the time the study was performed.
2.3 Deviations	No

3 MATERIALS AND METHODS

3.1 Test material		[redacted]
3.1.1	Lot/Batch number	Not specified
3.1.2	Specification	Not stated
3.1.3	Purity	Not specified
3.1.4	Composition of Product	Not specified
3.1.5	Further relevant properties	Light yellow liquid
3.1.6	Method of analysis	No data
3.2 Preparation of TS solution for poorly soluble or volatile test substances		Not relevant
3.3 Reference substance		No reference substance used
3.3.1	Method of analysis for reference substance	Not applicable
3.4 Testing procedure		

Section A7.4.1.2_04 **Acute toxicity to invertebrates**
Annex Point IIA7.2 **Marine species Mysid Shrimp (*Mysidopsis bahia*)**

3.4.1	Dilution water	See table A7_4_1_2-2
3.4.2	Test organisms	See table A7_4_1_2-3
3.4.3	Test system	See table A7_4_1_2-4
3.4.4	Test conditions	See table A7_4_1_2-5
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Mortality as well as physical characteristics of the test solutions (pH, dissolved oxygen, salinity, light intensity and temperature).
3.4.7	Sampling	No samples were taken.
3.4.8	Monitoring of TS concentration	No
3.4.9	Statistics	The 24, 48, 72 and 96-h LC50 values and their 95% confidence limits were determined by nonlinear interpolation.

4 RESULTS

4.1 Limit Test		Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2 Results test substance		
4.2.1	Initial concentrations of test substance	0, 130, 220, 360, 600 and 1000 mg [redacted]L
4.2.2	Actual concentrations of test substance	No analytical monitoring was performed
4.2.3	Effect data	See table A7_4_1_2-6 and table A7_4_1_2-7
4.2.4	Concentration / response curve	None
4.2.5	Other effects	None reported
4.3 Results of controls		See table A7_4_1_2-6
4.4 Test with reference substance		Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

Section A7.4.1.2_04 **Acute toxicity to invertebrates**
Annex Point IIA7.2 **Marine species Mysid Shrimp (*Mysidopsis bahia*)**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The acute toxicity of [redacted] to *Mysidopsis bahia* was determined under static conditions in a 96-h test compatible with guideline requirements of the US EPA. Mysids (10 in each of two vessels per treatment) were exposed to nominal concentrations of 0 (control), 130, 220, 360, 600 and 1000 mg [redacted]/L in filtered sea water. Observations of mortality and abnormal appearance and behaviour were made at daily intervals, together with checks on the condition of the test media. Measurements of water quality were made in all treatments at test initiation and termination at 96 h, or at earlier timepoints in treatments where 100% mortality occurred before 96 h.

5.2 Results and discussion

Mortality: zero mortality occured in the control group. No behavioural abnormalities were seen in the control or any of the groups exposed to [redacted]

Mean Mortality (%)				
Nominal concentration mg/L	**24 h**	**48 h**	**72 h**	**96h**
0	0	0	0	0
130	0	0	0	5
220	0	40	45	100
360	40	100	100	100
600	100	100	100	100
1000	100	100	100	100

Test parameters:

The water temperature was within the range of 21-22°C. Dissolved oxygen concentrations were 5.3 – 6.9 mg/l. The pH ranged from 7.1-7.9. Salinity was 31% in test water used but was not monitored throughout testing. The test was conducted under fluorescent lighting 14 hour light / 10 hour dark cycle.

5.2.1 NOEC — Not calculated

5.2.2 LC_{50} — The 96 hour LC50 was 160 mg/L

5.2.3 LC_{100} — Not reported

5.3 Conclusion

The testing of the acute toxicity of [redacted] to the marine mysid shrimp *Mysidopsis bahia* under static conditions resulted in an LC_{50} value (96 h) of 160 mg/L.

Section A7.4.1.2_04 **Acute toxicity to invertebrates**
Annex Point IIA7.2 **Marine species Mysid Shrimp (*Mysidopsis bahia*)**

5.3.1 Reliability [redacted]
5.3.2 Deficiencies [redacted]

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	19/03/2018
Materials and Methods	Agree
Results and discussion	Agree
Conclusion	Agree
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Natural seawater, [redacted] This water was pumped through sand-filled fibreglass filters and through 10-micrometer porosity poly-propylene core filters into an elevated fibreglass reservoir. Test water was additionally filtered through a S-micrometer porosity polypropylene core filter before distribution into the test chambers. Test water had a salinity of salinity 31%.
Alkalinity	Not specified
Hardness	Not specified
pH	Not specified
Oxygen content	Not specified
Conductance	Not specified
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Mysidopsis bahia*
Source	The mysids used were obtained from laboratory cultures [redacted]
Age	4 days old
Breeding method	Not specified
Kind of food	*Artemia salina nauplii*
Amount of food	Not specified
Feeding frequency	Daily during holding
Pretreatment	Not specified
Feeding of animals during test	Mysids were fed on day 0 and 2 during the test.

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	None
Volume of test vessels	1.6 L
Volume/animal	100 mL
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	Maintained at 21-22°C for all test solutions

Dissolved oxygen

Concentration (mg test mat./L)		Dissolved oxygen (mg/L) 0 h	24 h	48 h	96 h
0	A	6.8	-	-	6.1
	B	6.7	-	-	6.3
130	A	6.8	-		5.0
	B	6.6	-	-	5.5
220	A	6.7	-	-	5.3
	B	6.7	-	-	5.3
360	A	6.7	-	6.3	-
	B	6.6	-	6.0	
600	A	6.7	6.0	-	-
	B	6.7	5.7	-	
1000	A	6.7	6.3	-	-
	B	6.8	6.4	-	

pH

Concentration (mg test mat./L)		pH 0 h	24 h	48 h	96 h
0	A	7.7	-	-	7.9
	B	-	-	-	7.9
130	A	7.9	-	-	7.7
	B	-	-	-	7.9
220	A	7.8	-	-	7.8
	B	-	-	-	7.8
360	A	7.7	-	7.7	-
	B	-	-	7.6	
600	A	7.6	7.7	-	-
	B	-	7.6	-	
1000	A	7.6	7.5	-	-
	B	-	7.5	-	

Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Not specified
Photoperiod	14 hours light / 10 hours dark

Table A7_4_1_2-6: Cumulative mortality data (numbers per replicate)

Concentration (mg test mat./L)	Replicate	Cumulative Mortality			
		24 h	48 h	72 h	96 h
0	A	0	0	0	0
	B	0	0	0	0
130	A	0	0	0	0
	B	0	0	0	1
220	A	0	3	3	10
	B	0	5	6	10
360	A	4	10	10	10
	B	4	10	10	10
400	A	10	10	10	10
	B	10	10	10	10
1000	A	10	10	10	10
	B	10	10	10	10

Table A7_4_1_2-7: Effect data

Time hours	**LC_{50} (mg test material/L)**
24 (95% confidence limits)	380 220-600
48 hours (95% confidence limits)	230 130-360
72 (95% confidence limits)	230 130-360
96 hours (95% confidence limits)	160 130-220

Section A7.4.1.2_05 Acute toxicity to invertebrates
Annex Point IIA7.2 ***Daphnia magna***

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted]1984) Acute toxicity of [redacted] to Daphnids (*Daphnia magna*). [redacted]. BPD ID A7.4.1.2_05.
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	In-house protocol based on 'Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians' (US EPA, 1975). Comparable to OECD 203.
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Yes, no analytical monitoring of test concentrations was performed
		3 MATERIALS AND METHODS
3.1	**Test material**	[redacted]
3.1.1	Lot/Batch number	Not specified
3.1.2	Specification	Not stated
3.1.3	Purity	Not specified
3.1.4	Composition of Product	Not specified
3.1.5	Further relevant properties	Amber colored liquid
3.1.6	Method of analysis	No data
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant
3.3	**Reference substance**	No reference substance used
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_2-2
3.4.2	Test organisms	See table A7_4_1_2-3
3.4.3	Test system	See table A7_4_1_2-4
3.4.4	Test conditions	See table A7_4_1_2-5
3.4.5	Duration of the test	48 hours
3.4.6	Test parameter	Mortality and observations of physical characteristics of each replicate test solution.
3.4.7	**Sampling**	No samples were taken

Section A7.4.1.2_05 **Acute toxicity to invertebrates**
Annex Point IIA7.2 ***Daphnia magna***

3.4.8	**Monitoring of TS concentration**	No
3.4.9	**Statistics**	Not required.
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 130, 220, 360, 600 and 1000 mg test mat./L
4.2.2	Actual concentrations of test substance	No analytical monitoring was performed.
4.2.3	Effect data (Immobilisation)	See table A7_4_1_2-6 and table A7_4_1_2-7
4.2.4	Concentration / response curve	None
4.2.5	Other effects	None
4.3	**Results of controls**	No adverse effects were reported for the control animals
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The acute toxicity of [redacted] to *Daphnia magna* was determined under static conditions in a 48-h test according to methodology of the US EPA. Daphnids (5 in each of three vessels per treatment) were exposed to nominal concentrations of 0 (control), 78, 130, 220, 360, 600 and 1000 mg [redacted]L in a hard dilution water prepared by fortification of well water. Observations of mortality and abnormal appearance and behaviour were made at daily intervals, together with checks on the condition of the test media. Measurements of water quality were made in the control and the lowest, middle and highest exposure treatments at test initiation and termination.

Section A7.4.1.2_05 **Acute toxicity to invertebrates**
Annex Point IIA7.2 ***Daphnia magna***

5.2 Results and discussion

Mortality: There were no treatment-related sublethal effects. A single daphnid of the control was observed at the surface of the test medium after 48 h.

Mean Cumulative Mortality %		
Nominal concentration mg/l	**24 h**	**48 h**
0	0	0
130	0	0
220	0	0
360	0	0
600	0	0
1000	0	0

Temperature, dissolved oxygen and pH: The temperature of the test solutions in the control was between 19-20°C. The concentration of dissolved oxygen ranged between 8.3 and 8.9 mg/L. The pH value ranged betweeen 7.8 and 8.4.

5.2.1 NOEC >1000 mg test mat./L (nominal at 48 hours)

5.2.2 LC_{50} >1000 mg test mat./L (nominal at 48 hours)

5.2.3 LC_{100} >1000 mg test mat./L (nominal at 48 hours)

5.3 Conclusion The testing of the acute toxicity of [redacted] to the freshwater crustacean *Daphnia magna* under static conditions resulted in an LC_{50} value (48 h) of >1000 mg/L. No control mortalities occurred.

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Section A7.4.1.2_05 **Acute toxicity to invertebrates**
Annex Point IIA7.2 ***Daphnia magna***

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	19/03/2018
Materials and Methods	Agree
Results and discussion	Agree
Conclusion	Agree
Reliability	[redacted]
Acceptability	
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Fortified well water based on the formula for hard water (U.S. EPA, 1975) filtered through a carbon filter and an Amberlite XAD-7 resin column to remove any potential organic contaminants
Alkalinity	As $CaCO_3$ 120 mg/L
Hardness	As $CaCO_3$ 180 mg/L
pH	8.0
Oxygen content	>60% saturation maintained
Conductance	600 µmhos/cm
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	The daphnids used were obtained from laboratory cultures [redacted]
Age	< 24 hours old
Breeding method	Not specified
Kind of food	None
Amount of food	Not specified
Feeding frequency	Once daily
Pretreatment	Other than culture conditions, not specified
Feeding of animals during test	None

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	None
Volume of test vessels	250 mL
Volume/animal	200 mL/5 animals
Number of animals/vessel	5
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	19 to 20 °C
Dissolved oxygen	Concentration (mg test mat./L) / Dissolved oxygen (mg/L) after 0 h / 48 h 0 / 8.7 / 8.9 130 / 8.7 / 8.9 360 / 8.7 / 8.3 1000 / 8.8 / 8.4
pH	Concentration (mg test mat./L) / pH after 0 h / 48 h 0 / 8.2 / 8.4 130 / 8.1 / 8.3 360 / 8.0 / 8.1 1000 / 7.8 / 8.2
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Not specified for test chambers, culture area received a light intensity of 5-8 hectolux.
Photoperiod	16 hours light / 8 hours dark

Table A7_4_1_2-6: Cumulative mortality data

	Mortality	
Nominal concentration [mg test mat./L]	**Mean Percentage**	
	24 h	**48 h**
0	0	0
130	0	0
220	0	0
360	0	0
600	0	0
1000	-	-

Table A7_4_1_2-7: Effect data

	EC_{50}[1]	**95 % c.l.**	**NOEC**
24 h [mg test mat./L]	> 1000 (n)	-	-
48 h [mg test mat./L]	>1000 (n)	-	1000

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

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	**Not fullfilled**
Immobilisation of control animals <10%	**Yes**	
Control animals not staying at the surface	**Yes**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**Yes**	
Concentration of test substance ≥80% of initial concentration during test	**No analytical monitoring performed**	

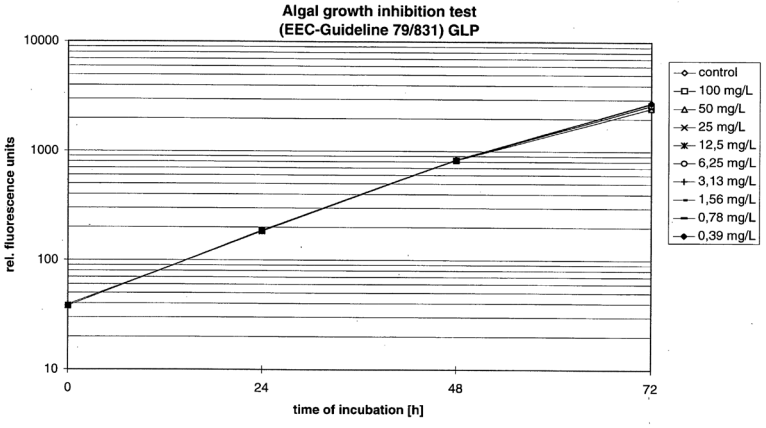
Criteria for poorly soluble test substances	**Not relevant**	

Section A7.4.1.3_01 Growth inhibition test on algae

Annex Point IIA7.3

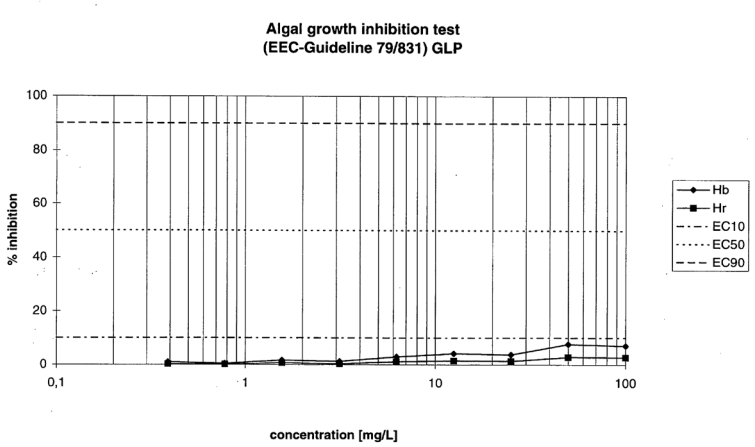
			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1997) Determination of the inhibitory effect of [redacted] on cell multiplication of unicellular green algae. [redacted] 1997, (unpublished), BPD ID A7.4.1.3_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, Directive 92/69/EEC, Annex V, C.3 (update of May 1988)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glyoxal (1,2-ethanedial)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	[redacted] active ingredient (i.e. glyoxal), [redacted] water	
3.1.5	Further relevant properties	None relevant	
3.1.6	Method of analysis	Reversed phase HPLC with UV-detection after derivatization with 2,4-dinitrophenylhydrazine; evaluation by external standard	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant since the TS is soluble in water up to 10 g/l.	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not relevant	
3.4	**Testing procedure**		
3.4.1	Culture medium	Mineral composition [mg/l]: 15 mg/l NH_4Cl, 12 mg/l $MgCl_2$ * 6 H_2O, 18 mg/l $CaCl_2$ *2 H_2O, 15 mg/l MgSO4 *7 H_2O; 1.6 mg/l KH_2PO_4, 0.08 mg/l $FeCl_3$ * 6 H_2O, 0.1 mg/l Na_2EDTA * 2 H_2O, 50 mg/l $NaHCO_3$, 0.185 mg/l H_3BO_3, 0.415 mg/l $MnCl_2$ * 4 H_2O, 0.003 mg/l $ZnCl_2$,0.0015 mg/l $CoCl_2$ * 6 H_2O, 0.00001 mg/l $CuCl_2$ * 2 H_2O, 0.007 mg/l Na_2MoO_4 * 2 H_2O pH approx. 8	
3.4.2	Test organisms	See table A7_4_1_3-2	

Section A7.4.1.3_01 Growth inhibition test on algae
Annex Point IIA7.3

3.4.3	Test system	See table A7_4_1_3-3	
3.4.4	Test conditions	See table A7_4_1_3-4	
3.4.5	Duration of the test	72 hours	
3.4.6	Test parameter	Cell multiplication inhibition	
3.4.7	Sampling	Fluorescence measurements were performed after 0, 24, 48, and 72 hours.	
3.4.8	Monitoring of TS concentration	Yes, at the start of the test the uninoculated replicates were analyzed, at the end of the test (after 72 h) both the uninoculated and inoculated replicates were analyzed. The analytical monitoring was performed for following nominal concentrations: 0 (control), 0.39, 6.25 and 100 mg/L.	X
3.4.9	Statistics	Biomass growth: calculation via the integral over the total duration of the test for each concentration. Growth rate: calculated over the total duration of the study for each concentration level and compared to control. The EC values are calculated from the concentration-response relationship. The LOEC was determined by comparing the means of the fluorescence measurement of the various concentration levels with the control. The Duncan multiple range test was performed at a 95% significance level. Every higher tested concentration must have at least the same or stronger effects then the LOEC.	
		4 RESULTS	
4.1	**Limit Test**	No	
4.1.1	Concentration	Not relevant	
4.1.2	Effect data	Not relevant	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25.0, 50.0, 100.0 mg/L	X
4.2.2	Actual concentrations of test substance	Analytical monitoring after 0 and 72 h in the uninoculated nominal test concentrations 0.39, 6.25 and 100 mg/l revealed mean recovery rates of 83 – 101 %. In the inoculated concentrations, the recovery after 72 h was between 56 and 85 %. Since the mean measured concentrations in the uninoculated samples were greater than 80%, the effect concentrations were based on nominal values.	X
4.2.3	Growth curves		

Section A7.4.1.3_01 Growth inhibition test on algae
Annex Point IIA7.3

4.2.4 Concentration / response curve



4.2.5 Cell concentration data — See table A7_4_1_3-5

4.2.6 Effect data (cell multiplication inhibition)

Effect concentrations (mg a.i./L) for 0-72 h		95 %-c.l. (mg a.i./L)
E_rC_{10}	≥ 100	n.d.
E_bC_{10}	≥ 100	n.d.
E_rC_{50}	> 100	n.d.
E_bC_{50}	> 100	n.d.
NOEC*	3.13	n.d.
LOEC*	6.25	n.d.

n.d.: not determined due to mathematical reasons
* NOEC and LOEC valid for growth rate and biomass integral

X

X

4.2.7 Other observed effects — None

4.3 Results of controls — The cell multiplication factor in the untreated control after 72 h was 74. For details see table A7_4_1_3-5

4.4 Test with reference substance — Not performed

4.4.1 Concentrations — Not relevant

4.4.2 Results — Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The inhibitory effect of [redacted] glyoxal) on the cell multiplication of the unicellular green algae *Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*) CHODAT SAG 86.81 was investigated. The test was carried out according to Directive 92/69/EEC, C.3 (1992) under GLP conditions.
Multiplication of cells was determined under the influence of [redacted] in relation to the untreated control at 23 °C. Following nominal concentrations were tested 0.0 (control), 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25.0, 50.0, and 100 mg/L. The initial cell density of *Desmodesmus subspicatus* was 10E+4 cells/ml. Fluorescence measurements were performed after 0, 24, 48, and 72 hours.
An analytical monitoring of the test concentrations was conducted at the start of the test (uninoculated replicates) and at test end (after 72 h; both uninoculated and inoculated replicates) by means of phase HPLC with

Section A7.4.1.3_01 Growth inhibition test on algae
Annex Point IIA7.3

UV-detection. The analytical monitoring was carried out for following nominal concentrations: 0, 0.39, 6.25 and 100 mg/L.

5.2 Results and discussion

Analytical monitoring:
The results of the analytical monitoring in the uninoculated samples revealed a mean recovery rate of > 80%; therefore the effect concentrations were based on nominal concentrations. X

Biological effects:

Concentration [mg/L]	Biomass		Growth rate	
	absolute	Inhibition [% of the Control]	absolute	Inhibition [% of the Control]
0 (control)	2293	0	0,060	0
100,00	2126	7,3	0,058	2,6
50,00	2115	7,8	0,058	2,8
25,00	2201	4,0	0,059	1,3
12,50	2194	4,3	0,059	1,4
6,25	2218	3,3	0,059	1,1
3,13	2264	1,3	0,060	0,3
1,56	2250	1,9	0,059	0,5
0,78	2273	0,9	0,059	0,7
0,39	2268	1,1	0,060	0,2

Inhibitory effects on algae growth were seen at higher test concentrations, but were only slighty exhibited.

5.2.1 NOEC 3.13 mg a.i./L X

5.2.2 ErC50 > 100 mg a.i./L

5.2.3 E_bC50 > 100 mg a.i./L

5.3 Conclusion The test resulted in an E_rC_{50} of > 100 mg a.i./L. Significant inhibitory effects on algae growth were observed at 6.25 mg a.i./L; therefore the NOEC is 3.13 mg a.i./L. The validity criteria for the algal growth inhibition test according to OECD Guideline 201 were fulfilled. X

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Section A7.4.1.3_01 **Growth inhibition test on algae**
Annex Point IIA7.3

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	2/03/2018
Materials and Methods	No data on a reference substance is available. 3.4.8: "The analytical monitoring was performed for following nominal concentrations: 0 (control), 0.39, 6.25 and 100 mg test mat./L"
Results and discussion	4.2.1: "0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25.0, 50.0, 100.0 mg test mat./L" 4.2.2: "The mean measured concentrations in the uninoculated samples were greater than 80% (averaged on the values at the start and the end of the test). However, note that the recovery in the uninoculated sample for the lowest concentration 0.39 mg/Lafter 72h is not within the range of ± 20 % (77%). In the inoculated samples, recovery rates after 72h for the two lowest measured concentrations 0.39 mg/L and 6.25 mg/L are 56 % and 62% respectively. Consequently, the effect concentrations were based on measured values". 4.2.6 in the table: "Effect concentrations (mg test mat./L) for 0-72 h" ; "95 %-c.l. (mg test mat./L)" 5.2: "The results of the analytical monitoring in the inoculated samples revealed a mean recovery rate of ≤80%; therefore the effect concentrations were based on measured concentrations." 4.2.6, 5.2: At the highest tested measured concentration of 85 mg test mat./L, only 2.6 % growth rate inhibition was detected. The E_rC_{50} and E_bC_{50} were both >85 mg test mat./L and the long term ErC_{10} value of 85 mg test mat./L was determined. In the final analytical report, concentrations were given for the test material [redacted] Consequently, the ErC_{10} = 34 mg a.i./L. 5.2.1: NOEC/ ErC_{10} = 85 mg test mat./L (measured concentration), equivalent to 34 mg a.i./L; 5.2.2: ErC_{50} >85 mg test mat.L (measured concentration), equivalent to 34 mg a.i./L; 5.2.3: EbC_{50}> 85 mg test mat./L (measured concentration), equivalent to 34 mg a.i./L;
Conclusion	NOEC/ ErC_{10}= 34 mg a.i./L
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	None

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	*Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*) CHODAT
Strain	SAG 86.81
Source	[redacted]
Laboratory culture	Yes
Method of cultivation	Liquid culture, weekly passage, 23 ±2 °C, 10000 cells/ml (volume 100 ml)
Pretreatment	Algae were precultured 72 h prior to test start. No further pretreatment was carried out
Initial cell concentration	10^4 cells/ml

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	250 ml (culture and test volume of algal suspension: 100 mL)
Culturing apparatus	According to guideline
Light quality	Artificial light, OSRAM L25 universal white, permanent illumination (about 120 µE/m² s) in the range of 400 – 700 nm
Procedure for suspending algae	Not specified, probably as prescribed by the guideline
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No (flasks were plugged with gas permeable silicone sponge caps)

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	23 ± 2 °C
pH	Start of the test, uninoculated: 7.7-7.8 End of test, uninoculated: 7.8-7.9 End of test, inoculated: 9.3 – 9.8 (control: 9.8)
Aeration of dilution water	-
Light intensity	About 120 µE/m²s
Photoperiod	Permanent illumination

Concentration [mg/L]	uninoculated 0 h	uninoculated 72 h	inoculated 72 h
0 (control)	7,7	7,8	9,8
100,00	7,8	7,9	9,3
50,00	7,8	7,9	9,5
25,00	7,8	7,9	9,7
12,50	7,8	7,9	9,8
6,25	7,8	7,9	9,7
3,13	7,8	7,9	9,8
1,56	7,8	7,9	9,7
0,78	7,8	7,9	9,7
0,39	7,7	7,8	9,7

Table A7_4_1_3-5: Cell concentration data

Nominal concentration [mg/L]	Cell concentrations (mean values) [relative fluormeter units]							
	Measured				Percent of control			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
0	38	188	839	2798	100	100	100	100
0.39	38	185	830	2771	100	98	99	99
0.78	39	184	835	2781	103	98	100	99
1.56	38	187	829	2733	100	99	99	98
3.13	38	185	830	2763	100	98	99	99
6.25	38	183	832	2672	100	97	99	95
12.50	38	184	824	2638	100	98	98	94
25.00	38	183	826	2649	100	97	98	95
50.00	38	186	821	2482	100	99	98	89
100.00	38	185	824	2500	100	98	98	89
Temperature [°C]	23 ± 2 °C							
pH	7.7 – 7.8	-	-	9.3 – 9.8				

Table A7_4_1_3-6: Validity criteria for algal growth inhibition test according to OECD Guideline 201 (March 2006)

	Fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**Yes**	
Coefficient of variation of average specific growth rates in control cultures < 7 %.	**Yes***	
Mean coefficient of variation of sectional growth rates calculated for the individual control replicates from 0 to 72 h < 35 %	**Yes***	
Concentration of test substance ≥80% of initial concentration during test		**X**

* = recalculated from raw data by the author of this summary

Criteria for poorly soluble test substances	**Not relevant**	

Section A7.4.1.3_02
Annex Point IIA, VII.7.3.

Growth inhibition test on algae

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		1 REFERENCE
1.1	**Reference**	[redacted] (1988) Algal growth inhibition test. [redacted] 1990 (unpublished), BPD ID A7.4.1.3_02
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, following the German Industrial Standard DIN 38412, part 9 (not specified in report)
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Glyoxal, purity not specified (likely [redacted] aqueous solution)
3.1.1	Lot/Batch number	Not specified
3.1.2	Specification	As given in section 2
3.1.3	Purity	No data
3.1.4	Composition of Product	No data
3.1.5	Further relevant properties	Slightly yellow test solution; Stock solution: 1000 mg/L; a white precipitate was observed
3.1.6	Method of analysis	Not applicable
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant since the TS is mixable with water up to 10 g/L.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not relevant
3.4	**Testing procedure**	
3.4.1	Culture medium	Mineral composition [mg/L]: 15 mg/L NH_4Cl, 12 mg/L $MgCl_2$ * 6 H_2O, 18 mg/L $CaCl_2$ * 2 H_2O, 15 mg/L MgSO4 *7 H_2O; 1.6 mg/L KH_2PO_4, 0.08

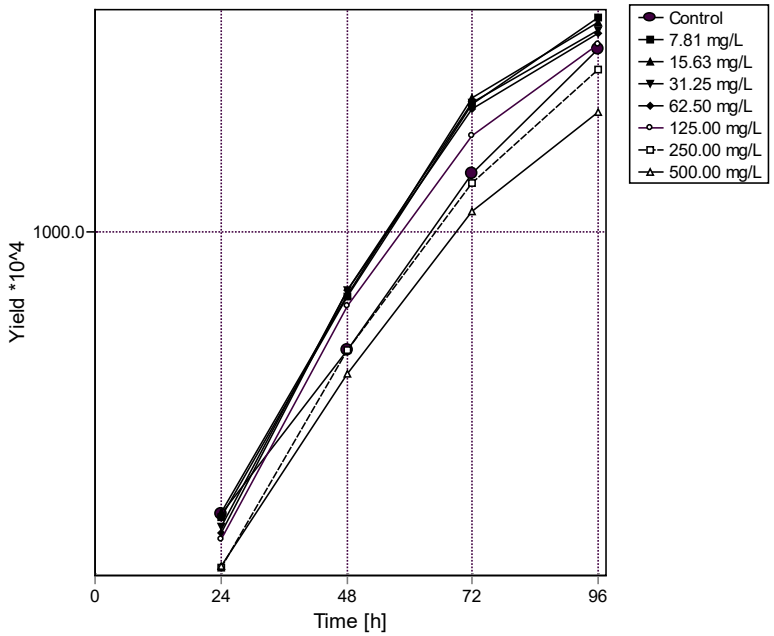
Section A7.4.1.3_02
Annex Point IIA, VII.7.3.

Growth inhibition test on algae

		mg/L $FeCl_3$ * 6 H_2O, 0.1 mg/L Na_2EDTA * 2 H_2O, 50 mg/L $NaHCO_3$, 0.185 mg/L H_3BO_3, 0.415 mg/L $MnCl_2$ * 4 H_2O, 0.003 mg/L $ZnCl_2$, 0.0015 mg/L $CoCl_2$ * 6 H_2O, 0.00001 mg/L $CuCl_2$ * 2 H_2O, 0.007 mg/L Na_2MoO_4 * 2 H_2O The pH after aerating was approx. 8. This nutrient solution compared with the algal nutrient solution prescribed by the OECD guideline 201.
3.4.2	Test organisms	See table A7_4_1_3-2
3.4.3	Test system	See table A7_4_1_3-3
3.4.4	Test conditions	See table A7_4_1_3-4
3.4.5	Duration of the test	72 hours (96 hours)
3.4.6	Test parameter	Cell multiplication inhibition; fluorescence values were equated with cell numbers
3.4.7	Sampling	Fluorescence measurements were performed after 0, 24, 48, 72, and 96 hours.
3.4.8	Monitoring of TS concentration	No
3.4.9	Statistics	The raw data from the original report were recently re-evaluated according to OECD TG 201 (March 2006) using the computer programme ToxRatPro (v2.09, 08. Nov. 2006). Therefore, the fluorescence values were equated with cell numbers ([redacted], 2006. Alga, Growth Inhibition Test (OECD 201; DIN 38412-L9): [redacted]; unpublished; attachment to the report). The EC values were recalculated by probit analysis using linear max. likelihood regression. The NOEC was determined by comparing the means of the calculated biomass or growth rate of the various concentration levels with the control (Williams t-test).
		4 RESULTS
4.1	**Preliminary test**	Not performed.
4.1.1	Concentration	Not relevant
4.1.2	Effect data	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250 and 500 mg test mat./L
4.2.2	Actual concentrations of test substance	No analytical monitoring performed.
4.2.3	Growth curves	

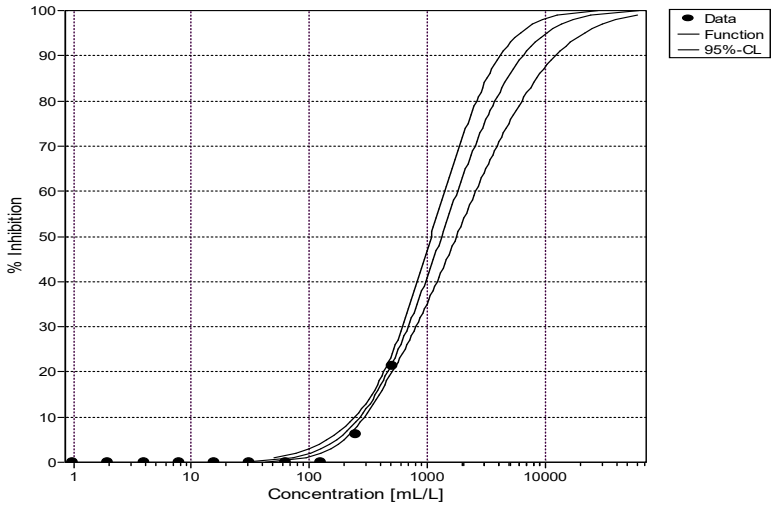
Section A7.4.1.3_02
Annex Point IIA, VII.7.3.

Growth inhibition test on algae



Yield (corrected cell number) in *Desmodesmus subspicatus* as dependent on test item concentration and time

4.2.4 Concentration / response curve



Concentration-effect curve showing the influence of the test item on yield of the introduced *Desmodesmus subspicatus* as observed after 72 h

4.2.5 Cell concentration data (72 h)

See table A7_4_1_3-5

Section A7.4.1.3_02
Annex Point IIA, VII.7.3.

Growth inhibition test on algae

4.2.6 Effect data (cell multiplication inhibition)

Effect concentrations (mg test mat./L) for 0-72 h		95 %-c.l. (mg test mat./L)
E_rC_{10}	662	642 – 684
E_bC_{10}	270	245 - 292
E_yC_{10}	268	242 - 290
E_rC_{50}	> 500	n.d.
E_bC_{50}	> 500	n.d.
E_yC_{50}	> 500	n.d.
NOEC*	250	
LOEC*	500	

n.d.: not determined due to mathematical reasons
* NOEC and LOEC valid for growth rate, biomass integral, and yield

4.2.7 Other observed effects: Compared to the control, algae growth was stimulated in concentrations of ca. 1 – 125 mg test mat./L.

4.3 Results of controls: See table at point 4.2.5 "Cell concentration data"

4.4 Test with reference substance: Not performed

4.4.1 Concentrations: Not relevant

4.4.2 Results: Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods: The inhibitory effect of glyoxal (most likely [redacted] aqueous solution) on cell multiplication of the unicellular green algae *Desmodesmus subspicatus* (formerly named *Scenedesmus subspicatus*) SAG 86.81 was studied.
The test was carried out according to German Industrial Standard DIN 38412, part 9; GLP was not compulsory at the time the study was performed.
Exponentially growing algae were cultured for several generations.
Multiplication of cells was determined under the influence of glyoxal in relation to the untreated control. The following concentrations were tested: 0 (control), 0.91, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250 and 500 mg test mat./L. Algal exposition was performed in 10 mL tubes with flat bottom at 20 °C. The initial cell density of *Desmodesmus subspicatus* was 10^4 cells/mL.
Fluorescence measurements were performed after 0, 24, 48, 72, and 96 hours (chlorophyll a fluorescence at 685 nm as a criterion for biomass).
No analytical monitoring of the test concentrations was conducted.
The raw data from the original report were recently re-evaluated according to OECD TG 201 (March 2006) using the computer programme ToxRatPro (v2.09, 08. Nov. 2006). Therefore, the fluorescence values were equated with cell numbers.
The EC values were recalculated by probit analysis using linear max. likelihood regression. The NOEC was determined by comparing the means of the calculated biomass or growth rate of the various concentration levels with the control (Williams t-test).

5.2 Results and discussion: An inhibitory effect on algae growth was seen with cell density starting at a test concentration of 250 mg test mat./L; in the lower concentrations algae growth rate was stimulated. X

Section A7.4.1.3_02 Annex Point IIA, VII.7.3.	Growth inhibition test on algae	
5.2.1 NOE_rC	250 mg test mat./L	
5.2.2 E_rC_{50}	> 500 mg test mat./L	
5.2.3 E_bC_{50}	> 500 mg test mat./L	
5.3 Conclusion	The treatment of the algae with glyoxal had stimulating effects on algae growth at concentrations of up to 125 mg test mat./L. Significant effects on algae growth rate were not determined. The E_rC_{10} was extrapolated to be 662 mg test mat./L. The E_rC_{50} is > 500 mg test mat./L. The NOE_rC is 250 mg test mat./L. The determined NOEC and EC values refer to the test material as such. The validity criteria for the algal growth inhibition test according to OECD Guideline 201 (2006) were fulfilled with respect to the 72-h test period.	X
5.3.1 Reliability	[redacted]	X
5.3.2 Deficiencies	[redacted]	

EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	19/03/2018
Materials and Methods	Agree
Results and discussion	5.2: *"*An inhibitory effect on algae growth was seen with cell density starting at a test concentration of 250 mg test mat./L (0.7% and 2.2% inhibition after 72h and 96h exposure respectively)". At the highest concentration of 500 mg test mat./L, only 5.6% and 8.36% growth rate inhibition was detected (after 72h and 96h exposure respectively).
Conclusion	Since the low inhibitory effects oberved at the highest tested concentration which remain lower than 10% of the control, a long term ErC_{10} value of 500 mg test mat./L, equivalent to 200 mg a.i./L was determined.
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	None

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	*Desmodesmus subspicatus* (formerly named *Scenedesmus subspicatus*)
Strain	SAG 86.81
Source	Not specified
Laboratory culture	Yes
Method of cultivation	250 mL Erlenmeyer flasks
Pretreatment	Not specified
Initial cell concentration	10^4 cells/mL

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	10 mL
Culturing apparatus	Not specified
Light quality	Not specified
Procedure for suspending algae	Not specified, probably as prescribed by the guideline
Number of vessels/ concentration	6 inoculated; 1 uninoculated
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	20 °C
pH	No data
Aeration of dilution water	Not specified
Light intensity	Not specified
Photoperiod	Not specified, but likely continuously illuminated in accordance with the guideline

Table A7_4_1_3-5: Cell concentration data

Nominal concentration [mg test mat./L]	Cell concentrations (mean values) [relative fluormeter units]									
	measured					Percent of control				
	0 h	24 h	48 h	72 h	96 h	0 h	24 h	48 h	72 h	96 h
0	40	207	513	1500	3260	100	100	100	100	100
0.98	41	223	760	2614	4277	100	108	148	174	131
1.95	40	221	726	2549	4181	100	107	141	170	128
3.91	41	217	740	2411	4059	101	105	144	161	124
7.81	36	200	700	2313	3976	90	97	137	154	122
15.63	36	204	711	2385	3870	88	99	139	159	119
31.25	39	193	733	2330	3676	97	93	143	155	113
62.5	40	188	729	2240	3620	98	91	142	149	111
125	37	178	665	1884	3367	90	86	130	126	103
250	39	157	511	1406	2866	96	76	100	94	88
500	39	159	447	1187	2185	97	77	87	79	67
Temperature [°C]	20 °C									
pH	no data									

Table A7_4_1_3-6: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fullfilled
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	**No analytical monitoring performed.**	**X**
Coefficient of variation of average specific growth rates in control cultures < 7 %.	**Yes**	
Mean coefficient of variation of sectional growth rates calculated for the individual control replicates from 0 to 72 h < 35 %	**Yes**	

Criteria for poorly soluble test substances	**Not relevant**	

Section A7.4.1.3_03 Growth inhibition test on algae

Annex Point IIA7.3

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (2009) Effect of [redacted] on the growth of the marine Diatom *Skeletonema costatum*. [redacted] 2009, (Unpublished), BPD ID A7.04.1.3_03
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s.] for [first entry to Annex I/ authorisation]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	OECD 201, OPPTS 850.5400
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Glyoxal [redacted] (CAS No.: 107-22-2)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	Test substance in aqueous solution
3.1.5	Further relevant properties	The test material was described as colourless homogeneous liquid. It was stored at room temperature under nitrogen.
3.1.6	Method of analysis	Reversed phase HPLC with UV/VIS detection after pre-column derivatization; determination by the method of external standards
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference	Not relevant

Section A7.4.1.3_03 Growth inhibition test on algae

Annex Point IIA7.3

substance

3.4 Testing procedure

3.4.1 Culture medium: The following nutrient, salts and vitamins were added to artificial sea water (30 g "HW sea salt professional"/L douple distilled water):

Nutrients	Concentration (mg/L)
$MnCl_2$• 4 H_2O	2.16
K_3PO_4	3.0
Na_2EDTA • 2 H_2O	15.0
$NaNO_3$	50.0
$FeCl_3$ •6 H_2O	0.72
Na_2SiO • 9 H_2O	20.0
$CoCl_2$ •6 H_2O	0.00606
H_3BO_3	17.1
$ZnSO_4$ • 7 H_2O	0.675
$CuSo_4$ • 5 H_2O	0.00236
Biotin	5×10^{-5}
Vitamin B_{12}	5×10^{-4}
Thiamin-Hydrochlorid	0.25

The pH was adjusted to 8.0 and the solution was sterile filtered (pore size about 0.2 µm).

3.4.2 Test organisms: See table A7_4_1_3-2

3.4.3 Test system: See table A7_4_1_3-3

3.4.4 Test conditions: See table A7_4_1_3-4

3.4.5 Duration of the test: 96 hours

3.4.6 Test parameter: Inhibition (%) of yield (y) and growth rate (r) compared to the control.

3.4.7 Sampling: Fluorescence measurements were performed after 24, 48, 72 and 96 hours.

3.4.8 Monitoring of TS concentration: A sample of each test concentration (0, 88, 132, 198, 296, 444, 667 and 1000 mg/L) was taken at the beginning and the end of the test.

3.4.9 Statistics: The EC values for the yield (Y) and the growth rate (R) were calculated using a probit analysis.

The Dunnett's Test was performed to determine the NOEC.

4 RESULTS

Section A7.4.1.3_03 Growth inhibition test on algae

Annex Point IIA7.3

4.1	**Limit Test**	Not relevant, the test concentrations were selected on the basis of a pre-test.
4.1.1	Concentration	Not relevant
4.1.2	Effect data	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 88, 132, 198, 296, 444, 667 and 1000 mg test material/L
4.2.2	Actual concentrations of test substance	

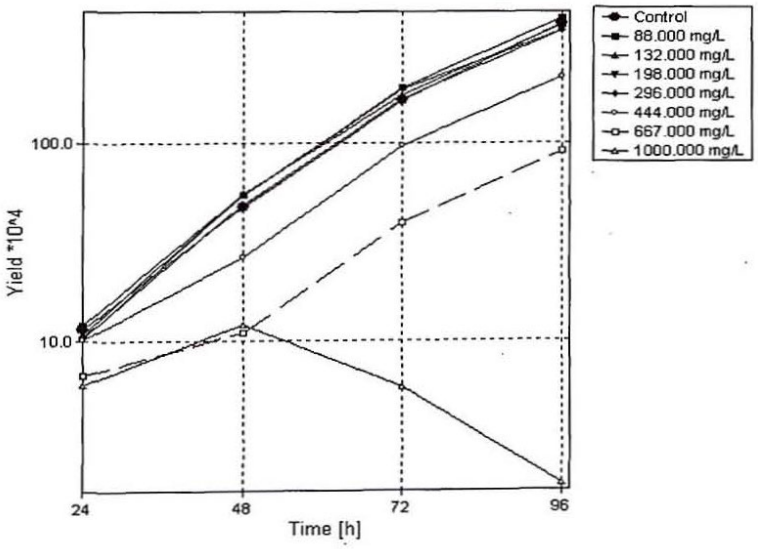
Test material concentration (mg/L)	
Nominal	**geometric mean measured**
88	69.6
132	111.2
198	170.9
296	261.5
444	409.1
667	626.8
1000	963.4

At the test beginning as well as at the test end, the recovery rate was greater than 80%, therefore the test results are based on the nominal concentrations.

X

4.2.3 Growth curves

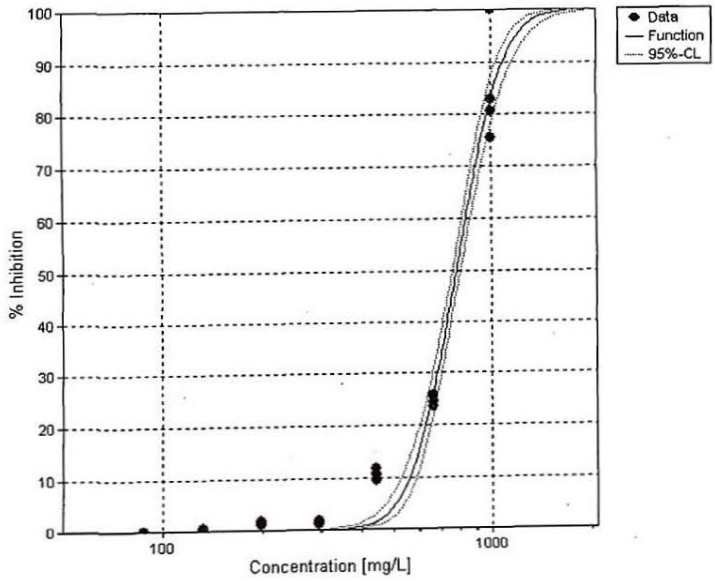
Algal growth rates:



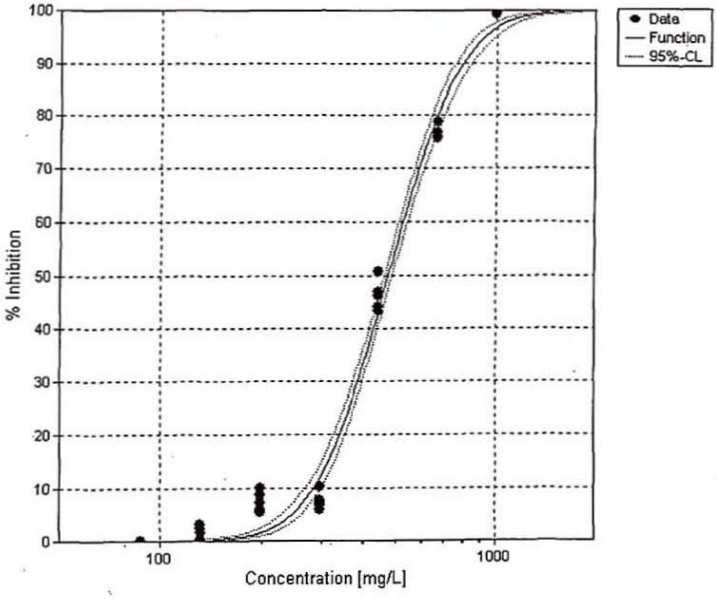
Section A7.4.1.3_03 Growth inhibition test on algae

Annex Point IIA7.3

4.2.4	Concentration / response curve	Concentration/effect-relationship on growth rate after 96h:



Concentration/effect-relationship on yield after 96h:



4.2.5	Cell concentration data (growth rate	See table A7_4_1_3-5

Section A7.4.1.3_03 Growth inhibition test on algae

Annex Point IIA7.3

and yield)

4.2.6 Effect data (cell multiplication inhibition)

Nominal test material concentration (mg/L)	% Inhibition	
	growth rate	yield
88	-1.1	-6.5
132	0.3	1.7
198	1.3	7.4
296	1.3	7.5
444	10.3	46.1
667	24.5	77.1
1000	83.9	99.5

X

4.2.7 Other observed effects: At 667 mg/L about one third of the observed cells and at 1000 mg/L all of the observed cells were smaller than those in the control.

4.3 Results of controls: For details see Table A7_4_1_3-5

4.4 Test with reference substance: Not performed

4.4.1 Concentrations: Not relevant

4.4.2 Results: Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to determine the effect of [redacted] on the growth of the marine algae *Skeletomena costatum* (SAG 19-99).

Test material: [redacted]

The test was carried out according to OPPTS-guideline 850.5400 and OECD guideline 201 under GLP.

Multiplication of cells was determined under the influence of [redacted] in relation to the untreated control.

The following nominal concentrations were tested 0.0 (control), 88, 132,198, 296, 444, 667 and 1000 mg test material/L. The initial cell density of *Skeletomena costatums* was 1×10^4 cells/mL. Fluorescence measurements were performed after 24, 48, 72 and 96 hours. Results were assessed with respect to inhibition of growth rate (r) and yield (y).

An analytical monitoring of the test concentrations was conducted at the start of the test and at test ending by reversed phase HPLC with UV/VIS-detection. The analytical monitoring was carried out for all test concentrations.

Section A7.4.1.3_03 Growth inhibition test on algae

Annex Point IIA7.3

5.2 Results and discussion

Analytical monitoring:

The results of the analytical monitoring revealed an overall recovery rate of > 80%, therefore the test results are based on nominal concentrations. X

Effect data: X

See Table 4.2.6

5.2.1 NOEC

	mg test material/L	mg glyoxal/L
72-h NOE_rC	296.0	118.4
96-h NOE_rC	296.0	118.4
72-h NOE_yC	296.0	118.4
96-h NOE_yC	132.0	52.8

5.2.2 E_rC50

	mg test material/L	mg glyoxal/L
72-h E_rC_{50} (95% conf. limits)	867.8 (856.0 – 880.1)	347.1 (342.4 – 352.0)
96-h E_rC_{50} (95% conf. limits)	784.6 (763.0 – 807.2)	313.8 (305.2 – 322.9)

5.2.3 E_yC50

	mg test material/L	mg glyoxal/L
72-h E_yC_{50} (95% conf. limits)	504.3 (492.4 – 516.6)	201.7 (197.0 – 206.6)
96-h E_yC_{50} (95% conf. limits)	476.5 (464.0 – 489.3)	190.6 (185.6 – 195.7)

5.3 Conclusion

The 72-h and 96-h EC_{50} values for effects on growth rate were 347.1 mg a.s./L and 313.8 mg a.s./L, respectively. The 72-h and 96-h NOE_rC was in both cases 118.4 mg a.s./L.

The validity criteria for the algal growth inhibition test according to OECD Guideline 201 were fulfilled.

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Section A7.4.1.3_03 Growth inhibition test on algae

Annex Point IIA7.3

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	19/03/2018
Materials and Methods	Agree
Results and discussion	4.2.2, 5.2: in the study report BPD ID A7.4.1.3_03, it is stated that the measured recoveries for [redacted] were 94.6-98.0% (average of 96.7%) of nominal at test initiation and 65.3-95.3% (average of 81.5%) of nominal at test termination. Furthermore, it is mentioned that the found values are indicating a concentration dependent decrease of the glyoxal concentration over a period of 96h. The recovery values for the nominal tested concentrations 88, 132 and 198 mg/L are respectively 65.3, 72.4 and 77.9% after 96h exposure. Consequently, the test results have to be based on the measured concentrations. "See Table Section 4.2.6" 4.2.6: the values correspond to the inhibition after 96h exposure. 5.2: geometric mean measured concentrations: 72-h E_rC_{10} (95% conf. limits) = 432.7 mg test mat./L (419.4-445.3 mg/L) 72-h E_rC_{10} (95% conf. limits) = 173 mg a.i./L (73-178 mg/L) 96-h E_rC_{10} (95% conf. limits) = 523.5 mg test mat./L (491.8-550.1mg/L) 96-h E_rC_{10} (95% conf. limits) = 209.4 mg a.i./L (196.7-220 mg/L) Table A7_4_1_3-5: percentage of inhibition are lacking in the table.
Conclusion	Agree
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_1_3-2: Dilution water

Criteria	Details
Source	Standard medium was used: Enriched Salt Water (ESW) according to OPPTS 850.5400 (referring to ASTM 1218)
Acid capacity (Ks) up to pH 4.3	Not specified
Hardness	Not specified
pH	7.9 – 8.3
Ca / Mg ratio	Not specified
Na / K ratio	Not specified
Oxygen content	Not specified
Conductance	Not specified
Holding water different from dilution water	No

Table A7_4_1_3-3: Test organisms

Criteria	Details
Species	*Marine diatom Skeletonema costatum*
Strain	SAG 19-99
Source	[redacted]
Age	7 days old
Laboratory culture	Yes
Method of cultivation	Not specified
Pretreatment	Not specified
Initial cell concentration	10^4 cells/mL

Table A7_4_1_3-4: Test system

Criteria	Details
Volume of culture flasks	100 mL
Culturing apparatus	Erlenmeyer dimple flasks
Light quality	cool white-type fluorescent
Procedure for suspending algae	Not specified, probably as prescribed by the guideline
Number of vessels/ concentration	5
Test performed in closed vessels due to significant volatility of TS	Not relevant

Table A7_4_1_3-5: Test conditions

Criteria	Details
Test temperature	20 ± 1 °C
pH	7.6-8.2
Aeration of dilution water	No specified
Light intensity	4300 lux
Photoperiod	14 h light : 10 h darkness

Table A7_4_1_3-5: Cell concentration data

Nominal concentration [mg test mat./L]	Cell numbers (mean values) [mg/L]								
	measured				Percent of control				
	24 h	48 h	72 h	96 h	0 h	24 h	48 h	72 h	96 h
0	0.033	0.118	0.375	0.872					
88	0.034	0.135	0.430	0.926					
132	0.031	0.138	0.402	0.857					
198	0.030	0.135	0.427	0.811					
296	0.030	0.123	0.381	0.810					
444	0.030	0.069	0.231	0.485					
667	0.021	0.032	0.099	0.217					
1000	0.019	0.034	0.019	0.008					
Temperature [°C]	20 °C								
pH	no data								

Table A7_4_1_3-6: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fullfilled

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	**X**	
Coefficient of variation of average specific growth rates in control cultures < 10 %.	**Yes**	
Mean coefficient of variation of sectional growth rates calculated for the individual control replicates from 0 to 72 h < 35 %	**Yes**	

Criteria for poorly soluble test substances	**Not relevant**	

Section A7.4.1.3_04 Growth inhibition test on algae *Selenastrum capricornutum*

Annex Point IIA7.3

Official use only

1 REFERENCE

1.1	**Reference**	Bollman et al. (1990) Report on Algal Toxicity tests on selected office of Toxic substances (OTS) Chemicals. III A7.4.1.3_04. Note the document on which this summary is based is a poor quality copy provided by the US National Technical Information Service (NTIS) which contains the following preface statement: ATTENTION AS NOTED IN THE NTIS ANNOUNCEMENT, PORTIONS OF THIS REPORT ARE NOT LEGIBLE. HOWEVER IT IS THE BEST REPRODUCTION AVAILABLE FROM THE COPY SENT TO NTIS. P. 5 is similarly overprinted with "KEINE BESSEREN KOPIEN VON DER VORLAGE MÖGLICH" (No better copies of the publication are possible). Moreover the NTIS copy is incomplete: p. 6 is missing, as are pp. 24-154 which appears to include the appendices containing details of the test design and all the relevant raw and transformed data used to derive the reported endpoints.
1.2	**Data protection**	None. The data constitute part of a published report released to the public by the US EPA.
1.2.1	Data owner	US Environmental Protection Agency.
1.2.2	Companies with letter of access	Not relevant
1.2.3	Criteria for data protection	Not relevant

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	In-house protocol of the US EPA's Environmental Research Laboratory–Cornwallis OR (ERL-C) modified according to a request of the Office for Toxic Substance (OTS). OTS modifications were based on the standard procedure from the Federal Register, (vol. 50; 188; Part 797; Sec 797.1050, Algal Toxicity Test) and further EPA publications (Greene et al., 1988 and Webber et al., 1989).

Section A7.4.1.3_04 Growth inhibition test on algae *Selenastrum capricornutum*

Annex Point IIA7.3

2.2	**GLP**	No
2.3	**Deviations**	None mentioned
		3 MATERIALS AND METHODS
3.1	**Test material**	Glyoxal. The report also contains results for 10 other substances that were similarly tested. This summary is limited to the information relevant to glyoxal.
3.1.1	Lot/Batch number	Not specified
3.1.2	Specification	Not stated
3.1.3	Purity	Not specified
3.1.4	Composition of Product	Not specified
3.1.5	Further relevant properties	Not specified
3.1.6	Method of analysis	Not specified
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant
3.3	**Reference substance**	No reference substance used
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Culture medium	Algal assay medium (AAM)
3.4.2	Test organisms	Pseudokirchneriella subcapitata, formerly known as *Selenastrum capricornutum.*
3.4.3	Test system	Static. The definitive test comprised five glyoxal concentrations ranging from 42.50 to 1000 mg/L, with 3 replicates per treatment.
3.4.4	Test conditions	Not stated. The report indicates that several initial attempts were unsuccessful because glyoxal caused the medium pH to fall below the range of tolerance of the test organism. The definitive test appears eventually to have been performed after pH adjustment, but no details of the adjustment procedure are given in the available source document.
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Cell counts were made
3.4.7	Sampling	A Coulter counter was used to count algal cells at 24, 48, 72 and 96

		hours. Samples were apparently taken at test initiation and termination and placed in refrigerated storage for possible confirmatory analysis of exposure concentrations.
3.4.8	Monitoring of TS concentration	Not performed
3.4.9	Statistics	Median effect levels and 95% fiducial limits were derived from a regression analysis using the Statgraphics program.
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not relevant
4.1.2	Effect data	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Five concentrations ranging from 42.50 to 1000 mg glyoxal/L (nominal). Intermediate concentrations not stated
4.2.2	Actual concentrations of test substance	Not specified
4.2.3	Growth curves	None given
4.2.4	Concentration / response curve	Not given
4.2.5	Cell concentration data (growth rate and yield)	Not given
4.2.6	Effect data (cell multiplication inhibition)	Not given
4.2.7	Other observed effects	Non specified
4.3	**Results of controls**	Not given
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

Section A7.4.1.3_04 **Growth inhibition test on algae *Selenastrum capricornutum***
Annex Point IIA7.3

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The effects of glyoxal on the growth of the unicellular alga P. subcapitata (formerly S. capricornutum) was determined under static conditions in a 96-h test according to methodology of the US EPA. Algal cell densities were determined at 24-h intervals with a particle counter. Reference is made to generic methodology of the US EPA, however the report makes mention of the need to adjust the pH of (some of) the media containing glyoxal, but no detail is provided on this key aspect of the test procedure. The report also contains some discussion of the desirability of adding EDTA to the test medium to achieve satisfactory growth in the untreated control, but no confirmation is available of the composition of the medium used to test glyoxal or of the achievement of satisfactory performance in the control treatment.

5.2 Results and discussion

5.2.1 NOEC

Not specified

5.2.2 EC50

The 96-h EC50 determined in the definitive test was 148.96 mg glyoxal/L

5.3 Conclusion

The 96-hour EC50 for inhibition of growth of *S. capricornutum* was 148.96 mg glyoxal/L, with lower and upper fiducial limits of 0.00 and 348.59 mg/L, respectively.

5.3.1 Reliability

[redacted]

5.3.2 Deficiencies

[redacted]

Section A7.4.1.3_04 **Growth inhibition test on algae *Selenastrum capricornutum***

Annex Point IIA7.3

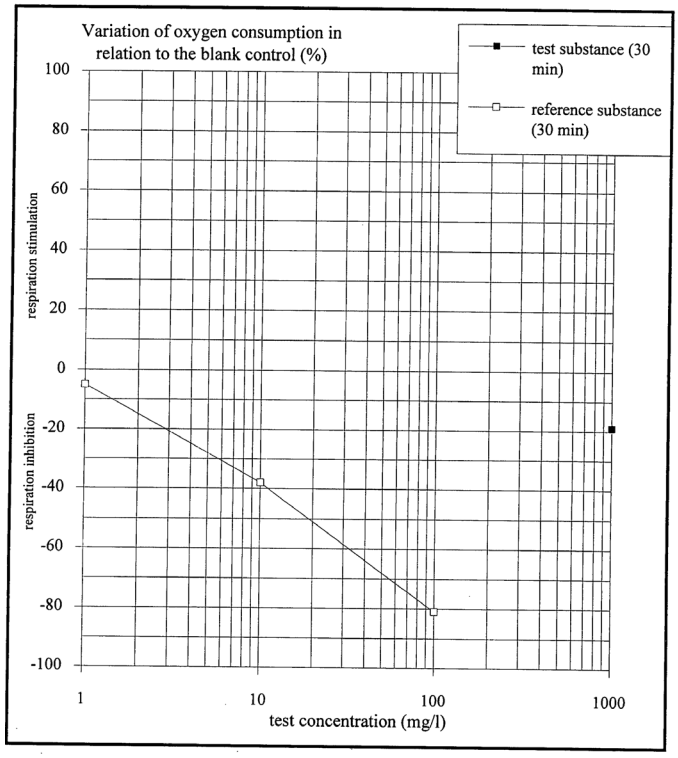
	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	19/03/2018
Materials and Methods	*Agree*
Results and discussion	*Agree*
Conclusion	*Agree*
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Section A7.4.1.4_01 Inhibition to microbial activity in activated sludge (aquatic)
Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1996) Determination of the inhibition of Oxygen Consumption by [redacted] in the Activated Sludge Respiration Inhibition Test. [redacted] 1996 (unpublished), BPD ID A7.4.1.4_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, following the Annex of EEC Directive 88/302; corresponds to OECD TG 209 and ISO Standard 8192	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, only one test concentration (limit test)	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]	
3.1.1	Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	[redacted]	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Not performed	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant	
3.3	**Reference substance**	Yes, 3,5-dichlorophenol	
3.3.1	Method of analysis for reference substance	Not relevant	
3.4	**Testing procedure**		
3.4.1	Synthetic medium	8 mL/vessel 100-fold concentrated OECD Medium	
3.4.2	Inoculum / test organism	For details on inoculum see table A7_4_1_4-2	
3.4.3	Test system	For details on test type, laboratory equipment etc. see table A7_4_1_4-3	
3.4.4	Test conditions	For relevant test conditions see table A7_4_1_4-4	
3.4.5	Duration of the test	30 minutes	
3.4.6	Test parameter	Respiration inhibition (by oxygen measurement)	
3.4.7	Analytical parameter	-	

Section A7.4.1.4_01
Annex Point IIA7.4

Inhibition to microbial activity in activated sludge (aquatic)

3.4.8	Sampling	The oxygen consumption was measured for 6-10 minutes after an incubation time of 30 minutes.
3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	Control without test substance (blank control), reference substance as positive control
3.4.11	Statistics	Not performed
	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 concentrations of test substance	1000 mg test mat./L (limit test)
4.2.2	Actual concentrations of test substance	Analysis was not performed, reported values refer to nominal concentrations
4.2.3	Growth curves	Not relevant
4.2.4	Cell concentration data	Not relevant
4.2.5	Concentration/ response curve	
4.2.6	Effect data	30-min EC_{20} = ca. 1000 mg test mat./L, corresponding to ca. 400 mg a.i./L
4.2.7	Other observed effects	No other inhibition phenomena were reported

Section A7.4.1.4_01 **Inhibition to microbial activity in activated sludge**
Annex Point IIA7.4 **(aquatic)**

4.3	**Results of controls**	The blank control (mean value of three replicates): Specific oxygen consumption rate: 21 mg O_2/g^*h (mean of 3 values)	
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	1, 10, 100 mg/L	
4.4.2	Results	30-min EC_{50} = ca. 20 mg/L	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The aim of the present study was to determine the inhibition of the oxygen consumption of activated sludge by [redacted] glyoxal). The test was performed according to the Annex of EEC Directive 88/302 (similar to OECD TG 209) under GLP conditions. [redacted] was tested in Erlenmeyer flasks (250 mL) at an incubation temperature of 20 ± 2°C as a limit test with 1000 mg test mat./L (400 mg a.i./L). The reference substance 3,5-dichlorophenol was tested at 1, 10 and 100 mg/L. Activated sludge from a laboratory wastewater plant fed with municipal and synthetic sewage was used as inoculum (final volume: 1 g/L dry substance). The blank control comprised 3 test vessels, whereas the test and reference substance included 1 vessel/concentration. After an incubation time of 30 minutes the oxygen consumption was measured with an O_2-electrode. The change in oxygen consumption compared to the control was the measure for respiration inhibition.	X
5.2	**Results and discussion**	The specific oxygen consumption rate of the blank control (mean value of three replicates) was determined to be 21 mg O_2/g^*h. The specific oxygen consumption rate of [redacted] at 1000 mg test mat./L was found to be 17 mg O_2/g^*h, respectively. Compared to the blank control, the inhibition was 19 %. The validity criteria for this test system were fulfilled, since the deviations of the blank controls are less then 15%. The EC_{50} of the reference substance 3,5-dichlorophenol is in the range of 5-30 mg/L. The test is valid.	
5.2.1	EC_{20}	ca. 1000 mg test mat./L, corresponding to ca. 400 mg a.i./L	X
5.2.2	EC_{50}	> 1000 mg test mat./L, corresponding to ca. 400 mg a.i./L	
5.2.3	EC_{80}	> 1000 mg test mat./L, corresponding to ca. 400 mg a.i./L	
5.3	**Conclusion**	The EC_{50} is > 1000 mg test mat./L, corresponding to ca. 400 mg a.i./L. Disturbances of the biodegradation process of activated sludge are not to be expected if the substance is correctly introduced into waste water treatment plants.	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	[redacted]	

Section A7.4.1.4_01 **Inhibition to microbial activity in activated sludge (aquatic)**
Annex Point IIA7.4

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	*23/03/2018*
Materials and Methods	5.1: The initial inoculum concentration used was 1g/L dry substance per vessel. The corresponding recommended concentration of 2 – 4 g/L suspended solids was not indicated.
Results and discussion	5.2.1: The EC_{20} can not be considered as a NOEC. Consequently, only the EC_{50} > 400 mg a.i./L can be determined.
Conclusion	
Reliability	[redacted]
Acceptability	
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_4-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Not applicable
Strain	Not applicable
Source	Laboratory wastewater plant municipal and synthetic sewage
Sampling site	Laboratory wastewater plant
Laboratory culture	Cultured in the laboratory wastewater plant
Method of cultivation	Laboratory wastewater plant
Preparation of inoculum for exposure/ Pretreatment	Not specified
Initial cell concentration	1 g/L dry substance

Table A7_4_1_4-3: Test system

Criteria	Details
Culturing apparatus	Erlenmeyer flasks (250 mL)
Number of culture flasks/concentration	Blank control: 3 vessels test and reference substance: 1 vessel/concentration
Aeration device	According to guideline
Measuring equipment	O_2-electrode
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_4-4: Test conditions

Criteria	Details
Test temperature	20 ± 2°C
pH	Not reported
Aeration of dilution water	According to guideline
Suspended solids concentration	1 g/L dry weight

Section A7.4.1.4_02
Annex Point IIA7.4

Inhibition to microbial activity (*Pseudomonas putida*)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1996) Determination of the inhibitory effect of [redacted] on the cell multiplication of the bacterium *Pseudomonas putida*. [redacted] 1996 (unpublished), BPD ID A7.4.1.4_02	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, German Industrial Standard DIN 38412, part 8 (March 1991)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]	
3.1.1	Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	[redacted]	
3.1.5	Further relevant properties	Protect against heat (> 50 °C)	
3.1.6	Method of analysis	Not performed	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant	
3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	Not relevant	
3.4	**Testing procedure**		
3.4.1	Synthetic medium	Medium as prescribed by the guideline	
3.4.2	Inoculum /	See table A7_4_1_4-2	

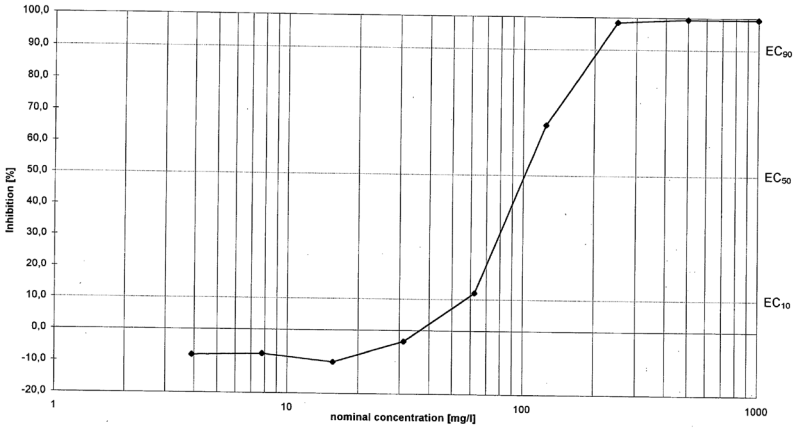
Section A7.4.1.4_02 **Inhibition to microbial activity (*Pseudomonas putida*)**
Annex Point IIA7.4

	test organism		
3.4.3	Test system	See table A7_4_1_4-3	
3.4.4	Test conditions	See table A7_4_1_4-4	
3.4.5	Duration of the test	16 hours	
3.4.6	Test parameter	Optical cell density at 436 nm	X
3.4.7	Analytical parameter	-	
3.4.8	Sampling	Measurement of the optical cell density after 16 hours (test end) Measurement of the pH value at test start and test end in an uninoculated replicate and at test end in all (inoculated) replicates	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Inoculated blank control	
3.4.11	Statistics	Mean, standard deviation, variation coefficient EC_{10}, EC_{50}, EC_{90}	
		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 concentrations of test substance	0 (blank control, inoculated), 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 mg test mat./L	
4.2.2	Actual concentrations of test substance	No analytical monitoring was done.	
4.2.3	Growth curves	None	
4.2.4	Cell concentration data		X

Test concentration (mg/l)	Optical density (mean ± sd)	% of Control
Control (inoculated)	0.332 ± 0.010	-
3.91	0.359 ± 0.003	108.2
7.81	0.358 ± 0.017	107.7
15.63	0.366 ± 0.006	110.1
31.25	0.344 ± 0.003	103.5
62.5	0.292 ± 0.017	87.9
125	0.114 ± 0.005	34.0
250	0.006 ± 0.000	1.6
500	0.004 ± 0.000	0.5
1000	0.004 ± 0.000	0.6

Section A7.4.1.4_02
Annex Point IIA7.4

Inhibition to microbial activity (*Pseudomonas putida*)

4.2.5	Concentration/ response curve	
4.2.6	Effect data	EC10 = 8.8 mg/l EC50 = 13.3 mg/l EC90 = 27.9 mg/l
4.2.7	Other observed effects	A slight stimulating effect on cell multiplication was observed in the concentrations 3.91 – 31.25 mg test mat./L.
4.3	**Results of controls**	Not relevant
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The aim of the present study was to determine the inhibitory effect of [redacted] glyoxal in aqueous solution) on cell multiplication of the sludge bacterium *Pseudomonas putida*. The test was performed according to the German Industrial Standard DIN 38412, part 8 under GLP conditions. [redacted] was tested at the following nominal concentrations: 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 mg test mat./L. The nutrient medium was inoculated with a bacterial suspension of *Pseudomonas putida* and the test solution was added; the test series was accompanied by a control blank, which was also inoculated. Four replicates were set up per concentration and control. The test was performed in 50 mL glass tubes with flat bottom and plugged with gas permeable silicone sponge caps. The test volume was 10 mL, the test temperature was 21 ± 1 °C and the incubation period was 16 hours. The optical cell density was measured at 436 nm at the end of the incubation period. Measurements of pH were conducted at test start as well as at test end in inoculated and uninoculated replicates. The validity criteria are: - cell density in the untreated control must increase by a factor of at

X

Section A7.4.1.4_02 **Inhibition to microbial activity (*Pseudomonas putida*)**
Annex Point IIA7.4

		least 100 - variation coefficients of means should be < 10%	
5.2	**Results and discussion**	A clear concentration-response relationship was seen; inhibitory effects on bacterial growth were determined at concentrations of ≥ 50 mg test mat./L. A slight stimulating effect was reported for the concentrations 3.91 – 31.25 mg test mat./L. The EC_{50} was 102 mg test mat./L corresponding to ca. 40 mg glyoxal/L. The validity criteria were fulfilled. The test is valid.	X
5.2.1	EC10	56.9 mg test mat./L	
5.2.2	EC50	102 mg test mat./L	
5.2.3	EC90	209 mg test mat./L	
5.3	**Conclusion**	Under the reported test conditions the cell multiplication of *Pseudomonas putida* is inhibited by [redacted] at concentrations of ≥ 50 mg test mat./L. The EC_{10} was 56.9 mg test mat./L and the EC_{50} was 102 mg test mat./L corresponding to 22.8 and 40.8 mg glyoxal/L, respectively.	
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	[redacted]	

Section A7.4.1.4_02 **Inhibition to microbial activity (*Pseudomonas putida*)**
Annex Point IIA7.4

EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	23/03/2018
Materials and Methods	3.4.6: The corresponding absorbance values in FTU were not given.
Results and discussion	4.2.6: "EC10 = 56.9 mg/l (nominal concentration) EC50 = 102 mg/l (nominal concentration) EC90 = 209 mg/l (nominal concentration)" 4.2.4 and 5.2: The table gives the the mean optical density measured at 436 nm in relative units after 16h exposure. It is not possible to assess the validity criterion in the control because results of optical density at the end of the test were not expressed in FTU contrary to initial cell concentration which is given only in FTU (initial concentration = 5 FTU, see Table A7_4_1_4-2). The corresponding Formazin Turbidity Units of the optical density measured at 436 nm were not given. 5.2: a decrease of pH values at test end in inoculated samples was observed from the 125 to 1000 mg/L (4.9<pH<5.5). This deviation may have a significant impact on the results of the study.
Conclusion	Agree
Reliability	[redacted]
Acceptability	
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_4_1_4-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
Nature	Cell culture
Species	*Pseudomonas putida*
Strain	DSM 50026
Source	[redacted]
Sampling site	Obtained in regular intervals
Laboratory culture	Yes
Method of cultivation	Agar slant culture tubes (nominal volume 20 mL) plugged with gaspermeable silicone sponge caps; weekly passage with inoculating loop; temperature: 25 ± 1°C.
Preparation of inoculum for exposure/ Pretreatment	Erlenmeyer flasks (nominal volume 250 mL) plugged with gaspermeable cellulose caps, in a pre-treatment culture medium solution (100 mL) prepared as prescribed by the guideline, and at a temperature of 21 ± 1°C.; incubation time: about 7 h with shaking; Inoculation density as optical density: 10 Formazine Nephelometric Units (FNU)
Initial cell concentration	Optical density: 5 FNU

Table A7_4_1_4-3: Test system

Criteria	Details
Culturing apparatus	50 mL glass tubes with flat bottom, plugged with gas permeable silicone sponge caps; test volume: 10 mL
Number of culture flasks/concentration	4 replicates/test concentration
Aeration device	Shaker
Measuring equipment	Photometer (optical cell density at 436 nm; dilution 1:5)
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_4-4: Test conditions

<table>
<tr><th>Criteria</th><th>Details</th></tr>
<tr><td>Test temperature</td><td>21 ± 1 °C</td></tr>
<tr><td>pH</td><td>In all uninoculated concentrations incl. control replicate: pH 7.1 (0 h), pH 7.0 (16 h)

Values at test end (inoculated, range of 4 replicates):

<table>
<tr><th>Test concentration (mg/l)</th><th>pH</th></tr>
<tr><td>Control</td><td>6.6 – 6.7</td></tr>
<tr><td>3.91</td><td>6.9 – 7.0</td></tr>
<tr><td>7.81</td><td>6.9 – 7.0</td></tr>
<tr><td>15.63</td><td>6.9 – 7.1</td></tr>
<tr><td>31.25</td><td>6.8 – 6.9</td></tr>
<tr><td>62.5</td><td>6.3 – 6.6</td></tr>
<tr><td>125</td><td>4.9 – 5.4</td></tr>
<tr><td>250</td><td>5.3 – 5.4</td></tr>
<tr><td>500</td><td>4.9 – 5.0</td></tr>
<tr><td>1000</td><td>5.4 – 5.5</td></tr>
</table>
</td></tr>
<tr><td>Aeration of dilution water</td><td>Not specified</td></tr>
<tr><td>Suspended solids concentration</td><td></td></tr>
</table>

Section A7.4.2_01 Bioconcentration in aquatic organisms
Annex Point IIA, VII.7.5. **Calculation, SRC BCFWIN v2.17**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2008) Glyoxal, BCFWIN v.2.17 calculations. [redacted] 2008, (unpublished), BPD ID A7.4.2_01	
1.2	**Data protection**	No	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s for first entry to Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	The data refer to an acknowledged calculation program: BCFWIN v2.17, a model included in the EPIWIN program, which was developed by the Syracuse Research Corporation, NY.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glyoxal	
3.1.1	Lot/Batch number	Not relevant	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	Not relevant	
3.1.4	Further relevant properties	Not relevant	
3.1.5	Radiolabelling	Not relevant	
3.1.6	Method of analysis	Not relevant	
3.2	**Reference substance**	Not relevant	
3.2.1	Method of analysis for reference substance	Not relevant	
3.3	**Testing/estimation procedure**		
3.3.1	Test system/ performance	Not relevant	
3.3.2	Estimation of bioconcentration	Model: BCFWIN v2.17 Data used for calculation: SMILES: O=CC=O log Kow used by BCF estimates: -1.15 (measured value; see reference) Correction factors not used for log Kow < 1	
		4 RESULTS	
4.1	**Experimental data**	Not relevant	
4.1.1	Mortality/behaviour	Not relevant	
4.1.2	Lipid content	Not relevant	
4.1.3	Concentrations of test material during test	Not relevant	

Section A7.4.2_01 **Bioconcentration in aquatic organisms**
Annex Point IIA, VII.7.5. **Calculation, SRC BCFWIN v2.17**

4.1.4	Bioconcentration factor (BCF)	Not relevant
4.1.5	Uptake and depuration rate constants	Not relevant
4.1.6	Depuration time	Not relevant
4.1.7	Metabolites	Not relevant
4.1.8	Other Observations	Not relevant
4.2	**Estimation of bioconcentration**	Estimated Log BCF = 0.500 (BCF = 3.162)
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	By means of the SRC BCF WIN (v2.17) EPIWIN program developed by the Syracuse Research Corporation (NY) the bioconcentration of glyoxal in aquatic organisms was estimated on the basis of a measured log Kow value of -1.15 (Reference #1).
5.2	**Results and discussion**	The estimated BCF is 3.2, indicating that significant bioconcentration in aquatic organisms is not to be expected.
5.3	**Conclusion**	The calculated BCF value of 3.2 does not indicate significant bioconcentration in aquatic organisms. This is in accordance with the hydrophilic nature of glyoxal as well as with the log Kow, which was ascertained experimentally for this substance (see reference #1).
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]
References		1) [redacted] (2002) Partition Coefficient n-Octanol / Water (log Pow) of "[redacted]". [redacted] 2002 (unpublished), BPD ID A3.09_01

EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	27/02/2018
Materials and Methods	Agree
Results and discussion	Agree
Conclusion	Agree
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from ...
Date	*Give date of 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*
Reliability	*Discuss if deviating from view of rapporteur member state*
Findings	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.4.3.1 **Annex Point IIIA, XIII.2.2.**	**Prolonged toxicity to an appropriate species of fish**	
	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 [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	23/03/2018	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.4.3.2_01 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (2009) Glyoxal [redacted] - Early Life-Stage Test on the fathead minnow (*Pimephales promelas*) in a flow through system. [redacted] (Unpublished), 02 Feb 2009, BPD ID A7.4.3.2 _01
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD 210 (1992) (U.S.) EPA-FIFRA 72-4 (a) (1982) (U.S.) EPA-OPPTS 850.1400 (1996)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 METHOD
3.1	**Test material**	Glyoxal [redacted]
3.1.1	Batch number	[redacted]
3.1.2	Specification	[redacted]
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	Aqueous solution
3.1.5	Further relevant properties	Liquid / colourless, clear, miscible with water Stable under storage conditions (at room temperature under N_2)
3.1.6	Method of analysis	The test substance was used with the given specification of the manufacturer. No further analyses were conducted.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable, since the test substance is soluble.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference	Not applicable

Section A7.4.3.2_01 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

	substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_3_2-2	
3.4.2	Test organisms	See table A7_4_3_2-3	
3.4.3	Handling of embryos and larvae (OECD 210/212)	The eggs were placed in the exposure chamber approx. less than 5 hours after fertilization (day 0), all embryos were in the stage before cleavage of the blastodisc commences. The transfer of animals from the glass levels into the steel aquaria took place on day 17.	
3.4.4	Test system	Flow-through; for details on test type, renewal of TS solution, laboratory equipment, loading, replicates etc. see table A7_4_3_2-4	X
3.4.5	Test conditions	For relevant test conditions see table A7_4_3_2-5	
3.4.6	Duration of the test	34 days	
3.4.7	Test parameter(s)	Survival, time to hatch and swim-up, toxic signs and abnormalities as well as body weight and length were examined.	
3.4.8	Examination / Sampling	Survival rates for a specific live stage (like “day 0 until hatch”) were determined. The Mortality was counted daily and the dead animals were removed. Once weekly the exact number of survivors was determined. The time span from study day 0 until hatch and the time span from hatch to end of swim-up were defined. Signs of toxicity and abnormalities were determined daily. The body weight and length were determined at the end of the exposure.	
3.4.9	Monitoring of TS concentration	Yes, samples were collected on day zero and subsequently at weekly intervals alternating from one test vessel per concentration group. On day 19 samples from each test vessels were taken and analyzed for the content of test substance. The analyses of the samples were carried out at [redacted]	
3.4.10	Statistics	For the body weights and lengths of the fish the statistical evaluation was carried out using Dunnett’s test for a simultaneous comparison of several dose groups with the control group. The test was performed two-sided. For the embryo, larvae, and fish survival, a pairwise comparison of each dose group with the control group was carried out via the Fisher’s exact test. The test was performed one-sided. Additionally the one-sided Wilcoxon-test was performed, with the replicate as the statistical unit to examine the variabilities between the replicates.	
		4 RESULTS	
4.1	**Range finding test**	Not performed	
4.1.1	Concentrations	Not relevant	
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant	

Section A7.4.3.2_01 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

4.1.3 Nature of adverse effects: Not relevant

4.2 Results test substance

4.2.1 Initial concentrations of test substance: Nominal: 0 (control), 3, 10, 32, 100 and 300 mg test material/L corresponding to 0, 1.2, 4.0, 12.7, 40 and 119 mg a.s./L

4.2.2 Actual concentrations of test substance: Analytically determined concentrations of the test material in the test water:

Day	Date	Replicate	Nominal concentration (mg/L)					
			0	3.0	10	32	100	300
-9[a]	06 Aug 08	B	<0.1	3.2	8.9	31	71	274
-2[a]	13 Aug 08	C	<0.1	4.1	10.2	32	88	255
0	15 Aug 08	A	<0.1	2.7	10.4	30	84	264 [m]
5	20 Aug 08	B	<0.1	2.8	9.5	30	90	382 [h]
7	22 Aug 08	C	-	-	-	-	-	258
12	27 Aug 08	D	<0.1	2.9	13.5 [d]	33	93	275
19		A	<0.1	2.6	8.7	34	100	321
19		B	<0.1	2.7	9.0	27 [e]	79 [f]	305 [i]
19	03 Sep 08	C	<0.1	2.5	8.9	33	105 [m]	220 [j]
19		D	<0.1	2.6	8.8	31 [m]	112 [i]	319 [k]
19		mean[b]	<0.1	2.6	8.9	31	99	291
26	10 Sep 08	D	<0.1	2.1 [c]	8.2	26	69 [g]	258
33	17 Sep 08	A	<0.1	2.4	8.4	26	89	252
Mean measured concentration (mg/L)				**2.6**	**9.8**	**29**	**87**	**283**
Standard deviation				0.29	1.96	2.8	10.2	45.6
Mean, % of Nominal				86	98	92	87	94
Lowest value,				2.1	8.2	26	69	252
% Nominal				70	82	81	69	84
Highest value,				2.9	13. 5	33	99	382
% Nominal				97	135	103	99	127

The mean measured concentrations of the test material were (% of nominal provided in parenthesis):

- 2.6 ± 0.29 mg/L (86%)
- 9.8 ± 1.96 mg/L (98%)
- 29 ± 2.8 mg/L (92%)
- 87 ± 10.2 mg/L (87%)
- 283 ± 45.6 mg/L (94%)

4.2.3 Effect data: Survival:

The mean survival rates for the groups exposed to the test material (nominal) and the control:

- embryo survival until hatch (= hatched larvae related to 100 individuals at the beginning)

Test group	Nominal concentration [mg/L]	Mean survival	Range#
0	0 (control)	98%	(96 – 100%)
1	3.0	97%	(92 – 100%)
2	10	98%	(96 – 100%)
3	32	98%	(96 – 100%)
4	100	97%	(96 – 100%)
5	300	97%	(92 – 100%)
The embyo survival until hatch was not statistically significantly decreased in comparison to the control group in any of the concentration groups.			

= Range for the 4 replicates (%)

Section A7.4.3.2_01 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

- Survival of larvae from hatch until day 7 (end of swim-up)
  (= number of survivors day 7 related to number of hatched larvae)

Test group	Nominal concentration [mg/L]	Mean survival	Range#
0	0 (control)	87%	(79 – 96%)
1	3.0	88%	(80 – 96%)
2	10	79%	(75 – 84%)
3	32	89%	(84 – 96%)
4	100	80%	(72 – 92%)
5	300	87%	(83 – 88%)
The larvae survival from hatch until end of swim-up was not statistically significant decreased in the concentration groups in comparison to the control group.			

- Survival of young fish days 7 – 34
  (= survivors at end of exposure related to day 7 survivors at end of swim-up)

Test group	Nominal concentration [mg/L]	Mean survival	Range#
0	0 (control)	96%	(95 – 100%)
1	3.0	96%	(92 – 100%)
2	10	97%	(90 – 100%)
3	32	97%	(95 – 100%)
4	100	94%	(84 – 100%)
5	300	95%	(95%)
The fish survival from end of swim-up (day 7) until the end of exposure (day 34) was not statistically significantly decreased in comparison to the control group in any of the concentration groups.			

- Survival of test organisms from day 0 to test termination (day 34)
  (= survivors at end of exposure related to 100 individuals at the beginning)

Test group	Nominal concentration [mg/L]	Mean survival	Range#
0	0 (control)	82%	(76 – 92%)
1	3.0	82%	(76 – 88%)
2	10	75%	(72 – 76%)
3	32	84%	(80 – 88%)
4	100	73%	(64 – 88%)
5	300	80%	(72 – 84%)
The survival during days 0 – 34 was not statistically significantly decreased in comparison to the control group in any of the concentration groups.			

= Range for the 4 replicates (%)

The survival until hatch, from the end of hatch to the end of swim-up (day 7), from the end of swim-up to the end of exposure (day 7 – 34) as well as over the whole exposure period (day 0 – 34) was not significantly impaired by the test substance in any of the concentration groups.

Time to hatch:

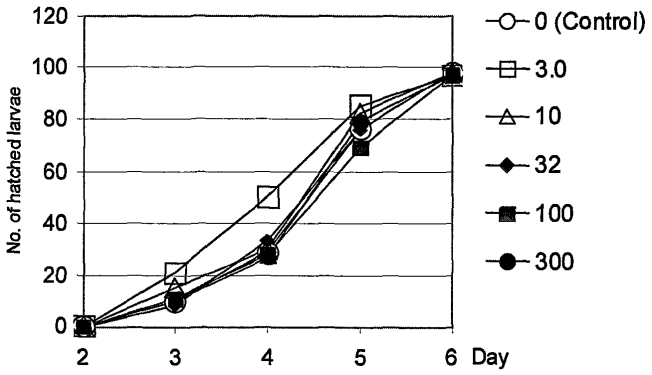
- *Time to hatch, duration of hatch (range of 4 replicates)*

Test group	Nominal concentration [mg/L]	start of hatch[a]	end of hatch[b]
0	0 (control)	Day 2	Day 6
1	3.0	Day 2	Day 6
2	10	Day 2	Day 6
3	32	Day 2	Day 6
4	100	Day 2	Day 6
5	300	Day 2	Day 6

a Start of hatch was defined as the day before the day on which the first hatched larva was observed, since hatch had taken place during the last day before the observation.

b End of hatch was defined as the day at which the last larva in a replicate of test group has hatched before hatch was terminated. Hatch was terminated after hatch of ≥ 95% of the surviving individuals of all test groups.

Section A7.4.3.2_01 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2



Hatch in the replicates of the control group and in all concentration groups started simultaneously at day 2 of exposure and was completed on day 6.

Time to swim-up:

- Time to swim-up, duration of swim-up (range of 4 replicates)

Test group	Nominal concentration [mg/L]	start of swim-up	end of swim-up[a]
0	0 (control)	Day 4	Day 6 - 7
1	3.0	Day 4	Day 6 - 7
2	10	Day 4	Day 6 - 7
3	32	Day 4	Day 6 – 7
4	100	Day 4	Day 6 - 7
5	300	Day 4	Day 6 - 7

a End of swim-up was defined as the day on which ≥ 95% of the surviving individuals of a replicate finished swim-up.

The start of swim-up (day 4) and the end of swim-up (day 6 – 7) occurred almost simultaneously in the control group and in all concentration groups.

Section A7.4.3.2_01 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

Sublethal effects (signs of toxicity) and abnormalities:

Table 6: Sublethal effects, individual data per replicate

Nominal concentration	0 (Control)				1 (3.0 mg/L)				2 (10 mg/L)				3 (32 mg/L)				4 (100 mg/L)				5 (300 mg/L)			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Day 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Day 4	Observation of symptoms started on day 7, 1 day after end of hatch, when the test organisms were large enough																							
Day 5																								
Day 6																								
Day 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 21	g1	0	0	0	0	0	0	0	0	0	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 22	g1	0	0	0	0	0	0	0	0	0	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 23	g1	0	0	0	0	0	0	0	0	0	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 24	0	0	0	0	g1	0	0	0	0	0	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 25	0	0	0	0	g1	0	0	0	0	0	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 26	0	0	0	0	g1	0	0	0	0	0	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 27	0	0	0	0	g1	0	0	0	0	0	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 28	0	0	0	0	g1	0	0	0	0	0	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 29	0	0	0	0	g1	0	0	0	0	0	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 30	0	0	0	0	g1	0	0	0	0	0	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 31	0	0	0	0	g1	0	0	0	0	g1	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 32	0	0	0	0	g1	0	0	0	0	g1	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 33	0	0	0	0	g1	0	0	0	0	g1	0	0	0	0	g1	0	0	0	0	0	0	0	0	0
Day 34	0	0	0	0	g1	0	0	0	0	g1	0	0	0	0	g1	0	0	0	0	0	0	0	0	0

Key to symptoms:
V = reduced activity
A = apathy
C = convulsions
F = reduced food consumption
W = swimming near the surfaces
B = swimming near the bottom
T = tumbling
D = discoloration
0 = no symptoms detectable
() = slight to very slight

Explanation of morphological abnormalities:
d = deformations
g = reduced growth
t = shortened tail
h = incomplete hatch
y = extended yolk sac
s = yolk sac not removed

Number behind symbol for symptom = number of affected fish.

No abnormalities were seen in any of the concentration groups or in the control group. No vertebral deformations were seen. In the control group and in the test groups (3, 10 and 32 mg/L) a markedly reduced growth was seen in single individuals. It was considered to be not a substance-related effect.

Body weight:

Mean wet weights of the exposure groups in comparison to the control group:

Section A7.4.3.2_01 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

Test group	Nominal concentration [mg/L]	Mean weight of individual fish [mg]	% of control[a]	Statistical significance[b]
0	0 (control)	212	100%	-
1	3.0	244	115.1%	$p \leq 0.01$
2	10	252	119.0%	$p \leq 0.01$
3	32	216	102.2%	--
4	100	220	104.1%	--
5	300	213	100.6%	--

-- = Not statistically significant
- = Not relevant
a = Calculated on the basis of the individual values
b = Compared to control

No adverse substance-related effects on the body weight development were observed up to the highest tested concentration.

Body length:

Mean body length of the exposure groups in comparison to the control group:

Test group	Nominal concentration [mg/L]	Mean length of individual fish [cm]	% of control[a]	Statistical significance[b]
0	0 (control)	2.7	100%	-
1	3.0	2.8	103.1%	$p \leq 0.05$
2	10	2.8	103.9%	$p \leq 0.01$
3	32	2.7	99.3%	--
4	100	2.7	99.6%	--
5	300	2.6	98.4%	--

-- = Not statistically significant
- = Not relevant
a = Calculated on the basis of the individual values
b = Compared to control

No substance-related effects on the body length development were observed up to the highest tested concentration.

4.2.4 Concentration / response curve: Not applicable

4.2.5 Other effects: None observed

4.3 Results of controls

4.3.1 Number/ percentage of survival/animals showing adverse effects:

The mean control survival was:
-at hatch (related to total of 100 fertilized eggs) = 98%
(96% - 100%)
-at end of swim-up (day 7, related to larvae hatched) = 87%
(79% - 96%)
-at end of exposure (day 34, related to day 7 survivors) = 96%
(95% - 100%)
-at end of exposure (day 34, related to eggs at start) = 82%
(76% - 92%)

Section A7.4.3.2_01 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

		The hatching success was > 66% of the test organisms. More than 70% of the hatched larvae survived until the end of the exposure period.
4.3.2	Nature of adverse effects	None observed
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 aim of the present study was to investigate the toxicity of Glyoxal [redacted] to early life-stages of the fathead minnow (*Pimephales promelas*). Test material: [redacted], (Glyoxal, [redacted]) The test was performed according to guideline OECD 210 (1992), EPA-FIFRA 72-4 (1982) and EPA OPPTS 850.1400 (1996) under GLP conditions. Fertilized eggs of the fathead minnow were exposed under flow-though conditions for 34 days. The eggs were placed in the exposure chamber approx. less than 5 hours after fertilization (day 0) and were then exposed to following concentrations of the test material: 0, 3, 10, 32, 100, 300 mg/L. The test parameters were survival, time to hatch and swim-up, toxic signs and abnormalities as well as body weight and length. The test temperature was generally 25 ± 1°C, dissolved oxygen was maintained in a range between 5.2 and 8.6 mg/L; the pH was in a range of 7.7 – 8.1. For monitoring the test substance concentrations, samples were taken on day zero and then subsequently at weekly intervals alternating from one test vessel per concentration group. On day 19, samples from each test vessels were taken and analyzed for content of test substance.
5.2	**Results and discussion**	<u>Survival:</u> Over the whole study period (day 0 - 34) survival and hatch were not impaired in any of the tested concentration groups in comparison to the control. In conclusion, the NOAEC and the LOAEC for survival are 300 mg/L (nominal concentration) and 283 mg/L (based on mean measured concentrations). <u>Time to hatch and swim-up:</u> The time to hatch (day 2 – 6) and swim up (day 4 – 7) was similar in all test groups and not affected by the test substance. In conclusion, the NOAEC and the LOAEC for the time to hatch and swim-up are 300 mg/L (nominal concentration) and 283 mg/L (based on mean measured concentrations). <u>Toxic signs (symptoms) and abnormalities:</u> No signs of toxicity or substance-related abnormalities were observed up to the highest concentration group. Thus, the NOAEC and the LOAEC for sublethal effects are 300 mg/L (nominal concentration) and 283 mg/L

Section A7.4.3.2_01 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

		(based on mean measured concentrations). Body weight and length: No substance-related adverse effect on body weight and total body length was observed up to the highest tested concentration. Thus, the NOAEC and the LOAEC for the body weight and length are 300 mg/L (nominal concentration) and 283 mg/L (based on mean measured concentrations).
5.2.1	NOAEC	In conclusion, under the conditions of this study, the overall no observed adverse effect concentration (NOAEC) was ≥ 300 mg/L (nominal concentration) and 283 mg/L (based on mean measured concentrations)
5.2.2	LOAEC	The lowest concentration with adverse effects (LOAEC) was ≥ 300 mg/L (nominal concentration) and 283 mg/L (based on mean measured concentrations).
5.3	**Conclusion**	The chronic treatment of early-life-stages of fish with [redacted] (Glyoxal [redacted]) resulted in no substance-related effects. Referring to the nominal concentrations of the active substance Glyoxal, the NOAEC- and LOAEC values are: NOAEC: 119 mg a.s./L LOAEC: 119 mg a.s./L. The validity criteria can be considered as fulfilled (see validity criteria summarized in tables A7_4_3_2-6)
5.3.1	Other Conclusions	None
5.3.2	Reliability	[redacted]
5.3.3	Deficiencies	[redacted]

Section A7.4.3.2_01 **Effects on reproduction and growth rate of fish**
Annex Point IIIA XIII 2.2

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date	2/03/2018
Materials and Methods	3.4.4: Data from the study report are not available to ensure that variation of the flow rates of stock solutions and dilution water is less than 10% throughout the test.
Results and discussion	Agree
Conclusion	Agree
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from other member state (specify)
Date	*Give date of 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*
Remarks	

Section A7.4.3.3.1 TNsG, Ch. 3, Part A	Bioaccumulation in an appropriate species of fish	
	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 relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	2/03/2018	
Evaluation of applicant's justification	Agree	
Conclusion	Agree	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A7_4_3_2-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_3_2-2: Dilution water

Criteria	Details
Source	municipal water works of the city [redacted]; water was purified through a charcoal filter and diluted with deionized
Alkalinity (CaCO3)	No information
Hardness (CaCO3)	(approx. 100 mg/L as CaCO3)
pH	7.7 to 8.1
Oxygen content	No information
Conductivity	253 to 279 µS
TOC Content	< 2.0 mg/L
Holding water different from dilution water	Not relevant

Table A7_4_3_2-3: Test organisms

Criteria	Details
Species	fathead minnow (*Pimephales promelas*)
Source	Parental fathead minnows: [redacted]
Wild caught	No
Age/size	Developing embryos at the start of the test
Kind of food	ewly hatched brine shrimp larvae
Amount of food	*Artemia nauplii*
Feeding frequency	Feeding was increased in quantity with the duration of the study and thus with the size of the fish, twice daily
Post-hatch transfer time	No information
Time to first feeding	From day 6
Feeding of animals during test	Yes
Treatment for disease within 2 weeks proceeding test	No

Table A7_4_3_2-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	The flow rates: 7.5 L/hour/test group and/or 1.9 liters test water/hour for each test vessel. Theoretical exchange rate of the water contents was approximately 5 fold per 24 hours.
Volume of test vessels	Eggs and larvae: approx. 1.7 liter Fish: 6 liter
Volume/animal	Not relevant
Number of animals/vessel	25 eggs/incubation cup
Number of vessels/ concentration	4 replicates per solvent control/dilution water control/test concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2-5: Test conditions

Criteria	Details
Test temperature	25 ±1°C
Dissolved oxygen	5.2 to 8.6 mg/L (> 60% saturation at 25 °C)
pH	7.7 to 8.1
Adjustment of pH	No
Aeration of dilution water	No data
Intensity of irradiation	95 - 241 Lux at a light cycle of 16 hours light and 8 hours darkness
Photoperiod	light cycle of 16 hours light and 8 hours darkness

Table A7_4_3_2-6 Validity criteria for an ELS fish test according to OECD Guidelines 210

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

Section A7.4.3.3.2 **TNsG, Ch. 3, Part A**	**Bioaccumulation in an appropriate invertebrate species**	
	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 relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	2/03/2018	
Evaluation of applicant's justification	Agree	
Conclusion	Agree	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.4.3.4_01 **Annex Point IIIA XIII 2.4**		**Effects on reproduction and growth rate with an invertebrate species**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] (2009) *Daphnia magna* Reproduction Test, [redacted] 2009, BPD ID A7.4.3.4_01	
1.1.1	Data protection	Yes	
1.1.2	Data owner	[redacted]	
1.1.3	Companies with letter of access	[redacted]	
1.1.4	Criteria for data protection	Data on new [a.s.] for first entry to Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, Commission Regulation (EC) No. 440/2008, C.20 OECD TG 211 (1998)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Glyoxal [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Substance No.: [redacted]	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Aqueous solution	
3.1.5	Further relevant properties	Liquid / colourless, clear, miscible in water	
3.1.6	Method of analysis	Reversed phase HPLC (flow: 0.4 ml/min., injection volume: 5 µl, temp. 45°C) with UV/VIS-detection (370 nm); determination by the method of external standards Gradient run: t(min) 0 35 40 41 55 %(v/v)A 60 18 18 60 stop %(v/v)B 40 82 82 40 A: Demineralized water; B: Acetonitile	
3.2	**Preparation of TS solution for poorly soluble or volatile**	Not relevant	

Section A7.4.3.4_01 Annex Point IIIA XIII 2.4		Effects on reproduction and growth rate with an invertebrate species	
	test substances		
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not relevant	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_3_4-2	
3.4.2	Test organisms	*Daphnia magna* STRAUS	
3.4.3	Handling of offspring	Counting and removing of offspring on each Monday, Thursday, and Friday.	
3.4.4	Test system	See table A7_4_3_4-4	
3.4.5	Test conditions	See table A7_4_3_4-5	
3.4.6	Duration of the test	21 days	
3.4.7	Test parameter	Mortality, reproduction and growth as length in mm.	
3.4.8	Examination / Sampling	Animals were examined daily for mortality and for reproduction; offspring was removed thrice a week at time of renewal of the test solutions. The body length of the parent animals was determined at the end of the exposure with a microscope.	
3.4.9	Monitoring of TS concentration	The test solutions were renewed three times weekly on Monday, Wednesday and Friday. Samples for analysis were taken in one representative test interval per week in each freshly prepared test solution (without daphnids) and in the 48-h or 72-h old test solutions before renewal (combined sample with daphnids). Test samples of each concentration and the control were analyzed from the following days: 0 (fresh), 2 (48-h old), 9 (fresh), 12 (72-h old), 19 (fresh), 21 (48-h old).	
3.4.10	Statistics	A statistical evaluation was performed to determine effect concentrations (EC_{50}) as well as LOEC and NOEC. The data were not sufficient to calculate EC_{50} values. For the statistical evaluation of the LOEC and NOEC Dunnett's test was used to analyze the parameters: - Reproduction as number of living young (one sided analysis) - Growth as length in mm (two sided analysis)	
		4 RESULTS	
4.1	**Range finding test**	Yes	
4.1.1	Concentrations	No data	
4.1.2	Number/ percentage of animals showing adverse effects	14-day LC_0: 32 – 300 mg/L 14-day LOEC for reproduction: 32 mg/L	
4.1.3	Nature of adverse	See above	

<table>
<tr><td colspan="2">Section A7.4.3.4_01
Annex Point IIIA XIII 2.4</td><td>Effects on reproduction and growth rate with an invertebrate species</td><td></td></tr>
<tr><td colspan="2"></td><td></td><td></td></tr>
<tr><td colspan="2">effects</td><td></td><td></td></tr>
<tr><td>4.2</td><td>Results test substance</td><td></td><td></td></tr>
<tr><td>4.2.1</td><td>Initial concentrations of test substance</td><td><u>Nominal:</u> 0, 3, 8, 12, 18, and 32 mg/l</td><td></td></tr>
<tr><td>4.2.2</td><td>Actual concentrations of test substance</td><td>Analytical measured concentration of Glyoxal in the test solutions:
<table>
<tr><th>Nominal Concentration [mg/L]</th><th>Time-weighted mean [mg/L]^a</th><th>% of mean initial measured^b</th><th>% of nominal</th></tr>
<tr><td>0 (control)</td><td>< 0.1</td><td>-</td><td>-</td></tr>
<tr><td>3</td><td>2.51</td><td>79.2</td><td>83.6</td></tr>
<tr><td>8</td><td>7.96</td><td>89.7</td><td>99.5</td></tr>
<tr><td>12</td><td>12.1</td><td>93.2</td><td>101</td></tr>
<tr><td>18</td><td>18.6</td><td>94.6</td><td>103</td></tr>
<tr><td>32</td><td>33.2</td><td>96.8</td><td>104</td></tr>
</table>
a- based on 7 measured renewal period days;
b- initial measured is the mean measured concentration at the start of each renewal period;
for detailed results see analytical report in the Appendix.

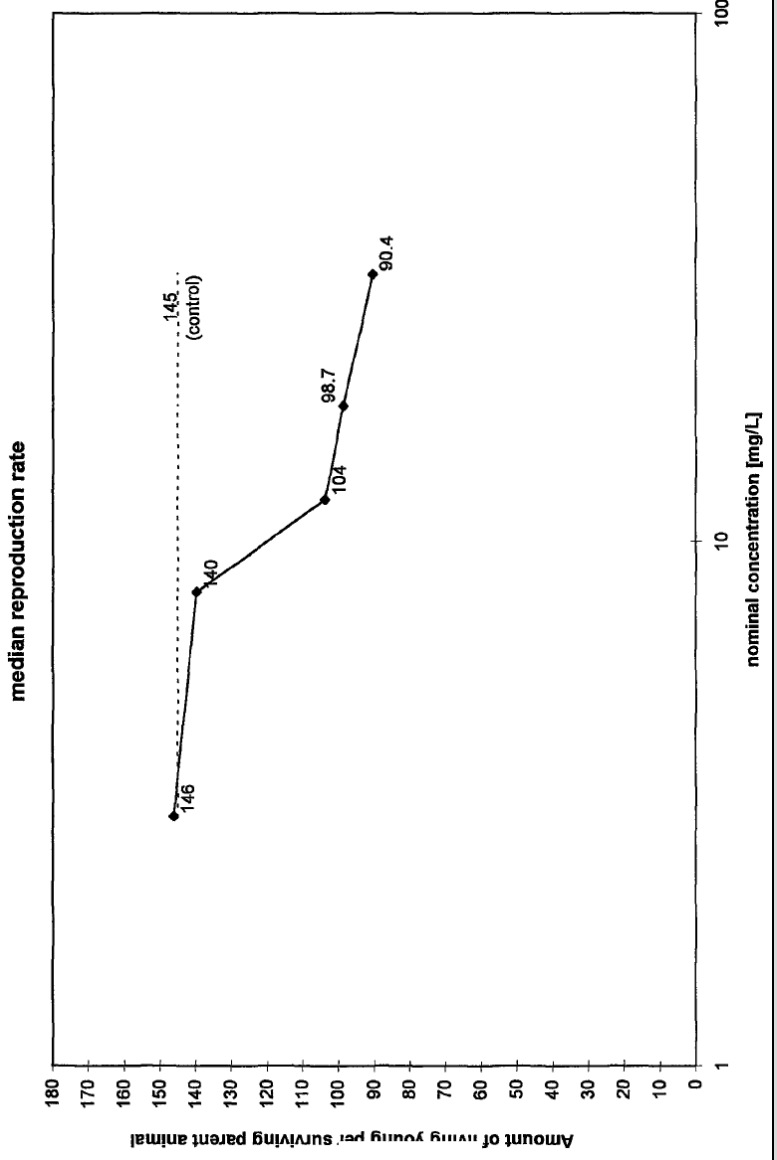
In 3 cases the measured values were slightly lower in old test solution then +/- 20% of nominal concentration.
<table>
<tr><th>Test day</th><th>Nominal concentration [mg/L]</th><th>Solution age at sample collection</th><th>% of mean initial measured</th><th>% of nominal</th></tr>
<tr><td>12</td><td>3</td><td>72h</td><td>52</td><td>50</td></tr>
<tr><td>12</td><td>8</td><td>72h</td><td>73</td><td>77</td></tr>
<tr><td>21</td><td>3</td><td>48h</td><td>45</td><td>47</td></tr>
</table>
</td><td></td></tr>
</table>

Section A7.4.3.4_01 Annex Point IIIA XIII 2.4	Effects on reproduction and growth rate with an invertebrate species	
4.2.3 Effect data (21 d)	Reproduction and Growth Summary (mean per surviving replicates after 21 days)	

Nominal Concentration [mg/L]	Reproduction		Growth	
	Mean Living Young	% effect[b]	Mean Length (mm)	% effect[b]
0 (control)	145 (7.2%[a])	-	4.3	-
3	146	-	4.2	-
8	140	-	4.3	-
12	**104****	**28%****	**4.1****	**5%****
18	**98****	**32%****	**4.0****	**7%****
32	**90****	**38%****	**3.9****	**9%****

*: p≤ 0.05; **: p≤ 0.01
a: Coefficient of variation for control fecundity.
b: Effect relative to control. Only calculated for statistically significant effects.

	Reproduction nominal	Reproduction measured	Length nominal	Length measured
LOEC [mg/L]	12	12.1	12	12.1
NOEC [mg/L]	8	7.96	8	7.96

Section A7.4.3.4_01 Annex Point IIIA XIII 2.4		Effects on reproduction and growth rate with an invertebrate species	
4.2.4	Concentration / response curve		
4.2.5	Other effects	No additional adverse effects or abnormal behaviour were observed in any of the test treatments.	
4.3	**Results of controls**	Controls were inconspicuous (see point 4.2.3)	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	

Section A7.4.3.4_01 Annex Point IIIA XIII 2.4		Effects on reproduction and growth rate with an invertebrate species	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	Materials and methods	The aim of the present study was to determine the chronic toxicity of Glyoxal [redacted] to *Daphnia magna*. The study was conducted according to an internationally harmonized guideline (e.g. OECD TG 211) under GLP conditions. Glyoxal [redacted] was tested under semi-static conditions for its effect on mortality, reproduction growth to *Daphnia magna* STRAUS. Ten neonates per concentration were exposed for 21 days to nominal concentrations of 0, 3, 8, 12, 18 and 32 mg Glyoxal/L. Animals were examined daily for mortality and for reproduction; offspring was removed three times a week. The test solutions were renewed three times weekly. Samples for analysis were taken in one representative test interval per week in each freshly prepared test solution and in the 48-h or 72-h old test solutions before renewal.	
5.2	Results and discussion	<u>Analytical monitoring:</u> The analytically determined time-weighted mean concentrations of the test substance in the test solutions during the exposure period were within the range of ± 20% of the nominal concentrations. The individually measured concentrations of the test substance in the test solutions were within ± 20% of the nominal concentration in all fresh test solutions. Measured values were slightly lower in old test solutions and only exceeded ± 20% of nominal in 3 cases (see Section 3.4.9). These low measured values are most likely due to the test substance binding to the increased amount of food (algal cells) used in the later days of the test. However, since they were outside the acceptable range of ±20% and following the recommendations of OECD TG 211, the results are evaluated based on the time-weighted mean measured concentrations. <u>Phys.-chem. data:</u> During the test the measured oxygen content of the test solutions was in the range of 8.7 to 9.3 mg/l, the pH value was 7.5 – 8.5. <u>Effect data (after 21 days):</u> No mortality was observed among parent animals over the 21 d exposure period. Significant effects on reproduction and growth were observed at ≥ 12 mg/L. Reproduction was affected to a greater degree than growth. Organisms in the highest test concentration (32 mg/L) had a 38% reduction in reproduction and a 9% reduction in growth. The data were not sufficient to calculate EC_{50} values for reproduction or growth. NOEC and LOEC values for reproduction (living young) and growth (length) of the parent animals after 21 d are based on nominal and mean measured concentrations.	
5.2.1	NOEC	8 mg/L (nominal) / 7.96 mg/L (mean measured)	
5.2.2	LOEC	12 mg/L (nominal) / 12.1 mg/L (mean measured)	

Section A7.4.3.4_01 **Annex Point IIIA XIII 2.4**	**Effects on reproduction and growth rate with an invertebrate species**	
5.2.3 LC0	≥ 32 mg/L (nominal) / ≥ 33.2 mg/L (mean measured)	
5.3 Conclusion	Based on mean measured concentrations, the 21-day NOEC for effects of Glyoxal on reproduction and growth of *Daphnia magna* is 7.96 mg a.s./L. The results in this study are consistent with all validity criteria and the test is valid according to the guidelines of this study.	X
5.3.1 Reliability	[redacted]	
5.3.2 Deficiencies	[redacted]	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by Rapporteur Member State	
Date	02/03/2018	
Materials and Methods	Agree	
Results and discussion	Agree In addition to the Dunnett's test used for the statistical evaluation of the LOEC and NOEC, a Williams test has been done and gave the same results.	
Conclusion	Based on mean measured concentrations, the 21-day NOEC for effects of Glyoxal on reproduction and growth of *Daphnia magna* is 7.96 mg test mat./L equivalent to 3.19 mg a.i./L.	
Reliability	[redacted]	
Acceptability	[redacted]	
Remarks		
	Comments from ... (SPECIFY)	
Date	*Give date of 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*	
Remarks		

Table A7_4_3_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not applicable
Vehicle	Not applicable
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	Not applicable

Table A7_4_3_4-2: Dilution water

Criteria	Details
Source	Synthetic fresh water (M4 medium)
Salinity	Not relevant
Hardness	freshly prepared test solutions: 2.43 to 2.48 mmol/L old test solutions (before renweal): 2.45 to 2.50 mmol/L
pH	7.5 – 8.5
Ca / Mg ratio	About 4:1
Na / K ratio	Not specified
Oxygen content	After preparation, the M4 medium is aerated for approx. 24 h until saturation with oxygen is reached.
Conductance	550 – 650 µS/cm
TOC	Not specified
Holding water different from dilution water	No

Table A7_4_3_4-3: Test organisms

Criteria	Details
Strain / Clone	*Daphnia magna* STRAUS 1820
Source	The clone was supplied by [redacted]
Age	2 - 24 hours at test start (starting with the 3rd breed of the parent animals)
Breeding method	Breeding conditions were similar to the test conditions. Temperature was 20 ± 2°C, changing of the breeding water was on Monday and Friday. Age of the stock animals for the test was 2 to 4 weeks.
Kind of food	Green algae (*Desmodesmus subspicatus*)
Amount of food	Feeding schedule, amount of food per parent animal and day: Day 0-3 0.10 mg TOC Day 4-5 0.12 mg TOC Day 6-7 0.12 mg TOC Day 8-21 0.20 mg COD An algal concentrate (centrifugation, resuspension in M4 medium) was fed (max. 0.3 mL/50 mL/day).
Feeding frequency	Daily
Pretreatment	No
Feeding of animals during test	Yes, daily.

Table A7_4_3_4-4: Test system

Criteria	Details
Test type	Semistatic
Renewal of test solution	Three times weekly
Volume of test vessels	Glass beakers, nominal volume 100 mL, test volume 50 mL
Volume/animal	50 mL
Number of animals/vessel	1 (the animals were placed impartially into the test vessels)
Number of vessels/ concentration	10
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_4-5: Test conditions

Criteria	Details
Test temperature	19°C
Dissolved oxygen (mg O_2/l)	8.7 to 9.3 mg/L
pH	7.5 to 8.5
Adjustment of pH	No
Aeration of dilution water	None
Quality/Intensity of irradiation	600 – 850 lux, wavelength 400 – 750 nm
Photoperiod	16 h light, 8 hours darkness

Table A7_4_3_4-6: Validity criteria for invertebrate reproduction test according to OECD Guideline 211

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

Section A7.4.3.5.1 **Annex Point IIIA, XIII.3.4.**	**Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk** **Effects on sediment dwelling organisms**	
	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 endpoint is not of concern for glyoxal as it is only required for product type 21 (product type specific requirement).	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*23/03/208*	
Evaluation of applicant's justification	*Agree*	
Conclusion	*Agree*	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.4.3.5.2 **Annex Point IIIA, XIII.3.4.**	**Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk** **Aquatic plant toxicity**	
	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 endpoint is not of concern for glyoxal as it is only required for product type 21 (product type specific requirement).	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*23/03/2018*	
Evaluation of applicant's justification	*Agree*	
Conclusion	*Agree*	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section III A7.4.1.3 **Annex Point IIA7.1**	**Acute toxicity to fish**	
	STATEMENT	Official use only
Comment of the RMS	Two studies are provided. However important deficiencies have been detected in each study (concentration of the active substance has not been measured, load of fish more than two fold higher than maximum accepted, too low temperature for the second test). Required action: please provide a new study without any important deficiency.	
Response of the Notifier	[redacted]	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	

Section III A7.4.1.3 **Annex Point IIA7.1**	**Acute toxicity to fish**
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A 7.4.1.3 **Annex Point IIA4.1/4.2 & IIIA-IV.1**	**Growth inhibition test on algae**	
	STATEMENT	Official use only
Comments of the RMS	[redacted]	
Response of the Notifiant	[redacted]	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	

Section A 7.4.1.3	Growth inhibition test on algae
Annex Point IIA4.1/4.2 & IIIA-IV.1	
Remarks	

Section A7.4_ JNS **Annex Point IIA7**	**General _ Glyoxal Hydrates** **Justification of non-submission**	
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]Scientifically unjustified [ ]** **Other justification [X]**	Official use only
Detailed justification:	[redacted]	

[redacted]				

Section A7.4_ JNS **Annex Point IIA7**	**General _ Glyoxal Hydrates** **Justification of non-submission**

[redacted]

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	09/2018
Evaluation of applicant's justification	[redacted]
Conclusion	[redacted]
Remarks	

COMMENTS FROM OTHER MEMBER STATE *(specify)*

Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.1.1_01
Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)
Nitrogen Transformation Test

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (2009) [redacted] Soil Microorganisms – Nitrogen Transformation Test. [redacted] (Unpublished), BPD ID A7.5.1.1_01
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	OECD TG 216
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Glyoxal [redacted] (CAS No: 107-22-2)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	Test substance diluted in water
3.1.5	Further relevant properties	The test material was described as colourless clear homogenous liquid and miscible with water at ca. 20°C. The stability under storage conditions (room temperature, under nitrogen) over the exposure period was guaranteed by the sponsor.
3.1.6	Method of analysis	Not carried out
3.2	**Reference substance**	No reference substance was tested
3.2.1	Method of analysis for reference substance	Not applicable.
3.3	**Testing procedure**	
3.3.1	Soil sample / inoculum / test organism	See table A7_5_1_1-1
3.3.2	Test system	See table A7_5_1_1-3
3.3.3	Application of TS	See table A7_5_1_1-4
3.3.4	Test conditions	See table A7_5_1_1-5

Section A7.5.1.1_01 **Inhibition to microbial activity (terrestrial) Nitrogen Transformation Test**
Annex Point IIA7.4

3.3.5	Test parameter	Inhibition of microbial nitrogen transformation Luzerne meal was used as source of nitrogen and was supplied by [redacted] Luzerne meal contained 42.0 g Carbon /100 g and 3.4 g Nitrogen/100 g; the C/N ratio was 12:1 [redacted]	
3.3.6	Analytical parameter	Distilled water was added to the soil samples taken at each sampling time point and the soil suspensions were shaken. Following centrifugation, the supernatant of each suspension was stored frozen until nitrate determination. The nitrate determination was based on ion chromatography using an IC system [redacted] apparatus; the reagents were deionised water, a solution of sodium carbonate and sodium hydroxide as well as a standard solution of nitrate. The analytical monitoring of the nitrate concentrations was performed [redacted]	
3.3.7	Duration of the test	28 days	
3.3.8	Sampling	Samples were taken on days 0, 7 and 28 of incubation and were examined for nitrate concentration. For each test concentration and sampling time point, 3 samples were considered (each about 24 g)	X
3.3.9	Monitoring of TS concentration	Not performed as not of importance for the present type of study and not required by the guideline	
3.3.10	Controls	Controls without test material were added to the test series.	
3.3.11	Statistics	Probit analysis was not possible (no clear dose-response relationship)	
		4 RESULTS	
4.1	**Range finding test**	Not performed	
4.1.1	Concentration	Not applicable	
4.1.2	Effect data	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0, 62.5, 125, 250, 500 and 1000 mg test material/kg dry matter soil	
4.2.2	Actual concentrations of test substance	Not determined (no correction for purity)	
4.2.3	Concentration/ response curve	Not applicable	

Section A7.5.1.1_01 **Inhibition to microbial activity (terrestrial)**
Annex Point IIA7.4 **Nitrogen Transformation Test**

4.2.4 Nitrate content

Nitrate concentration in test mixture (mg/kg dry matter of soil):

Nitrate concentrations (mg/kg dry matter of soil) for each test mixture, at each sampling time point (3 samples)				
Test material concentration (nominal) [mg/kg dry matter of soil]	**Sample**	**Day 0**	**Day 7**	**Day 28**
0 (control)	1	80	95	220
	2	80	95	220
	3	80	95	220
63	1	75	105	235
	2	80	105	230
	3	80	105	228
127	1	80	80	190
	2	80	80	184
	3	75	79	185
250	1	75	144	260
	2	80	134	260
	3	80	144	253
501	1	75	134	300
	2	75	134	293
	3	75	134	295
1001	1	75	70	265
	2	75	70	265
	3	75	70	260

4.2.5 Percentage of Inhibition

Mean percentage of inhibition of nitrate production in test mixture: X

Inhibition of nitrate production (%) (mean of 3 samples per test concentration and time point)			
Test material concentration (nominal) [mg/kg dry matter of soil]	**At day 0**	**At day 7**	**At day 28**
0 (control)	-	-	-
63	2	-11	-5
127	2	16	15
250	2	-48	-17
501	6	-41	-34
1001	6	26	-19

4.2.6 Summary of the effect concentrations of the test substance

Time point	EC_{10} (mg test material/kg dry matter of soil)	EC_{50} (mg test material/kg dry matter of soil)
Day 0	>1001	>1001
Day 7	900	>1001
Day 28	>1001	>1001

4.3 Results of controls See 4.2.4 and 4.2.5

4.4 Test with reference substance Not performed

4.4.1 Concentrations Not applicable

4.4.2 Results Not applicable

Section A7.5.1.1_01 **Inhibition to microbial activity (terrestrial) Nitrogen Transformation Test**

Annex Point IIA7.4

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The aim of the present study was to investigate the adverse effects of Glyoxal [redacted] on nitrate production by aerobic soil microorganisms using the Nitrate Transformation Test. Luzerne meal was used as source of nitrogen. Test material: [redacted] The test was conducted according to OECD 216, and followed GLP. About 70 kg of soil were collected [redacted] the weather conditions were cloudy and 21°C. [redacted] The soil was defined as silty sand according to German DIN. The soil sample was stored in a closed plastic sack at 4 ± 2°C in the dark until test initiation. For testing, the soil sample was dried for two days at room temperature. The sample was pre-sieved < 10 mm and then < 2 mm. For the nitrogen transformation test, portions of soil were mixed with luzerne meal. The required aliquots of the test material stock solution were blended with demineralised water to adjust the scheduled test concentrations and a water content of 45 ± 5% of the water holding capacity of the soil. Afterwards the pH was determined in two replicates from every batch. The test mixtures were incubated for 28 days in the dark at a mean temperature of 20 ± 2°C. The test vessels were closed with a perforated aluminium cap. The water content was controlled by weighing the test samples, and water loss was regulated by addition of demineralised water. Controls consisted of the test medium (i.e. soil) without test material. Following nominal concentrations of test material in soil were tested: 0, 63, 127, 250, 501 and 1001 mg/kg dry matter soil. Sampling time points were day 0, day 7 and day 28; at each time point 3 samples per test concentration were considered. The content of nitrate in aqueous soil extracts obtained from the test mixture samples was determined by means of ion chromatography using an IC system [redacted] apparatus. The reagents were deionized water, a solution of sodium carbonate and sodium hydroxide and a standard solution of nitrate. The nitrate contents in the test mixtures were compared to those of the controls, and the percentage of inhibition of the nitrate production in the treated soil samples was calculated.	
5.2	**Results and discussion**	The microbial nitrogen transformation process in soil was not affected by Glyoxal [redacted] when applied at a concentration of 1001 mg test material/kg dry matter of soil.	X
5.2.1	NOEC	Not stated	
5.2.2	EC_{10}	After 0 days: > 1001 mg test material/kg dry matter of soil (dm), corresponding to > 400 mg a.s./kg dm. After 7 days: 900 mg test material/kg dm, corresponding to 360 mg a.s./kg dm. After 28 days: > 1001 mg test material /kg dm, corresponding to > 400 mg a.s./kg dm.	
5.2.3	EC_{50}	After 0 days: > 1001 mg test material /kg dm, corresponding to > 400 mg a.s./kg dm.	

Section A7.5.1.1_01 **Inhibition to microbial activity (terrestrial)**
Annex Point IIA7.4 **Nitrogen Transformation Test**

		After 7 days: >1001 mg test material /kg dm, corresponding to > 400 mg a.s./kg dm. After 28 days: > 1001 mg test material /kg dm, corresponding to > 400 mg a.s./kg dm.	
5.3	**Conclusion**	The microbial nitrogen transformation process in soil was not affected by Glyoxal [redacted] when applied at a concentration of 1001 mg test material/kg dry matter of soil. Based on nominal concentrations, the EC_{10} and EC_{50}, respectively, after 28 days are greater than 400 mg a.s./kg dry matter soil. The deviation of formed nitrate in the blank controls was < 15% at the end of the exposure, confirming the validity of the test.	
5.3.1	Reliability	[redacted]	X
5.3.2	Deficiencies	[redacted]	

Section A7.5.1.1_01 **Inhibition to microbial activity (terrestrial)**
Annex Point IIA7.4 **Nitrogen Transformation Test**

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date	03/2018
Materials and Methods	There is three samples per concentrations considered at each time point, and no further information is available on replicates. It is not possible to know if the samples referenced in the results are from true replicates, or sub-samples from the same replicate.
Results and discussion	The results presented in section 4.2.5 indicate that there are no dose-response relationships at day 7 and day 28. Indeed at day 7, it should be noted that an inhibition of the nitrate production higher than 15% (until 26%) at the tested concentration of 127 mg test mat/kg and at the highest concentration (1001 mg test mat/kg) were shown whereas an increase of nitrate production largely higher than 15% were observed at both previous concentrations (48% and 41% respectively). Moreover, according the infobox 11 of the Guidance on BPR Vol IV Part B+C (and also previous guidance), any significant deviation (decrease/increase) from the control in a soil nitrification inhibition test should be considered as a relevant effect. At day 28, increases of 17% - 34% and 19% of the nitrate production was observed at the highest concentrations 250 – 501 and 1001 mg test mat/kg respectively. These increases are largely higher than 15%. Regarding these high variations of the obtained results, no confidence limits can be derived due to the bad fit. Then, statistical analysis are not possible.
Conclusion	No reliable EC_{10} and/or EC_{50} can be derived from this study.
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_5_1_1-1: Microbial sample / Inoculum

Criteria	Details
Nature	Silty sand, defined as soil type 5 M [redacted]
Sampling site:	The soil sample was prepared conformely to the specifications of the guideline.
Geographical reference on the sampling site	[redacted]
Data on the history of the site	Sampling date was the 20 Aug 2008
Use pattern	Not specified
Depth of sampling [cm]	About 20 cm
Sand / Silt / Clay content [% dry weight]	Soil defined as silty sand according to German DIN Percentage of sand (i.e. particles > 0.063-2.0 mm) 55.2 +/- 2.1 %
pH	7.2 +- 0.1
Organic carbon content [% dry weight]	1.29 +/- 0.20
Maximal water holding capacity (WHC_{max}; g/100 g)	42.1 +/- 4.0
Nitrogen content [% dry weight]	Not specified
Cation exchange capacity [mval/100g]	15 +/-3
Initial microbial biomass	173.3 mg/kg dry soil matter
Water content of the delivered soil at test initiation (WC; g/100 g dry matter)	10.2
Reference of methods	Determination of the initial microbial biomass according to ISO 14240-1 Determination of the initial water content according to ISO 11465
Collection / storage of samples	The soil sample was stored in a closed plastic sack at 4 +/- 2 °C in the dark until test initiation
Preparation of inoculum for exposure	Portions of about 326 g of soil and about 1.5 g Luzerne meal were mixed. Soil was adjusted to 45 +/+ WHC
Pretreatment	None

Table A7_5_1_1-2: Test organism

Criteria	Details
Species	Not relevant, see table A7_5_1_1-1
Strain	“
Source	“
Sampling site	“
Laboratory culture	“
Method of cultivation	“
Preparation of inoculum for exposure	“
Pretreatment	“
Initial cell concentration	“

Table A7_5_1_1-3: Test system

Criteria	Details
Culturing apparatus	Test pots closed with a perforated aluminium cap
Number of vessels / concentration	3 samples per test concentration
Aeration device	Aeration was assured by the perforations in the caps.
Measuring equipment	Analyse of nitrate contents in aqueous soil extracts: Content of nitrate was determined by means of ion chromatography using an IC system [redacted] apparatus. The reagents were deionized water, a solution of sodium carbonate and sodium hydroxide and a standard solution of nitrate
Test performed in closed vessels	See above

Table A7_5_1_1-4: Application of test substance

Criteria	Details
Application procedure	A suitable quantity of soil was placed in a mixer. A defined amount of test substance was added without carrier material to adjust the scheduled test concentrations. One g of Luzerne meal was added as wells 16.4 ml distilled water (to get 45 +/- 5% of the WHC_{max}).
Carrier	None
Concentration of liquid carrier [% v/v]	Not relevant as no carrier was used.
Liquid carrier control	Not relevant as no carrier was used.
Other procedures	None

Table A7_5_1_1-5: Test conditions

Criteria	Details
Organic substrate	For the nitrogen transformation test, the soil samples were amended with about 1 g lucerne meal a source of nitrogen.
Incubation temperature	20 +/-2°C (mean: 20.6 °C)
Soil moisture	18.9 g /100 g dry soil matter
Method of soil incubation	The test samples were incubated up to 28 days in the dark; the water content was controlled by weighing of the test samples and water loss was regulated by addition of demineralised water.
Aeration	Aeration was assured by the perforations in the caps.
pH in test mixtures at test initiation	7.2 – 7.4

Section A7.5.1.1_02 Inhibition to microbial activity (terrestrial) Carbon Transformation Test

Annex Point IIA7.4

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (2009) [redacted] Soil microorganisms – Carbon Transformation Test. [redacted] 2009 (Unpublished), BPD ID A7.5.1.1_02
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s.] for first entry to Annex I authorisation
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD 217
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Glyoxal [redacted] (CAS No. 107-22-2)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
1.1.1	Purity	[redacted]
3.1.3	Composition of Product	Test substance in aqueous solution
3.1.4	Further relevant properties	The test material described as colourless clear, homogenous liquid, and miscible with water at ca. 20 °C. The stability under storage conditions (room temperature, under nitrogen) over the exposure period was guaranteed by the manufacturer.
3.1.5	Method of analysis	Not carried out
3.2	**Reference substance**	No reference substance was tested
3.2.1	Method of analysis for reference substance	Not applicable
3.3	**Testing procedure**	

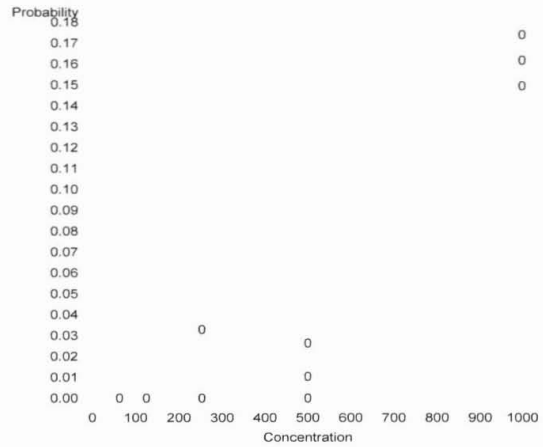
3.3.1	Soil sample / inoculum / test organism	See table A7_5_1_1-1
3.3.2	Test system	See table A7_5_1_1-3
3.3.3	Application of TS	See table A7_5_1_1-4
3.3.4	Test conditions	See table A7_5_1_1-5
3.3.5	Test parameter	Inhibition of microbial carbon transformation
3.3.6	Analytical parameter	The respiration rates induced by glucose were measured hourly up to 12 hours in each test sample. The degradation of glucose in the soil samples was determined by absorption of the CO_2 produced by the glucose; the absorption of CO_2 induced a negative pressure in the test pots, which was detected with the OxiTop pressure heads. The calculation of glucose induced soil respiration (BA) was based on following formula: $BA = M_{O2}/R\mathbf{x}T \quad \mathbf{x}\ V_{fr}/m_{Bt}\ \mathbf{x}\ I\ \Delta P\ I$ BA = glucose-induced soil respiration (mg O_2/kg DM soil) M_{O2} = molecular weight of O_2 (31998.8 mg/mol) R = gas constant (8.314 hPa/mol/K) T = test temperature (K) V_{fr} = free gas volume in the test assay (L) m_{Bt} = mass of dry substance soil (kg) I ΔP I = absolute value of the pressure alternation (hPA) The calculated respiration rate was expressed as mg O_2 released/kg DM soil/h). The mean respiration rate of 3 single samples of test mixture per test concentration was determined and was compared with the control value; the percent of deviation from control was calculated.
3.3.7	Duration of the test	28 days
3.3.8	Sampling	Samples were taken on day 0, 7 and 28 of incubation and were examined for glucose induced respiration rates. For each test concentration and sampling time point, 3 samples were considered (each about 118.9 g)
3.3.9	Monitoring of TS concentration	Not performed as not of importance for the present type of study and not required by the guideline
3.3.10	Controls	Controls without test material were added to the test series.
3.3.11	Statistics	To estimate the EC_{10} and the EC_{50} and its confident limits (95%), a dose-response curve was fitted using the probit model to the inhibition values.

4 RESULTS

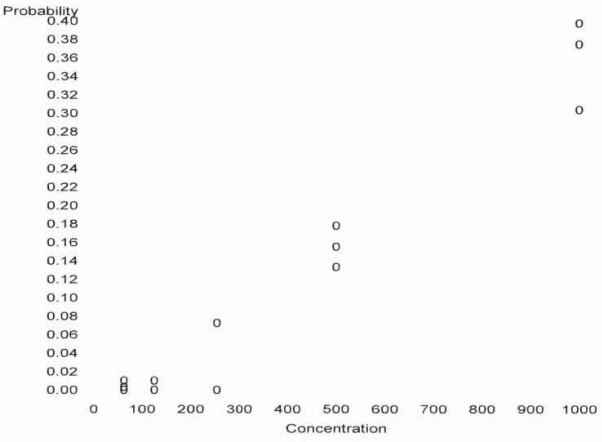
4.1	**Range finding test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Effect data	Not applicable
4.2	**Results test**	

substance

4.2.1	Initial concentrations of test substance	0, 62.5, 125, 250, 500 and 1000 mg test material/kg DM soil
4.2.2	Actual concentrations of test substance	62.7, 125, 253, 500 and 1000 mg test material/kg DM soil
4.2.3	Concentration/ response curve	See graphs below
4.2.4	At test initiation	Figure 1: Graphical illustration of the probit analysis of the test substance at the start of exposure period

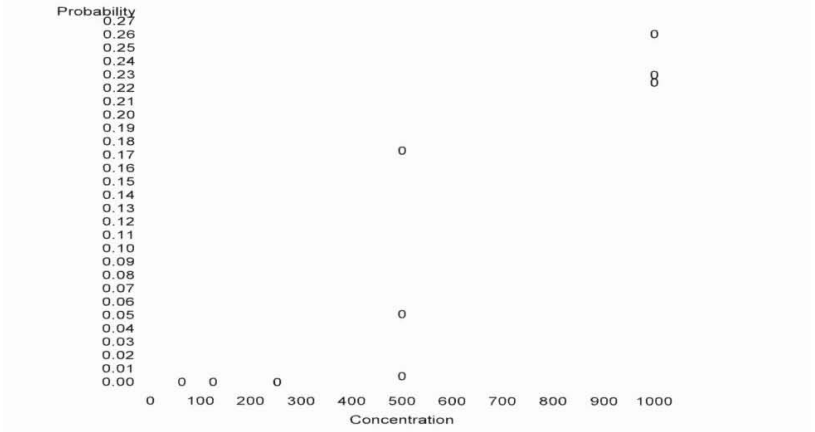


4.2.5	At sampling time point 7 days	Figure 2: Graphical illustration of the probit analysis of the test substance after an exposure period of 7 days



4.2.6 At sampling time point 28 days

Figure 3: Graphical illustration of the probit analysis of the test substance after an exposure period of 28 days



4.2.7 Effect data

Comparative means (3 samples per test concentration) of the glucose induced soil respiration after 12 hours (mg O_2/kg DM soil/h); samples taken after day 0, 7 and 28:

Nominal concentration (mg test material/kg DM soil)	Mean values of glucose induced soil respiration (mg O_2/kg DM soil)		
	At day 0	At day 7	At day 28
0 (control)	88.7	98.8	73.5
62.7	97.4	98.8	84.1
125	95.3	101.4	83.9
253	93.3	97.5	80.0
500	88.2	83.4	67.9
1000	74.3	63.3	56.0

Inhibition (mean values, %) of the glucose induced soil respiration:

Nominal concentration (mg test material/kg DM soil)	Inhibition of glucose induced soil respiration (mean values, %)		
	At day 0	At day 7	At day 28
62.7	0	0.4	0
125	0	0.5	0
253	1.1	2.4	0
500	1.2	15.5	7.6
1000	16.2	35.9	23.8

Summary of the effect concentrations of the test material:

Time point	EC_{10} (mg test material/kg DM soil)	EC_{50} (mg test material/kg DM soil)
Day 0	850 (CL: 798-903)	>1000
Day 7	400 (CL: 345-473)	>1000
Day 28	600 (CL: 485-731)	>1000

CL: confidence Limits (p=0.95)

Summary of the effect concentrations of the test material with regard to the active substance (a.s.):

Time point	EC_{10} (mg a.s./kg DM soil)	EC_{50} (mg a.s./kg DM soil)
Day 0	340 (CL: 319-361)	>400
Day 7	160 (CL: 138-189)	>400

Day 28	240 (CL: 194-292)	>400

CL: confidence Limits (p=0.95)

4.2.8	Other observed effects	None
4.3	**Results of controls**	See 4.2.7
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

5.1 Materials and methods

The aim of the present study was to investigate the long term potential effects of [redacted] on aerobic soil microorganisms by means of the Carbon Transformation Test.
Test material: [redacted]
The test was conducted according to OECD 217 under GLP conditions.
About 70 kg of soil [redacted] the weather conditions were cloudy and about 21°C. [redacted] The soil was defined as silty sand according to German DIN. The soil sample was stored in a closed plastic sack at 4 ± 2°C in the dark until test initiation.
For testing, the soil sample was dried for two days at room temperature. The sample was then sieved < 10 mm and < 2 mm.
For preparation of the test mixture, suitable quantities of soil were placed in mixers. A stock solution of the test substance was prepared. For each test concentration a defined amount of the stock solution was mixed with water and the specific mixtures were added to the test substrate. The controls were prepared in the same way without the test substance. The water content of each mixture was 45 ± 5% of the WHC_{max}. The pH values were determined in two samples per test concentration at test initiation.
The test mixtures were incubated up to 28 days in the dark, in test pots closed with a perforated aluminium cap, at a temperature of 20.5 – 21.5°C; the water content was controlled by weighing of the test samples and water loss was regulated by addition of demineralised water.
The following concentrations of the test material in soil were tested: 0, 62.5, 125, 250, 500 and 1000 mg test material/kg dry matter (DM) soil.
Sampling time points were day 0, day 7 and day 28. At each time point, 3 samples of 118.9 g test mixture per test concentration were taken and were supplemented with glucose (about 250 mg/sample) and 1 g quartz sand.
Glucose induced respiration rates were measured in the dark for up to 12 hours at hourly intervals in respiration measurement units (OxiTop) by

		measuring the negative pressure resulting from absorbed CO_2. CO_2 is produced by the microorganisms when glucose is degraded. The calculated respiration rate was expressed as mg O_2 released/kg DM soil/h. The mean respiration rate of 3 single samples of test mixture per test concentration was determined and was compared with the control value; the percent of deviation from the control was calculated. To the inhibition values a dose response curve was fitted using the probit model. This curve was used for the estimation of EC_{10} and EC_{50} values and its confidence limits (p=0.95).	
5.2	**Results and discussion**		
5.2.1	NOEC		
5.2.2	EC_{10}	After 0 days: 850 mg test material/kg DM soil, corresponding to 340 mg a.s./kg DM soil After 7 days: 400 mg test material/kg DM soil, corresponding to 160 mg a.s./kg DM soil After 28 days: 600 mg test material/kg DM soil, corresponding to 240 mg a.s./kg DM soil	
5.2.3	EC_{50}	After 0 days: >1000 mg test material/kg DM soil, corresponding to >400 mg a.s./kg DM soil After 7 days: >1000 mg test material/kg DM soil, corresponding to >400 mg a.s./kg DM soil After 28 days: >1000 mg test material/kg DM soil, corresponding to >400 mg a.s./kg DM soil	
5.3	**Conclusion**	The carbon transformation test with [redacted] resulted in an EC_{10} after 28 days of 240 mg a.s./kg DM soil; the EC50 after 28 days was >400 mg a.s./kg DM soil. The deviation of glucose induced respiration in the blank controls was < 15% at the end of the exposure (2 values from 3 replicates, with one outlier), confirming the validity of the test.	X
5.3.1	Reliability	[redacted]	
5.3.2	Deficiencies	[redacted]	

EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date	03/2018
Materials and Methods	Agree with the applicant version.
Results and discussion	
Conclusion	On days 0 and 28, one value of each control group was far away from the other two values of the same group. Indeed, the variation of glucose induced respiration in the blank controls is equal to 45% at day 0 and 37% at day 28. In the report, these values are considered as outliers and then are excluded from the calculation. The control values influence strongly the test results, because the % inhibition was calculated on the basis of the control mean. As stated in the EU OECD guidance, so large variations in the controls can lead to false results. Therefore, the variation between replicate control samples should be less than ± 15 %. Consequently, the

	validity criteria of the test is not fulfilled and the study should be rejected.
Reliability	[redacted]
Acceptability	[redacted]
	[redacted]
Remarks	

	Comments from ...
Date	*Give date of 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*
Remarks	

Table A7_5_1_1-1: Microbial sample / Inoculum

Criteria	Details
Nature	Silty sand, defined as soil type 5 M ([redacted])
Sampling site:	The soil sample was prepared conformely to the specifications of the guideline.
Geographical reference on the sampling site	[redacted]
Data on the history of the site	[redacted]
Use pattern	Not specified
Depth of sampling [cm]	About 20 cm
Sand / Silt / Clay content [% dry weight]	Soil defined as silty sand according to German DIN Percentage of sand (i.e. particles > 0.063-2.0 mm) 55.2 +/- 2.1 %
pH	~~7.1~~7.2 +- 0.1
Organic carbon content [% dry weight]	1.29 +/- 0.20
Maximal water holding capacity (WHC_{max}; g/100 g)	42.1 +/- 4.0
Nitrogen content [% dry weight]	Not specified
Cation exchange capacity [mmol/kg]	15 +/-3
Initial microbial biomass	173.3 mg/kg dry soil matter
Water content of the delivered soil at test initiation (WC; g/100 g dry matter)	~~10.2~~ 8.8
Reference of methods	Determination of the initial microbial biomass: The initial microbiological biomass of the soil was determined by means of the OxiTop according to ISO 14240-1 and [redacted]: Atmungsaktivität AT4“. Determination of the initial water content: According to ISO 11465
Collection / storage of samples	The soil sample was stored in a closed plastic sack at 4 +/- 2 °C in the dark until test initiation
Preparation of inoculum for exposure	~~Portions of about 326 g of soil and about 1.5 g Luzerne meal were mixed. Soil was adjusted to 45 +/+ WHC~~ Portion of about 1632 g of the soil were given to a mixer
Pretreatment	None

Table A7_5_1_1-2: Test organism

Criteria	Details
Species	Not relevant, see table A7_5_1_1-1
Strain	“
Source	“
Sampling site	“
Laboratory culture	“
Method of cultivation	“
Preparation of inoculum for exposure	“
Pretreatment	“
Initial cell concentration	“

Table A7_5_1_1-3: Test system

Criteria	Details
Culturing apparatus	Test pots closed with a perforated aluminium cap
Number of vessels / concentration	3 samples per test concentration
Aeration device	Aeration was assured by the perforations in the caps.
Measuring equipment	Glucose induced respiration rates were measured for 12 consecutive hours in respiration measurement units OxiTop; in fact, the OxiTop pressure heads measure the negative pressure resulting from absorbed CO_2 produced by glucose.
Test performed in closed vessels	See above

Table A7_5_1_1-4: Application of test substance

Criteria	Details
Application procedure	A suitable quantity of soil was placed in a mixer. A defined amount of test substance was added without carrier material, and the mixture was blended. Water was added to 45 +/- 5% of the WHC_{max} and the mixture was mixed again.
Carrier	None
Concentration of liquid carrier [% v/v]	Not relevant as no carrier was used.
Liquid carrier control	Not relevant as no carrier was used.
Other procedures	None

Table A7_5_1_1-5: Test conditions

Criteria	Details
Organic substrate	For the carbon transformation test, the soil samples were amended with ~~400~~ 250 mg glucose per 118 g of test mixture; 1 g quarz sand was used as carrier..
Incubation temperature	20 +/- 2 °C
Soil moisture	During the test: 45% of the WHC_{max}
Method of soil incubation	The test samples were incubated up to 28 days in the dark; the water content was controlled by weighing of the test samples and water loss was regulated by addition of demineralised water.
Aeration	Aeration was assured by the perforations in the caps.
pH in test mixtures at test initiation	7.3 – 7.4

Section A7.5.1.2_01 **Annex Point IIIA XIII 3.2**		**Earthworm, acute toxicity test**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] (2009). [redacted] Determination of the acute effect of chemicals on the mortality of earthworms. [redacted] 2009, BPD ID A7.5.1.2_01.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 207, Commission Regulation (EC) No 440/2008	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Glyoxal [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	[redacted]	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Aqueous solution	
3.1.5	Further relevant properties	Miscible in water, homogenous	
3.1.6	Method of analysis	Not carried out	
3.2	**Reference substance**	Yes, 2-Chloroacetamide	
3.2.1	Method of analysis for reference substance	Not carried out	
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	See table A7_5_1_2-1	X

<table>
<tr><td colspan="2">Section A7.5.1.2_01
Annex Point IIIA XIII 3.2</td><td>Earthworm, acute toxicity test</td><td></td></tr>
<tr><td colspan="2"></td><td></td><td></td></tr>
<tr><td>3.3.2</td><td>Application of the test substance</td><td>The test material was mixed to the test substrate as a stock solution prepared with demineralised water, at a ratio of 1000 ml stock solution to 3000 g dry test substrate (no correction for purity).</td><td></td></tr>
<tr><td>3.3.3</td><td>Test organisms</td><td>See table A7_5_1_2-2</td><td>X</td></tr>
<tr><td>3.3.4</td><td>Test system</td><td>See table A7_5_1_2-3</td><td>X</td></tr>
<tr><td>3.3.5</td><td>Test conditions</td><td>See table A7_5_1_2-4</td><td>X</td></tr>
<tr><td>3.3.6</td><td>Test duration</td><td>14 days</td><td></td></tr>
<tr><td>3.3.7</td><td>Test parameter</td><td>Mortality, body weight</td><td></td></tr>
<tr><td>3.3.8</td><td>Examination</td><td>Examination was performed after 7 and 14 days</td><td></td></tr>
<tr><td>3.3.9</td><td>Monitoring of test substance concentration</td><td>No</td><td></td></tr>
<tr><td>3.3.10</td><td>Statistics</td><td>Probit analysis according to Finney [1] was performed using the SAS-System</td><td></td></tr>
<tr><td colspan="2"></td><td>4 RESULTS</td><td></td></tr>
<tr><td>4.1</td><td>Filter paper test</td><td>Not performed</td><td></td></tr>
<tr><td>4.1.1</td><td>Concentration</td><td>Not applicable</td><td></td></tr>
<tr><td>4.1.2</td><td>Number/ percentage of animals showing adverse effects</td><td>Not applicable</td><td></td></tr>
<tr><td>4.1.3</td><td>Nature of adverse effects</td><td>Not applicable</td><td></td></tr>
<tr><td>4.2</td><td>Soil test</td><td></td><td></td></tr>
<tr><td>4.2.1</td><td>Initial concentrations of test substance</td><td>0, 63, 126, 250, 502 and 1003 mg test material/kg dry weight artificial soil</td><td></td></tr>
<tr><td>4.2.2</td><td>Effect data (Mortality)</td><td>See table A7_5_1_2-5 and table A7_5_1_2-6</td><td></td></tr>
<tr><td>4.2.3</td><td>Concentration / effect curve</td><td>Not applicable</td><td></td></tr>
<tr><td>4.2.4</td><td>Other effects</td><td><u>Earthworm body weight</u>:
<table>
<tr><td>Test material concentration (mg/kg)</td><td>Total weight of the added worms [±0.01g]</td><td>Total weight of the added worms [±0.01g]</td></tr>
<tr><td>Control</td><td>13.51 (Ni=40*)</td><td>13.27 (Ne=40**)</td></tr>
</table></td><td></td></tr>
</table>

Section A7.5.1.2_01 Annex Point IIIA XIII 3.2	Earthworm, acute toxicity test	

63	12.56 (Ni=40)	11.13 (Ne=40)
126	13.67 (Ni=40)	12.35 (Ne=40)
250	13.03 (Ni=40)	13.02 (Ne=40)
502	13.49 (Ni=40)	12.25 (Ne=39)
1003	13.50 (Ni=40)	11.46 (Ne=40)

*, Ni = Number of worms at test initiation.

**, Ne = Number of worms at test end, i.e. after 14 days of exposure.

Inhibition of the biomass (not stated in the report; calculated by the author of this summary):

Inhibition (%) = 100 – ((day 14/day 0)*100)

Test material conc. (mg/kg)	Total weight of the added worms at test start [±0.01g]	Total weight of the added worms at test end [±0.01g]	Inhibition of biomass (%)
Control	13.51 (Ni=40*)	13.27 (Ne=40**)	1.8
62.5	12.56 (Ni=40)	11.13 (Ne=40)	11.7
125	13.67 (Ni=40)	12.35 (Ne=40)	9.7
250	13.03 (Ni=40)	13.02 (Ne=40)	0.1
500	13.49 (Ni=40)	12.25 (Ne=39)	9.2
1000	13.50 (Ni=40)	11.46 (Ne=40)	15.1
Mean*	13.25	12.03	
S.D.*	0.56	0.75	
Max val.*	13.67	13.02	
Min. val.*	12.56	11.13	

* Mean, standard deviation, maximal and minimal values refer to the treated samples (i.e. without the control group)

The values of the table above indicate that the exposure of the earthworms to the test material ([redacted]) had no significant impact on the earthworm biomass at the tested concentrations.

No further behavioural or morphological effects were reported.

4.3	**Results of controls**	
4.3.1	Mortality	No mortality observed

Section A7.5.1.2_01 Annex Point IIIA XIII 3.2		Earthworm, acute toxicity test	
4.3.2	Number/ percentage of earthworms showing adverse effects	No adverse effects observed	
4.3.3	Nature of adverse effects	Not applicable	
4.4	**Test with reference substance**		
4.4.1	Concentrations	0, 6.3, 12.7, 25.0, 50.3, 100 mg/ kg	
4.4.2	Results	LC50 (14 days) = 22.0 mg/kg (nominal)	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The aim of the present study was to investigate the toxicity of Glyoxal [redacted] to the earthworm *Eisenia foetida*. Test material: [redacted] Guideline: OECD 207, GLP Clitellated adult earthworms (*Eisenia fetida*; age ≥ 2 months; individual body weight > 300 mg, < 600 mg) were exposed to Glyoxal [redacted] over a 14-days period. The test concentrations were 0, 62.5, 125, 250, 500 and 1000 mg/kg dry weight artificial soil. Four replicates/concentration with 10 worms each were set up per concentration. The animals were checked after 7 and after 14 days for mortality. Further observed sublethal parameters were behaviour and body weight. The physico-chemical parameters over the testing period were as follows:	
5.2	**Results and discussion**	Mortality:	

Temperature [°C]	20.5 to 20.8 °C
pH	5.8 (dry test substrate)
Moisture content in the substrate	33.0-33.5 g /100 g dry weight

Test material concentration (nominal) [mg/kg artificial soil]	Mortality (day 14)	
0 (control)	0/40*	0%
62.5	0/40	0%
125	0/40	0%
250	0/40	0%
500	1/40	4%
1000	0/40	0%

Body weight and inhibition of biomass after 14 days:

<table>
<tr><td>Section A7.5.1.2_01
Annex Point IIIA XIII 3.2</td><td>Earthworm, acute toxicity test</td><td></td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td></td><td>
<table>
<tr><th>Test material concentration (nominal) [mg/kg artificial soil]</th><th>Total weight of the added worms [±0.01g]</th><th>Total weight of the added worms [±0.01g]</th><th>Inhibition (%)</th></tr>
<tr><td>Control</td><td>13.51 (Ni=40*)</td><td>13.27 (Ne=40**)</td><td>1.8</td></tr>
<tr><td>62.5</td><td>12.56 (Ni=40)</td><td>11.13 (Ne=40)</td><td>11.7</td></tr>
<tr><td>125</td><td>13.67 (Ni=40)</td><td>12.35 (Ne=40)</td><td>9.7</td></tr>
<tr><td>250</td><td>13.03 (Ni=40)</td><td>12.02 (Ne=40)</td><td>0.1</td></tr>
<tr><td>500</td><td>13.49 (Ni=40)</td><td>12.25 (Ne=39)</td><td>9.2</td></tr>
<tr><td>1000</td><td>13.50 (Ni=40)</td><td>11.46 (Ne=40)</td><td>15.1</td></tr>
</table>
* Ni = Number of worms at test initiation.
** Ne = Number of worms at test end
</td><td>X</td></tr>
<tr><td>5.2.1 LC_0</td><td>1000 mg test material/kg soil dry weight (dw) (nominal), similar to 397 mg a.s./kg dw (nominal)</td><td></td></tr>
<tr><td>5.2.2 LC_{50}</td><td>> 1000 mg test material/kg soil dw (nominal), similar to
> 397 mg a.s./kg dw (nominal)</td><td></td></tr>
<tr><td>5.2.3 LC_{100}</td><td>> 1000 mg test material/kg soil dw (nominal), similar to
> 397 mg a.s./kg dw (nominal)</td><td></td></tr>
<tr><td>5.3 Conclusion</td><td>The exposure of earthworms to test substrate containing the test material ([redacted] had no significant impact on the earthworm biomass at the tested concentrations.
No further behavioural or morphological effects were seen. No test substance-related mortality was observed. The single mortality observed at 500 mg/kg soil is not considered to be test substance-related. Mortality within the control group was < 10% and therefore the validity criterion for earthworm acute toxicity tests according to OECD 207 is fulfilled. Based on nominal concentrations, the LC50 was > 397 mg Glyoxal/kg soil dry weight.</td><td>X</td></tr>
<tr><td>5.3.1 Other Conclusions</td><td>None</td><td></td></tr>
<tr><td>5.3.2 Reliability</td><td>[redacted]</td><td></td></tr>
<tr><td>5.3.3 Deficiencies</td><td>[redacted]</td><td></td></tr>
<tr><td></td><td></td><td></td></tr>
</table>

Section A7.5.1.2_01 **Annex Point IIIA XIII 3.2**	**Earthworm, acute toxicity test**	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	03/2018	
Materials and Methods	Tables A7_5_1_2-1, A7_5_1_2-2, A7_5_1_2-3, A7_5_1_2-4 are missing despite our request. These tables were added here below by the eCA.	
Results and discussion	4.2.2: table A7_5_1_2-5 and table A7-5-1-2-6 are missing. For results of mortality, see table in section 5.2.	
Conclusion	No statistical analysis are available to investigate whether effects on biomass are significant or not. Data on replicate are not available. Then, the sentence should be corrected as follow: "*The exposure of earthworms to test substrate containing the test material ([redacted]) ~~had no significant impact~~ showed some effects on the earthworm biomass at the tested concentrations (up to 15% inhibition at the highest tested concentration). However no statistical analysis are available to investigate whether effects on biomass are significant or not."* LC_{50} > 1000 mg test material/kg soil dw (nominal), equivalent to > 397 mg a.s./kg dw (nominal) Agreed with the applicant proposal	
Reliability	[redacted]	
Acceptability	[redacted]	
Remarks		
	Comments from ... *(specify)*	
Date	*Give date of 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*	
Remarks		

Table A 7.5.1.2-2: Test organisms

Criteria	Details
Species/strain	*Eisenia foetida* (Michaelsen).
Source of the initial stock	[redacted]
Culturing techniques	The worm were bred in horse manure
Age/weight	> 2 months (guaranteed by the supplier)
Pre-treatment	One day to test substrate and test conditions.

Table A 7.5.1.2-3: Test system

Criteria	Details
Artificial soil test substrate	Quartz sand: 70% Kaolin clay: 20% Sphagnum peat: 10% Calcium carbonate to pH adjustment
Test mixture	concentrations applied in the study were: 0 (control), 63, 126, 250, 502 and 1003 mg/kg dry weight artificial soil. Test material was mixed to the test substance as a stock solution prepared with demineralised water, at a ratio of 1000 ml stock solution to 3000 g dry test substrate
Size, volume and material of test container	1 L glass container covered with a lid
Amount of artificial soil (kg)/ container	Approx. 750 g (wet substrate)
Number of replicates/concentration	4 test vessels per concentration
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Continuous light (400 – 800 [Lux])
Test performed in closed vessels due to significant volatility of test substrate	Volatilisation not likely under test conditions, sealed vessels not necessary

Table A 7.5.1.2-4: Test conditions

Criteria	Details
Test temperature	20°C ± 2%
Moisture content	35 % of dry matter artificial soil
pH	6.0 ± 0.5
Adjustment of pH	No information
Light intensity / photoperiod	Continuous light (400 – 800 [Lux])

Section 7.5.1.3_01 Terrestrial plant toxicity

Annex Point IIIA XIII 3.4

Official use only

1 REFERENCE

1.1 Reference [redacted] (2009) [redacted] Determination of the effect of

Section 7.5.1.3_01 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

		chemicals on the emergence and growth of higher plants. [redacted] 2009 (Unpublished), BPD ID A7.5.1.3_01
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD-Guideline 208 (2006)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 METHOD
3.1	**Test material**	Glyoxal [redacted] (CAS no 107-22-2), [redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	[redacted] aqueous solution
3.1.5	Further relevant properties	Colourless, homogeneous liquid stored at room temperature under nitrogen
3.1.6	Method of analysis	The verification of all test concentrations was performed by analysis of the highest test solution concentration used for the test with documentation on the dilution and use of the calibrated application equipment. The highest concentration of the test material was measured using a TOC-analyzer equipped with an auto sampler.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant
3.3	**Reference substance**	Not relevant
3.3.1	Method of analysis for reference substance	Not relevant
3.4	**Testing procedure**	
3.4.1	Dilution water	see table A7_5_1_3-2

Section 7.5.1.3_01 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

3.4.2	Test plants	*Avena sativa, Brassica napus*, *Vicia sativa; for details* see table A7_5_1_3-3
3.4.3	Test system	see table A7_5_1_3-4
3.4.4	Test conditions	See table A7_5_1_3-5
3.4.5	Test duration	21 days
3.4.6	Test parameter	Emergence, shoot length, fresh and dry matter
3.4.7	Sampling	After 21 days of exposure
3.4.8	Method of analysis of the plant material	After 21 days of exposure the shoot lengths and the wet matters of the plants were determined. For the determination of the dry matter the plants were dried for 4 days at 60°C until their weight was constant.
3.4.9	Quality control	Yes
3.4.10	Statistics	The calculation of the NOEC/LOEC was carried out using the Dunnett´s test except for the emergence rate which has been calculated using the Wilcoxon test. EC_x values were determined via the program BMDS (Benchmark Dose Software released by EPA), when significant effects occurred.

4 RESULTS

4.1 Results test substance

4.1.1	Applied initial concentration	0, 62.5, 125, 250, 500, 1000 mg test material/kg dry matter (DM) soil
4.1.2	Phytotoxicity rating	The sensitivity of the used plant species to the applied test material concentrations decreased from *Brassica napus*, *Vicia sativa* to *Avena sativa*.
4.1.3	Shoot length	Shoot length of *Avena sativa* after 21 days of exposure.

Test material concentration [mg/kg DM soil]	Average shoot length [mm]	Maximum shoot length [mm]	Minimum shoot length [mm]
0	258.1	305	121
62.4	267.2	312	162
125	264.9	318	144
250	271.4	307	206
508	277.4	317	233
1001	282.0	316	255

No effects were observed.

Shoot length of *Brassica napus* after 21 days of exposure.

Test material concentration [mg/kg DM soil]	Average shoot length [mm]	Maximum shoot length [mm]	Minimum shoot length [mm]
0	87.5	102	69
62.4	84.8	108	68
125	89.7	101	65
249	86.3	108	54
508	89.1	106	75
1000	77.1	93	65

Section 7.5.1.3_01 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

Decrease in shoot length of *B. napus* was observed at 1000 mg test material/kg soil.

Shoot length of *Vicia sativa* after 21 days of exposure.

Test material concentration [mg/kg DM soil]	Average shoot length [mm]	Maximum shoot length [mm]	Minimum shoot length [mm]
0	452.3	523	390
62.4	446.9	538	370
125	447.8	578	352
250	448.4	545	392
508	462.4	513	410
1001	434.7	495	378

No effects were observed.

4.1.4 Plant dry matters

Dry matter of *Avena sativa* after 21 days of exposure.

Test material concentration [mg/kg DM soil]	Average dry matter [g]	Maximum dry matter [g]	Minimum dry matter [g]
0	0.3210	0.3453	0.2936
62.4	0.3164	0.3605	0.2908
125	0.2834	0.3062	0.2566
250	0.3149	0.3734	0.2668
508	0.3238	0.3695	0.2484
1001	0.3273	0.3487	0.3034

No effects were observed.

Dry matter of *Brassica napus* after 21 days of exposure.

Test material concentration [mg/kg DM soil]	Average dry matter [g]	Maximum dry matter [g]	Minimum dry matter [g]
0	0.2012	0.2125	0.1877
62.4	0.2174	0.2645	0.1635
125	0.2098	0.2255	0.1976
249	0.2065	0.2335	0.1760
508	0.2244	0.2532	0.1786
1000	0.1530	0.1625	0.1235

Decrease in dry matter of *B. napus* was observed at 1001 mg test material/kg soil.

Dry matter of *Vicia sativa* after 21 days of exposure.

Test material concentration [mg/kg DM soil]	Average dry matter [g]	Maximum dry matter [g]	Minimum dry matter [g]
0	0.7167	0.7525	0.6966
62.4	0.6301	0.6410	0.6069
125	0.6125	0.7517	0.5180
250	0.6775	0.7109	0.6374
508	0.6799	0.7232	0.6425
1001	0.5040	0.5317	0.4696

Decrease in dry matter of *V. sativa* was observed at 62.4, 125 and 1001 mg test material/kg soil.

4.1.5 Plant fresh matters

Fresh matter of *Avena sativa* after 21 days of exposure.

Test material concentration [mg/kg DM soil]	Average fresh matter [g]	Maximum fresh matter [g]	Minimum fresh matter [g]
0	2.1876	2.3364	2.0159
62.4	2.1804	2.5205	1.9856
125	2.0484	2.2306	1.8935
250	2.2572	2.5554	2.0101

Section 7.5.1.3_01 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

508	2.3637	2.6203	1.9693
1001	2.3139	2.5302	2.1845

No effects were observed.

X

Dry matter of *Brassica napus* after 21 days of exposure.

Test material concentration [mg/kg DM soil]	Average fresh matter [g]	Maximum fresh matter [g]	Minimum fresh matter [g]
0	2.0391	2.1467	1.8986
62.4	2.1641	2.3531	2.0578
125	2.1041	2.2115	1.9912
249	2.2713	2.3350	2.2176
508	2.4643	2.6659	2.1966
1000	1.6188	1.7520	1.4233

Decrease in fresh matter of *B. napus* was observed at 1001 mg test material/kg soil.

Fresh matter of *Vicia sativa* after 21 days of exposure.

Test material concentration [mg/kg DM soil]	Average fresh matter [g]	Maximum fresh matter [g]	Minimum fresh matter [g]
0	3.7041	4.0228	3.5453
62.4	3.5392	3.5942	3.4923
125	3.4763	4.1727	3.1733
250	3.7706	3.9469	3.5626
508	3.8197	4.0452	3.5338
1001	3.1920	3.3485	2.9990

Decrease in fresh matter of *V. sativa* was observed at 1001 mg test material/kg soil.

4.1.6 Root — Not analysed.

4.1.7 Number of dead plants — None

4.1.8 Effect data — See table A7_5_1_3-6

X

No EC_x calculations were performed for endpoints without significant results.

Avena sativa:
No effects occurred.

Brassica napus:
For the shoot length of *B. napus* an
EC_{10} = 939 mg test material/kg DM soil with a lower confidence limit of 564 mg test material/kg DM soil was calculated.

For the dry matter of *B. napus* an
EC_{10} = 622 mg test material/kg DM soil with a lower confidence limit of 448 mg test material/kg DM soil was calculated.

For the fresh matter of *B. napus* an
EC_{10} = 664 mg test material/kg DM soil with a lower confidence limit of 557 mg test material/kg DM soil was calculated.

Section 7.5.1.3_01 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

Vicia sativa:
For the dry matter of *V. sativa* an
EC_{10} = 659 mg test material/kg DM soil with a lower confidence limit of 570 mg test material/kg DM soil was calculated.

For the dry matter of *V. sativa* an
EC_{25} = 1042 mg test material/kg DM soil with a lower confidence limit of 901 mg test material/kg DM soil was calculated.

For the fresh matter of *V. sativa* an
EC_{10} = 941 mg test material/kg DM soil with a lower confidence limit of 746 mg test material/kg DM soil was calculated.

NOEC [mg test material/kg DM soil]

Parameter	*Avena sativa*	*Brassica napus*	*Vicia sativa*
Emergence rate	≥1001	≥1000	≥1001°
Shoot length	≥1001	508**	≥1001
Dry matter	≥1001	508*	508**
Fresh matter	≥1001	508**	508*

LOEC [mg test material/kg DM soil]

Parameter	*Avena sativa*	*Brassica napus*	*Vicia sativa*
Emergence rate	>1001	>1000	>1001°
Shoot length	>1001	1000**	>1001
Dry matter	>1001	1000*	1001**
Fresh matter	>1001	1000**	1001*

° = p≤0.05 (Wilkoxon-test one-sided)
* = p≤0.05 (Dunnett's test one-sided)
** = p≤0.01 (Dunnett's test one-sided)

Although there were significant results for the emergence of *V. sativa* at a concentration of 508 mg/kg DM soil, the NOEC was set to 1001 mg/kg DM soil, because no monotone dose concentration relationship could be observed. X

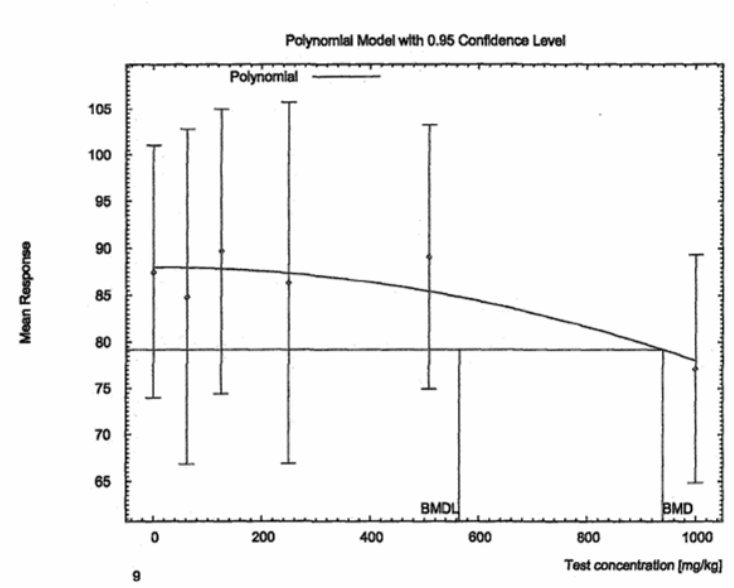
Although there were significant results for the dry matter of *V. sativa* at the concentrations of 62.4 and 125 mg/kg DM soil, the NOEC was set to 508 mg/kg DM soil, because no monotone dose concentration relationship could be observed. X

4.1.9 Concentration / response curve

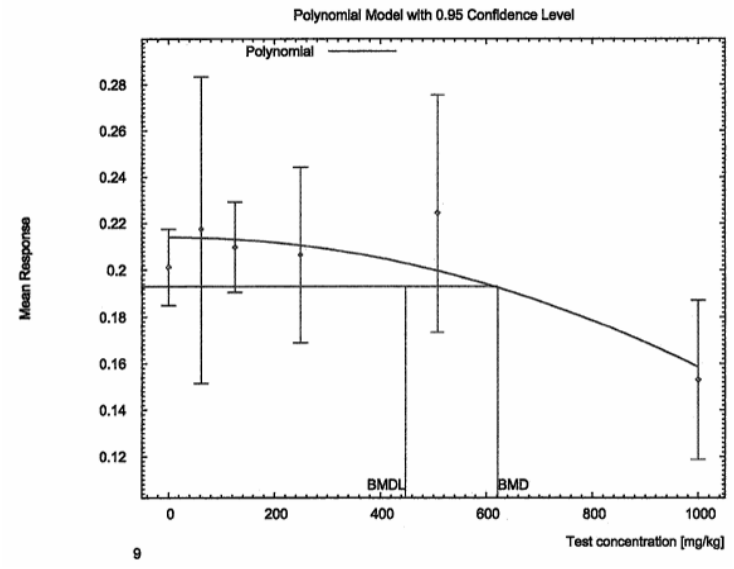
Avena sativa:
No concentration response effects were observed.

Brassica napus: Shoot length (EC_{10})

Section 7.5.1.3_01 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

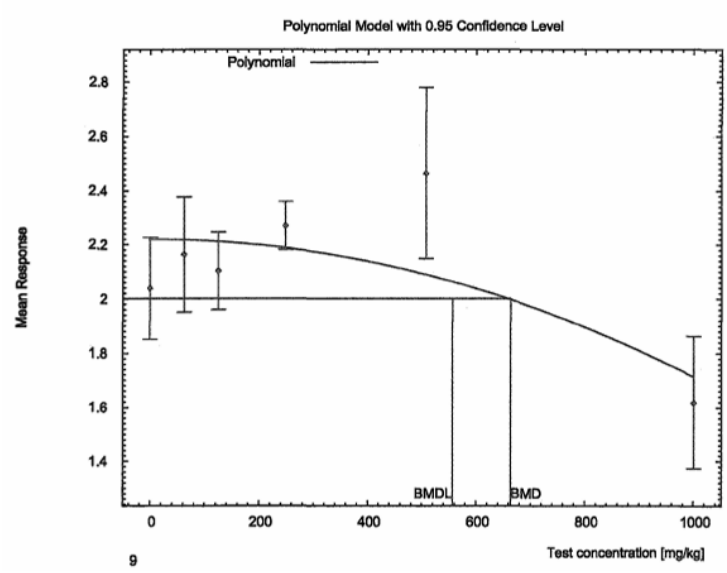


Brassica napus: Dry matter (EC_{10})

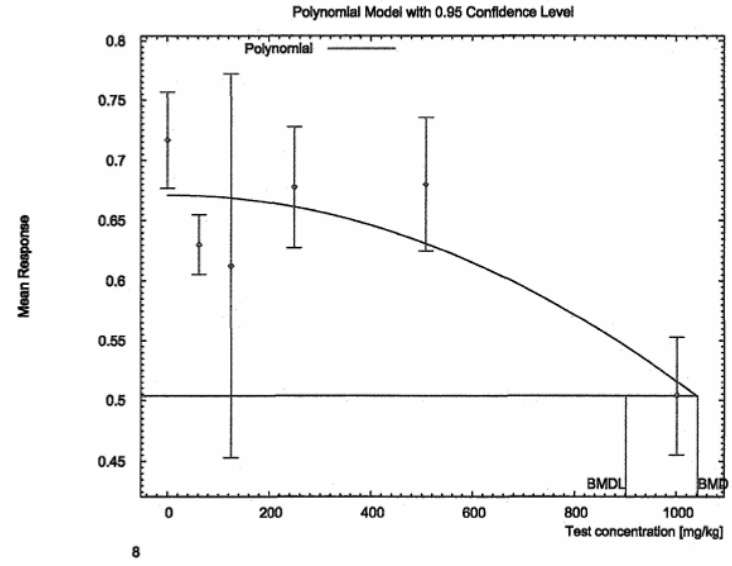


Brassica napus: Fresh matter (EC_{10})

Section 7.5.1.3_01 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

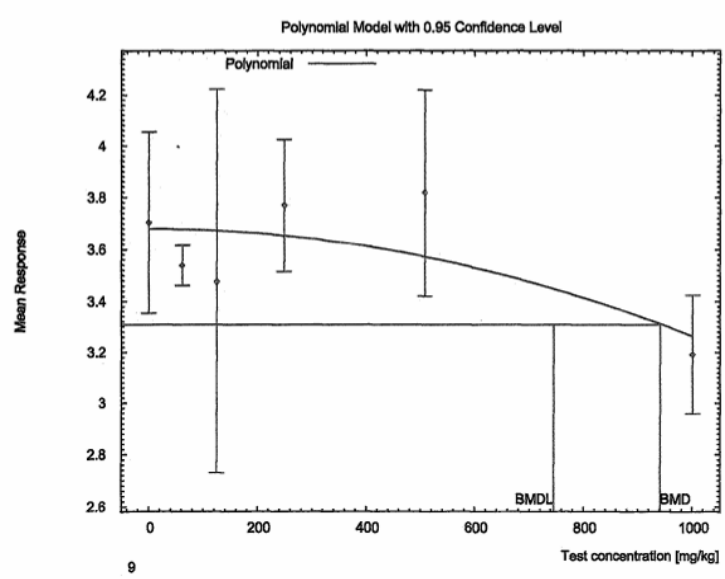


Vicia sativa: Dry matter (EC_{25})



Vicia sativa: Fresh matter (EC_{10})

Section 7.5.1.3_01 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4



4.1.10	Other effects	None observed.
4.2	**Results of controls**	
4.2.1	Number/ percentage of plants showing adverse effects	None of the control plants showed adverse effects.
4.2.2	Nature of adverse effects	-
4.3	**Test with reference substance**	A reference test assay was not set up.
4.3.1	Concentrations	-
4.3.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the study was the determination of effects from [redacted] on higher plants. This test was carried out according to OECD-Guideline 208 (2006) under GLP conditions.

Test material: [redacted]

The following plant seeds were tested: *Avena sativa*, *Brassica napus*, *Vicia sativa*. [redacted] According to the German DIN the soil type was silty sand (5M), [redacted] sieved to 2 mm and was stored until the beginning of the test in a plastic sack at a temperature of 4-6°C. Two days before use in the test, the soil was stored at room temperature.

The following concentrations were tested: 0, 62.5, 125, 250, 500 and

Section 7.5.1.3_01 **Terrestrial plant toxicity**
Annex Point IIIA XIII 3.4

1000 mg test material/kg DM soil.

Analytic: The highest concentration of the test material solution was verified using a TOC-analyzer ([redacted]) equipped with an auto sampler. All concentrations were verified by the documentation of the subsequent dilution and use of calibrated application equipment.

A stock solution of Glyoxal [redacted] was prepared. Required amounts of the stock solution were diluted with demineralised water and each solution was added to the soil portions. Portions of the soil/solution mixtures and untreated soil (control) were weighed into the pots and 10 seeds were sowed per pot. The soil was adjusted to a water content of 45% WHC_{max} with demineralised water. The pots were covered with plastic dishes, placed under controlled test conditions in an ecophyte (test chamber). Watering and randomisation of the pots was done daily.

Test conditions: Temperature: 20±10°C; Light intensity: 7000±500 lux; Light period: 16 hours light/8 hours dark; Air humidity: 70±25%.

The numbers of seeds that emerged were recorded daily.

After 4 days of exposure the number of plants per pot was thinned out to 5 plants. The test was finished and all plants were harvested 17 days after 50% of the control seeds had emerged.

The shoot length, fresh and dry matter of each plant was determined.

The calculation of the NOEC/LOEC for each parameter was carried out using the Dunnett´s test except for the emergence rate where the Wilcoxon test was used. EC_x values were determined via the program BMDS (Benchmark Dose Software released by EPA), when significant effects occurred.

5.2 Results and discussion

Analytic:

A recovery of 95% confirmed the correct initial weight for preparation of the test material stock solution and the calculated test concentrations by adding the aliquots to each soil portion.

Actual concentrations:

For *Avena sativa*: 0, 62.4, 125, 250, 508 and 1001 mg test material/kg DM soil.

For *Brassica napus*: 0, 62.4, 125, 249, 508 and 1000 mg test material/kg DM soil.

For *Vicia sativa*: 0, 62.4, 125, 249, 508 and 1001 mg test material/kg DM soil.

The NOEC/LOEC of *Avena sativa*:

Parameter	NOEC (mg test material/kg DM soil)	NOEC (mg a.s./kg DM soil)	LOEC (mg test material/kg DM soil)	LOEC (mg a.s./kg DM soil)
Emergence rate	≥1001	≥400.4	>1001	>400.4
Shoot length	≥1001	≥400.4	>1001	>400.4
Dry matter	≥1001	≥400.4	>1001	>400.4
Fresh matter	≥1001	≥400.4	>1001	>400.4

The NOEC/LOEC of *Brassica napus*:

Parameter	NOEC	NOEC	LOEC	LOEC

Section 7.5.1.3_01 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

	(mg test material/kg DM soil)	(mg a.s./kg DM soil)	(mg test material/kg DM soil)	(mg a.s./kg DM soil)
Emergence rate	≥1001	≥400.4	>1001	>400.4
Shoot length	508**	203.2	1000**	400
Dry matter	508*	203.2	1000*	400
Fresh matter	508**	203.2	1000**	400

The NOEC/LOEC of *Vicia sativa*:

Parameter	NOEC (mg test material/kg DM soil)	NOEC (mg a.s./kg DM soil)	LOEC (mg test material/kg DM soil)	LOEC (mg a.s./kg DM soil)
Emergence rate	≥1001°	≥400.4	>1001°	>400.4
Shoot length	≥1001	≥400.4	>1001	>400.4
Dry matter	508**	203.2	1001**	400.4
Fresh matter	508*	203.2	1001*	400.4

* Dunnett´s test, one-sided, p≤0.05

** Dunnett´s test, one-sided, p≤0.01

° Wilcoxon test, one sided, p≤0.05

5.2.1 EC_{10}

Brassica napus:

Shoot length: 939 mg test material/kg DM soil corresponding 375.6 a.s. mg/kg DM soil

Dry matter: 622 mg test material/kg DM soil corresponding 248.8 a.s. mg/kg DM soil

Fresh matter: 664 mg test material/kg DM soil corresponding 275.6 a.s. mg/kg DM soil

Vivia sativa:

Dry matter: 659 mg test material/kg DM soil corresponding 263.6 a.s. mg/kg DM soil

Fresh matter: 941 mg test material/kg DM soil corresponding 376.4 a.s. mg/kg DM soil

5.2.2 EC_{25}

Vicia sativa:

Dry matter: 1024 mg test material/kg DM soil corresponding 409.6 a.s. mg/kg DM soil

X

5.2.3 EC_{50}

Avena sativa:

Parameter	EC50 (mg test material/kg DM soil)	EC50 (mg a.s./kg DM soil)
Emergence rate	>1001	>400.4
Shoot length	>1001	>400.4
Dry matter	>1001	>400.4
Fresh matter	>1001	>400.4

Brassica napus:

Parameter	EC50	EC50

Section 7.5.1.3_01 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

	(mg test material/kg DM soil)	(mg a.s./kg DM soil)
Emergence rate	>1000	>400
Shoot length	>1000	>400
Dry matter	>1000	>400
Fresh matter	>1000	>400

Vicia sativa:

Parameter	EC50 (mg test material/kg DM soil)	EC50 (mg a.s./kg DM soil)
Emergence rate	>1001	>400.4
Shoot length	>1001	>400.4
Dry matter	>1001	>400.4
Fresh matter	>1001	>400.4

5.3 Conclusion

The test material [redacted] showed no toxic effects to the plant *Avena sativa*. All test parameters resulted in an EC_{50} of >400.4 mg a.s./kg DM soil. The NOEC was ≥400.4 mg a.s./kg DM soil and the LOEC was >400.4 mg a.s./kg DM soil.

For *Brassica napus*, all test parameters resulted in an EC_{50} of >400 mg a.s./kg DM soil. A NOEC of >203.2 mg a.s./kg DM soil was found for dry as well as for fresh weight of the plants. The LOEC for dry and fresh weight was 400.4 mg a.s./kg DM soil. X

For *Vicia sativa*, all test parameters resulted in an EC_{50} of >400.4 mg a.s./kg DM soil. A NOEC of >203.2 mg a.s./kg DM soil was found for dry as well as for fresh matter of the plants. The LOEC for the dry and fresh matter was 400.4 mg a.s./kg DM soil. X

The validity criteria for the seedling emergence and seedling growth test of terrestrial plants according to OECD Guideline 208 (2006) were fulfilled (see table A 7.5.1.3.-7). X

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Section 7.5.1.3_01 Terrestrial plant toxicity

Annex Point IIIA XIII 3.4

EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date	18/05/2018
Materials and Methods	5.1: after 4 days of exposure the number of plants per pot was thinned out to 5 plants without any explanations. Then, it is not possible to calculate the mean survival of emerged control seedlings necessary to validate the study.
Results and discussion	4.1.5: "Fresh matter of *Brassica napus* after 21 days of exposure". 4.1.8 and 5.2: significant results were detected in *B. napus* and *V. sativa*. In *B. napus*, shoot length, dry and fresh matter were significantly inhibited at 1000 mg test mat./kg DM soil. In *V. sativa*, significant inhibition of emergence (508 mg test mat./kg DM soil), dry matter (62.4 and 125 mg test mat./kg DM soil) and fresh matter (1001 mg/kg DM soil) were observed. It worth noting that there were no dose response relationships for all endpoints studied. Consequently, fit was bad and no reliable EC_x can be derived from this study. As significant results for the **emergence** of *V. sativa* at a concentration of 508 mg test mat./kg DM soil were observed, the NOEC has to be set to 249 mg test mat./kg DM soil instead of >1001 mg test mat./kg DM soil and the LOEC should be 508 mg test mat./kg DM soil. Also, as significant results for the **dry matter** of *V. sativa* at the concentrations of 62.4 and 125 mg test mat./kg DM soil, the NOEC is lower than 62.4 mg test mat./kg DM soil, and the LOEC should be 62.4 mg test mat./kg DM soil. 5.2.2 : "Dry matter EC_{25} = 1042 mg test mat/kg DM soil"

Section 7.5.1.3_01 Terrestrial plant toxicity

Annex Point IIIA XIII 3.4

Conclusion	5.3 : No dose response relationships were observed for any endpoints followed in this test for the three studied plant species and no reliable EC_x can be derived from this study. The NOEC for *A. sativa* is >1001 mg test mat./kg DM soil (or > 400.4 mg a.i./kg DM soil). The NOEC for *B. napus* is 508 mg test mat./kg DM soil (or 203.2 mg a.i./kg DM soil). For *V. sativa*, since significant effects were observed as soon as 62.4 mg test mat./kg DM soil for the dry matter, the LOEC = 62.4 mg test mat./kg DM soil (or 25 4 mg a.i./kg DM soil) and no NOEC can be determined. The mean survival of emerged control seedlings should be at least 90% for the duration of the study according to the EU guidance 208. This criterion can not be checked since the number of plants per pot was thinned out to 5 plants after 4 days of exposure. ~~Table A 7.5.1.3. 7 for validity criteria is not available~~. "The validity criteria for the seedling emergence and seedling growth test of terrestrial plants according to OECD Guideline 208 (2006) were fulfilled as follow: 1. Seedling emergence is at least 70 %in the controls. 2. There were no visible phytotoxic effects during the exposure in the controls. 3. The mean survival of emerged control seedlings is at least >90% at the end of exposure. *4. The environments conditions for all test pots were identical*"
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

	Comments from ... *(specify)*
Date	*Give date of 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*
Remarks	

Validity criteria1. Seedling emergence is at least 70 %in the controls.

2. There were no visible phytotoxic effects during the exposure in the controls.

3. The mean survival of emerged control seedlings is at least >90% at the end of exposure.

4. The environments conditions for all test pots were identical.

Table A7_5_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	-

Table A7_5_1_3-2: Dilution water

Criteria	Details
Source	-
Alkalinity / Salinity	-
Hardness	-
pH	-
Oxygen content	-
Conductance	-
Holding water different from dilution water	-

Table A7_5_1_3-3: Test plants

	Family	Species	Common name	Source (seed/plant)
Monocotyledonae	Poaceae	*Avena sativa L.*	Oat	-
Dicotyledonae	Brassicaceae	*Brassica napus L.*	Oilseed rape	-
Dicotyledonae	Fabaceae	*Vicia sativa L.*	Vetch	-

Table A7_5_1_3-4: Test system

Criteria	Details
Test type	Field study environmental chamber
Container type	PVC plant pots, upper internal diameter: 95 mm, 250 mL; covered with plastic dishes until the begin of emergence
Seed germination potential	Rate of emergence in the control was > 70%
Identification of the plant species	All pots were labelled with the study No. and sample code
Number of replicates	4/treatment and control
Numbers of plants per replicate per dose	10 seeds were sowed in each replicate. Pots were thinned to five uniform plants per pot
Date of planting	Not given
Plant density	Five plants per replicate
Date of test substance application	[redacted]
Heigh of plants at application	The test seeds were sowed in soil incorporated with the test item
Date of phytotoxicity rating or harvest	- emergence: daily, beginning with the emergence of the first seedlings after 2 days and ending after 19 days of exposure - growth (plant length, fresh weight, dry weight): after 21 d of exposure
Dates of analysis	See above

Table A7_5_1_3-5: Test conditions

Criteria	Details
Test type	Terrestrial plants, seed germination and growth test according to a OECD 208
Method of application	The active substance was mixed into the soil
Application levels	-
Dose rates	nominal: 0 (control), 62.5, 125, 250, 500, and 1000 mg test material/kg of dry weight soil nominal: 0 (control), 25, 50, 100, 200, and 400 mg a.i./kg of dry weight soil
Substrate characteristics	[redacted] pH 7.2±0.1; 1.29% organic carbon content; CEC = 15.3 mval/100 g
Watering of the plants	Water content was adjusted to 45 % WHC(max) with demineralized water
Temperature	Day/night temperatures: 20±10 °C
Thermoperiod	-
Light regime	16 h light : 8 h dark (7150 Lux (mean value))
Relative humidity	49.8 to 72.5%
Wind volatility	-
Observation periods and duration of test	Duration: 21 days
Pest control	No
Any other treatments and procedures	-

Table A7_5_1_3-6: Effects data

A – Total germinated plants of *Avena sativa*, *Brassica napus* and *vicia sativa*. Data per treatment series at the end of the exposure.

Plant	Test concentration [mg/kg soil] actual value	Mean value, no. of seedlings	Inhibition versus control [%]
Avena sativa	0	9.25	---
Avena sativa	62.4	9.25	0
Avena sativa	125	9.00	2.70
Avena sativa	250	9.00	2.70
Avena sativa	508	9.50	-2.70
Avena sativa	1001	8.75	5.41
Brassica napus	0	8.50	---
Brassica napus	62.4	7.25	14.71
Brassica napus	125	8.25	2.94
Brassica napus	249	8.25	2.94
Brassica napus	508	8.00	5.88
Brassica napus	1000	9.25	-8.82
Vicia sativa	0	9.25	---
Vicia sativa	62.4	8.25	10.81
Vicia sativa	125	9.25	0
Vicia sativa	249	9.00	2.70
Vicia sativa	508	7.00	24.32*
Vicia sativa	1001	9.25	0

Statistical significant (Wilcoxon-test (one-sided) : *p ≤0.05

B - Fresh matter of *Avena sativa, Brassica napus* and *vicia sativa*. Data per treatment series at the end of the exposure.

Plant	Test concentration [mg/kg soil] actual value	Fresh matter mean value [g]	Inhibition versus control [%]
Avena sativa	0	2.1876	---
Avena sativa	62.4	2.1804	0.33
Avena sativa	125	2.0484	6.36
Avena sativa	250	2.2572	-3.18
Avena sativa	508	2.3637	-8.05
Avena sativa	1001	2.3139	-5.78
Brassica napus	0	2.0391	---
Brassica napus	62.4	2.1641	-6.13
Brassica napus	125	2.1041	-3.19
Brassica napus	249	2.2713	-11.39
Brassica napus	508	2.4643	-20.86
Brassica napus	1000	1.6188	20.61**
Vicia sativa	0	3.7041	---
Vicia sativa	62.4	3.5392	4.45
Vicia sativa	125	3.4763	6.15
Vicia sativa	249	3.7706	-1.80
Vicia sativa	508	3.8197	-3.12
Vicia sativa	1001	3.1920	13.83*

Statistical significant (Dunnett's test (one-sided) : **p ≤ 0.01; *p ≤0.05

C - Dry matter of *Avena sativa*, *Brassica napus* and *vicia sativa*. Data per treatment series at the end of the exposure

Plant	Test concentration [mg/kg soil] actual value	Dry matter mean value [g]	Inhibition versus control [%]
Avena sativa	0	0.3210	---
Avena sativa	62.4	0.3164	1.16
Avena sativa	125	0.2834	11.47
Avena sativa	250	0.3149	1.62
Avena sativa	508	0.3238	-1.14
Avena sativa	1001	0.3273	-2.25
Brassica napus	0	0.2012	---
Brassica napus	62.4	0.2174	-8.08
Brassica napus	125	0.2098	-4.27
Brassica napus	249	0.2065	-2.66
Brassica napus	508	0.2244	-11.54
Brassica napus	1000	0.1530	23.95*
Vicia sativa	0	0.7167	---
Vicia sativa	62.4	0.6301	12.09*
Vicia sativa	125	0.6125	14.54*
Vicia sativa	249	0.6775	5.47
Vicia sativa	508	0.6799	5.13
Vicia sativa	1001	0.5040	29.68**

Section A7.5.2.1 TNsG, Ch. 3, Part A	Reproduction study with other soil non-target macro-organisms	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	X X
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
Date	**Evaluation by rapporteur member state** 25/05/2018	

Section A7.5.2.1 **TNsG, Ch. 3, Part A**	**Reproduction study with other soil non-target macro-organisms**
Evaluation of applicant's justification	[redacted]
Conclusion	[redacted]
Remarks	
	Comments from other member state *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.2.2 TNsG, Ch. 3, Part A	Long-term test with terrestrial plants	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	X X
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
Date	**Evaluation by rapporteur member state** 25/05/18	

Section A7.5.2.2 **TNsG, Ch. 3, Part A**	**Long-term test with terrestrial plants**
Evaluation of applicant's justification	[redacted]
Conclusion	[redacted]
Remarks	
	Comments from other member state *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 7.5.3.1.1 **Annex Point IIIA, XIII.1.1.**	**Acute oral toxicity on birds**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	From the intended uses of [redacted] no direct exposure to birds is expected. Therefore, unnecessary vertebrate testing can be avoided.	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	25/05/18	
Evaluation of applicant's justification	Agree	
Conclusion	Agree	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.5.3.1.2 Annex Point IIIA, XIII.1.2.	Effects on birds, Short-term toxicity	
	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 endpoint is not of concern for glyoxal as it is only required for product type 14.	X
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*25/05/18*	
Evaluation of applicant's justification	From the intended uses of glyoxal no direct exposure to birds is expected as they are strictly in-house uses. These studies are not required	
Conclusion	*Agree*	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.5.3.1.3 Annex Point IIIA, XIII.1.3.	Effects on birds, effects on reproduction	
	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 endpoint is not of concern for glyoxal as it is only required for product types 14, 16, 19 and 23.	X
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*25/05/18*	
Evaluation of applicant's justification	From the intended uses of glyoxal no direct exposure to birds is expected as they are strictly in-house uses These studies are not required.	
Conclusion	*Agree*	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.5.4.1 Annex Point IIIA, XIII.3.1.	Acute toxicity to honeybees and other beneficial arthropods, for example predators	
	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 endpoint is not of concern for glyoxal as it is only required for product type 19.	X
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*25/05/18*	
Evaluation of applicant's justification	A test on bees and/or other beneficial arthropods may be required for insecticides, acaricides and substances in products to control other arthropods which are used outdoors, i.e. for large scale-outdoor applications like fogging (e.g. product-type 18 - products against mosquitoes for human health reasons). Additionally, for systemic insecticides exposure to bees should also be quantified. Effects on arthropods do not usually have to be assessed for uses with indoor applications only. *Then this endpoint is not of concern for glyoxal.*	
Conclusion	*Agree*	
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.5.5 **Annex Point IIIA**	**Bioconcentration, terrestrial**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	Not relevant	

Section A7.5.5 Annex Point IIIA	Bioconcentration, terrestrial
	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by rapporteur member state
Date	*25/05/18*
Evaluation of applicant's justification	*Agree*
Conclusion	*Agree*
Remarks	
	Comments from other member state *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.5.1 Annex Point IIIA	Bioconcentration, further studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
Date	**Evaluation by rapporteur member state** *25/05/18*	

Section A7.5.5.1 **Annex Point IIIA**	**Bioconcentration, further studies**
Evaluation of applicant's justification	*Agree*
Conclusion	*Agree*
Remarks	
	Comments from other member state *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.6 Annex Point IIIA	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:	[redacted]	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	

Section A7.5.6 **Annex Point IIIA**	**Effects on other terrestrial non-target organisms**
Date	*25/05/18*
Evaluation of applicant's justification	*Agree*
Conclusion	*Agree*
Remarks	
	Comments from other member state *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.7.1.1 TNsG, Ch. 3, Part A	Acute oral toxicity to mammals	
	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:	[redacted]	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*25/05/18*	
Evaluation of applicant's justification	Agree	
Conclusion	*Agree*	

Section A7.5.7.1.1 **TNsG, Ch. 3, Part A**	**Acute oral toxicity to mammals**
Remarks	
	Comments from other member state *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.7.1.2 **TNsG, Ch. 3, Part A**	**Short term toxicity to mammals**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*25/05/18*	
Evaluation of applicant's justification	*Agree*	
Conclusion	*Agree*	
Remarks		

Section A7.5.7.1.2 **TNsG, Ch. 3, Part A**	**Short term toxicity to mammals**
	Comments from other member state *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.7.1.3 TNsG, Ch. 3, Part A	Effects on reproduction to mammals	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*25/05/18*	
Evaluation of applicant's justification	*Agree*	
Conclusion	*Agree*	
Remarks		

Section A7.5.7.1.3 **TNsG, Ch. 3, Part A**	**Effects on reproduction to mammals**
	Comments from other member state *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.6 **Annex Points IIA, VII.7.8.; IIIA, XII.4.; IIIA, XIII.5.**	**Summary of ecotoxicological effects and fate and behaviour in the environment**	
	Cross Reference to Doc IIA and Doc I	**Official use only**
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	For summary of ecotoxicological effects and fate and behaviour in the environment please see Doc IIA and Doc I (List of Endpoints).	
Undertaking of intended data submission [ ]	Not relevant	
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by rapporteur member state	
Date	*03/2018*	
Evaluation of applicant's justification	*agree*	
Conclusion		
Remarks		
	Comments from other member state *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A8 Measures necessary to protect man, animals and the environment

This information was previously submitted in support of Product Types 12. The data has therefore not been re-submitted but is cross referenced to Doc IIIA Section A8 of the Part D dossier.

Section A9 Classification and labelling

This information was previously submitted in support of Product Types 12. The data has therefore not been re-submitted but is cross referenced to Doc IIIA Section A8 of the Part D dossier.