Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Skeletonema costatum*

Official use only

1 REFERENCE

1.1	**Reference**	Scheerbaum, D. (2004): Preventol A4-S. Alga growth inhibition test with *Skeletonema costatum*, 96 h. DR. U. NOACK-LABORATORIUM FÜR ANGEWANDTE BIOLOGIE, unpublished Report-No. SSC91231, Project-No. 021031BK, date: 2004-08-19.
1.2	**Data protection**	Yes
1.2.1	Data owner	Lanxess Deutschland GmbH
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, DIN EN ISO-Guideline 10253 (1998) OECD-Guideline No. 201 (1994) EPA OPPTS 850.4400 Algal Toxicity, Tiers I and II (1996)
2.2	**GLP**	Yes
2.3	**Deviations**	Taking into account the validity criteria of the guidelines the following decisions were made: - pH adjustment to improve the stability of the a.i. in the test medium. - The temperature was reduced from 20 ± 2 °C to 18 ± 2 °C. - Double concentrated medium according to the recommendation of the CCAP Administration was used.

3 MATERIALS AND METHODS

3.1	**Test material**	Preventol A 4-S
3.1.1	Lot/Batch number	Batch number: [redacted]
3.1.2	Specification	As given in section 2 of dossier
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	Dichlofluanid is the active ingredient of Preventol A 4-S (content: [redacted] a.i.)
3.1.5	Further relevant properties	The test item is known to be hydrolytically not stable under exposure conditions: $t_{1/2}$ = 1.2 h at pH = 8.2, 20 °C. The water solubility was reported to be 1.3 mg/l at 20 °C. pH–value in water = 10.7
3.1.6	Method of analysis	Reverse phase HPLC –DAD using external standards, method validated
3.2	**Preparation of TS solution for poorly soluble or volatile**	A saturated solution (=stock solution) of the test item was prepared using 10 mg test item/L (3L) in dilution water. The dispersion was mixed with an ultraturrax (15 min with 20500 rpm, cooled in an ice

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	test substances	bath). Undissolved particles of the test item were removed by filtration (pore size 0.45 µm). The preparation of the stock solution was carried out in the dark to prevent photolysis of the test item. The time between preparing the stock solution and the addition of the algae to the test concentration did not exceed 30 min.	
3.3	**Reference substance**	Yes, 3,5-Dichlorophenol	
3.3.1	Method of analysis for reference substance	No analysis performed	
3.4	**Testing procedure**		
3.4.1	Culture medium	F/2 + Si double concentrated with natural seawater (salinity of 30 ± 5 S), according to the CCAP administration. Components and concentration: $NaPO_4$ 75 mg/L $NaH_2PO_4 \cdot H_2O$ 5 mg/L $Na_2SiO_3 \cdot 9\ H_2O$ 30 mg/L Titripex III 4.16 mg/L $FeCl_3 \cdot 6\ H_2O$ 3.15 mg/L $CuSO4 \cdot 5\ H_2O$ 0.01 mg/L $ZnSO4 \cdot 7\ H_2O$ 0.022 mg/L $CoCl2 \cdot 6\ H_2O$ 0.01 mg/L $MnCl2 \cdot 4\ H_2O$ 0.18 mg/L $Na_2MoO_4 \cdot 4\ H_2O$ 0.006 mg/L Vitamin B1 (Thiamin hydrochloride) 0.1 mg/L Vitamin H (Biotin) 0.5 µg/L Vitamin B12 (Cyanocobalamine) 0.405 µg/L pH 8.0 ± 0.2	
3.4.2	Test organisms	See Table A7_4_1_3-1	
3.4.3	Test system	See Table A7_4_1_3-2	X
3.4.4	Test conditions	See Table A7_4_1_3-3	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Effects of Dichlofluanid on the growth of the marine diatom *Skeletonema costatum*: - The concentration at which there was 50% inhibition of growth of biomass (E_bC_{50}) and - The concentration at which there was 50% inhibition of the growth rate (E_rC_{50}) Also detected were the lowest concentration at which there was an observable effect (LOEC) and the concentration at which there was no observed effect (NOEC).	
3.4.7	Sampling	Cell density was measured via Chlorophyll-a-fluorescence, excitation at 435 nm, emission at 685 nm. Each replicate was measured 6-fold. The cell density was measured at the beginning of the test and every 24 h. Microscopic evaluation of the cells at the start and at the end of the incubation was determined. Also any unusual cell shapes, colour	

		differences, differences in chloroplasts morphology, flocculation, adherence of algae to test containers or aggregation of alga cell were observed. Additionally, after 96h 10 mL algae suspension from the initially measured concentrations 109 – 219 µg a.i. /L and 5 mL from the control were transferred to 100 mL untreated dilution water and allowed to grow for further 3 d to determine whether the effect of the test item was reversible. Water quality parameters were determined to be within the acceptable limits: pH value was measured at 0 and 96 h, temperature and salinity were measured continuously throughout the test (every 30 min.). Light intensity was measured prior to the start.
3.4.8	Monitoring of TS concentration	Yes; Analytical concentrations of test substance and its metabolite DMSA in the test medium were determined for all nominal concentration levels (control, 7.11, 15.3, 29.2, 55.0, 109, 219 µg Dichlofluanid plus DMSA/l) at 0, 2, 4, 24 and 96 h via HPLC.
3.4.9	Statistics	The NOEC and LOEC values were determined by calculation of statistical significance of biomass integrals and growth rates. One Way Analysis of Variance and DUNNETT's test (growth rate and biomass integrals) were carried out for the determination of statically significant differences compared to control replicates. When running a One Way Analysis of Variance a Normality Test and an Equal Variance Test were done first. P-values for both Normality and Equal Variance Tests were 0.05. The α -value for ANOVA was α = 0.05. EC_{50}-values and confidence intervals after 72h and 96h were calculated by probit analysis. Probit values were taken from WEBER (1986). Confidence intervals were calculated according to a standard procedure (BREITIG & TRÜMPLING 1982).
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Based on the results of a preliminary range finding test, and with regard to the low solubility, a definitive test was performed with 6 dilution levels in a geometrical series with a dilution factor 2: 1:4 – 1:8 – 1:16 – 1:32 – 1:64 – 1:128. These dilution levels correspond to the following initially measured total concentrations: 219, 109, 55.0, 29.2, 15.3, and 7.11 µg/L. A control with dilution water was run in parallel.

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4.2.2	Actual concentrations of test substance	The test item showed a rapid hydrolysis with a half-life of less than 2 hours in the test. These findings are in line with the known half-life of hydrolysis. Therefore, the results are based on the initially measured concentrations (7.11, 15.3, 29.2, 55.0, 109, 219 µg Dichlofluanid plus DMSA/l). The Dichlofluanid concentration decreased markedly within 24 hours, whereas increasing concentrations of the metabolite DMSA were found. The recovery rates based on the sum of the results gained for the active ingredient Dichlofluanid and the metabolite DMSA (expressed as Dichlofluanid equivalents) exceed 80 % after 2, 4, 24, and 96 h compared to the respective initial concentration, except for the initial concentration 7.11 µg/l after 4h. Furthermore, the sum of the initially measured concentrations of the active ingredient Dichlofluanid and its metabolite DMSA (expressed as Dichlofluanid equivalents) is called "total concentration". All effect levels are given based on the total concentrations. Additionally, the effect levels are presented based on the initially measured concentration of the active ingredient Dichlofluanid.	X
4.2.3	Growth curves	A growth curve (number of cells vs. time) is given in the report (p. 30)	
4.2.4	Concentration / response curve	Growth inhibition curves (effect of test substance on amount of algal biomass vs. test substance concentration as well as effect of test substance on the algal growth rate vs. test substance concentration, respectively) are plotted in the report (pp. 28-29).	
4.2.5	Cell concentration data	See Table A7_4_1_3-4	
4.2.6	Effect data (cell multiplication inhibition)	See Table A7_4_1_3-5, Table A7_4_1_3-6 and Table A7_4_1_3-7	
4.2.7	Other observed effects	After 96 h algae were transferred from the total concentrations 109 – 219 µg/l and control to fresh-untreated medium and allowed to grow for further 3 days under test conditions. The test item effect was observed to be reversible at these concentration levels.	
4.3	**Results of controls**	See Table A7_4_1_3-5 and Table A7_4_1_3-6	
4.4	**Test with reference substance**		
4.4.1	Concentrations	0.25 – 0.5 – 1 – 2 – 4 mg/L	
4.4.2	Results	The 96 h-EC_{50} value of 3, 5-Dichlorophenol determined for algal biomass and growth rate were 1.04 and 1.19 mg/L, respectively, based on nominal concentrations. Thus, the reference substance presented results within the prescribed range according to the DIN EN ISO-Guideline No. 10253.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The influence of Preventol A 4-S on the growth of the diatom *Skeletonema costatum* was investigated in a 96 h hours static test according to current guidelines (DIN EN ISO-Guideline 10253 , OECD-	

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		Guideline 201, and EPA OPPTS 850.4400 Algal Toxicity Test, Tiers I and II).	
5.2	**Results and discussion**	In this study Preventol A 4-S was found to inhibit the growth of the marine diatom algae *Skeletonema costatum* after 96 h (growth rate) at the total concentrations (sum of the initially measured concentrations of the a.i. Dichlofluanid and its metabolite DMSA, expressed as Dichlofluanid equivalents) LOE_rC = 55.0 µg/L, corresponding to = 29.3 µg a.i./L. The EC_{50}-value with 95 % confidence intervals for inhibition of the specific growth rate (E_rC_{50}) after 96 h was 218 (190 - > 219) µg/L, corresponding to 114 (99.9 - > 115) µg a.i./L, respectively. However, the endpoint biomass presented lower values with a LOE_bC of 15.7 µg a.i./l and E_bC_{50} of 46.1 µg a.i./l. Inhibition effects were found to be reversible up to the top total concentration of 219 µg/L.	
5.2.1	NOEC	96h-NOE_bC = 9.08 µg/l, related to the a.i. Dichlofluanid 96h-NOE_rC = 15.7 µg/l, related to the a.i. Dichlofluanid	X
5.2.2	EC_{50}	96h-E_bC_{50} = 46.1 µg./l, related to the a.i. Dichlofluanid 96h-E_rC_{50} = 114 µg/l, related to the a.i. Dichlofluanid	X
5.3	**Conclusion**	Validity criteria are summarised in Table A7_4_1_3-8. A clear dose – response relationship can be derived from the cell concentration data.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	18/11/13
Materials and Methods	Applicant's version is acceptable noting the following: 3.4.3 – Temperature deviated below the recommended guideline range.
Results and discussion	Applicant's version is acceptable noting the following: 4.2.2 – Measured concentrations of dichlofluanid were not maintained throughout the study. This result is to be expected given the rapid rate of hydrolysis of the active substance. Concentrations of the metabolite DMSA were also analytically determined. Calculating equivalent dichlofluanid concentrations from DMSA levels, total concentrations of dichlofluanid were maintained at > 80 % of nominal levels during the study. Study endpoints have also been calculated based on mean measured concentrations of dichlofluanid only. However, due to a lack of a dose response relationship when concentrations are expressed in terms of mean measured values for dichlofluanid, no EC_{50} values could be determined.
Conclusion	Applicant's version is acceptable noting the following: 5.2.1 – Based on mean measured concentrations of dichlofluanid only a NOEC = 0.64 µg a.s./L is derived. 5.2.2 – The use of the lower 72 h EC50 values based on initial dichlofluanid concentrations is considered more appropriate. The 72 h-EbC50 = 36.9 (95% CI: 33.8-40.2) µg a.s./l and the 72 h-ErC50 = 83.0 (95% CI: 73.6-93.7) µg a.s./l
Reliability	1
Acceptability	Acceptable The study conformed to the guideline OECD 201, was conducted to GLP and met all guideline validity criteria. It is noted that the study was conducted at a temperature range below that recommended in the guideline, however, as exponential growth was demonstrated in the control group, this is not considered to impact the reliability of the study. The study is considered suitable for use in regulatory risk assessment.
Remarks	All endpoints and data presented in the summary and tables have been checked against the original summary and are correct. Given that continuous release is possible for PT21 products and since the metabolite DMSA may be less toxic to aquatic organisms than dichlofluanid, it is considered appropriate to use study endpoints based on mean measured concentrations of dichlofluanid only in the risk assessment. It is noted that this is potentially conservative as it assumes that the metabolite does not contribute to the toxicity seen in the study, i.e. that all effects are due to dichlofluanid exposure.
	COMMENTS FROM ...
Date	*Give date of comments submitted*

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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: Test organisms

Criteria	**Details**
Species	Marine diatom *Skeletonema costatum*
Strain	CCAP 1077/1C
Source	CCAP Administration, Institute of freshwater ecology, Windermere laboratory, Far Sawrey, AMBLESIDE, Cumbria LA22 OLP, UK
Laboratory culture	Yes
Method of cultivation	Stock cultures are prepared occasionally in dilution water. Light intensity amounted 35-710 µE/m^2*s for 24 h per day. Medium of culture: was F/2 + Si double concentrated with natural seawater (salinity of 30 ± 5 S), according to the CCAP administration. A four day old preculture was used as inoculum for the definitive test. All algae were from the same source and have not been used in a previous study.
Pre-treatment	-
Initial cell concentration	Test started with a biomass of approximately 3 x 10^4 cells/mL nutrient solution

Table A7_4_1_3-2: Test system

Criteria	Details
Volume of culture flasks	The medium was divided into 100 ml aliquots and these were poured into 250 ml Erlenmeyer flasks
Culturing apparatus	Incubation was performed under standardised conditions according to the mentioned guidelines
Light quality	Continuous illumination; light intensity: 60 – 120 µE/m^2*s.
Procedure for suspending algae	Test containers were placed on a rotary shaker and oscillated at approximately 40 rpm.
Number of vessels/ concentration	Control: 6 flasks Each test substance concentration: 3 flasks
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-3: Test conditions

Criteria	Details
Test temperature	18 ± 2 °C
pH	The pH was adjusted before the beginning of the test. At the beginning of the test the control cultures had a pH of 8.09 to 8.15; after 96 h the pH value had varied a little 8.28 to 8-83.
Aeration of dilution water	No data
Light intensity	66.9 µE/m^2*s (range 60.2 – 71.9 µE/m^2*s)
Photoperiod	Continuous illumination in the incubator (24 h/day)

Table A7_4_1_3-4: Cell densities at different test substance concentrations during test

Dilution Level	Total Concentration [µg/L]	RE. No.	Cell Density [cells/mL]				
			0 h	24 h	48 h	72 h	96 h
1:4	219	1	27972	15868	27660	69713	173131
		2	25925	14444	26770	59878	153373
		3	25435	15645	27260	62637	149190
		Mean	26444	15319	27230	64076	158565
1:8	109	1	31443	40209	94499	245087	723284
		2	30241	38741	89871	278551	732629
		3	29485	39942	96235	283802	716609
		Mean	30390	39631	93535	269147	724174
1:16	55.0	1	34780	60724	179227	429228	1245714
		2	32155	65975	169615	442667	1172734
		3	32644	66998	166856	496245	1261289
		Mean	33193	64566	171899	456047	1226579
1:32	29.2	1	30909	82217	205393	488057	1471329
		2	35715	84665	204592	527929	1505149
		3	35270	85421	215984	589784	1507819
		Mean	33965	84101	208656	535257	1494766
1.64	15.3	1	31932	85466	218654	614615	1579909
		2	31754	91918	224217	595658	1484679
		3	32822	92541	219411	643985	1634199
		Mean	32169	89975	220761	618086	1566262
1:128	7.11	1	29351	82484	234363	640781	1542084
		2	32466	86623	222125	609631	1553654
		3	31754	88536	248781	580083	1501144
		Mean	31190	85881	235090	610165	1532294
Control		1	33490	90405	225151	588360	1525619
		2	35225	84754	214649	583376	1510489
		3	34202	93031	246778	650749	1494469
		4	34024	86489	240860	665523	1691604
		5	33223	85199	218031	630368	1561219
		6	32511	92408	217097	638556	1477559
		Mean	33779	88714	227094	626155	1543493

Total Concentration = Sum of the initially measured concentrations of the active ingredient Dichlofluanid and its metabolite DMSA (expressed as Dichlofluanid equivalents)

RE. = Replicate

Table A7_4_1_3-5: Areas under the growth curves ("biomass integrals") and growth rates at different test substance concentrations, and their % deviation from controls (= 100%) after 72h

Dilution Level	Total Concentration [µg/L]	RE. No.	Biomass Integral	Inhibition of Biomass [%]	Growth Rate	Rate-related Inhibition [%]	Doubling Time [d]
1:4	219	1	8455	98.45	0.30	68.72	2.28
		2	6341	98.84	0.28	71.33	2.48
		3	10636	98.05	0.30	69.13	2.31
		Mean	(+) 8477	98.44	(+) 0.30	69.69	2.35
1:8	109	1	178644	67.19	0.68	29.67	1.01
		2	192285	64.68	0.74	23.95	0.94
		3	204366	62.46	0.75	22.45	0.92
		Mean	(+) 191765	64.78	(+) 0.73	25.30	0.95
1:16	55.0	1	367615	32.48	0.84	13.93	0.83
		2	376536	30.84	0.87	10.19	0.79
		3	400367	26.46	0.91	6.79	0.76
		Mean	(+) 381506	29.93	(+) 0.87	10.26	0.79
1:32	29.2	1	454366	16.54	0.92	5.49	0.75
		2	463934	14.79	0.90	7.75	0.77
		3	508122	6.67	0.94	3.53	0.74
		Mean	(+) 475473	12.67	(+) 0.92	5.56	0.75
1:64	15.3	1	531598	2.36	0.99	- 1.29	0.70
		2	534579	1.81	0.98	- 0.41	0.71
		3	551890	- 1.37	0.99	- 1.95	0.70
		Mean	(-) 539357	0.93	(-) 0.99	- 1.23	0.70
1:128	7.11	1	563860	- 3.57	1.03	- 5.60	0.67
		2	532399	2.21	0.98	- 0.44	0.71
		3	547974	- 0.65	0.97	0.50	0.72
		Mean	(-) 548079	- 0.67	(-) 0.99	- 1.85	0.70
Control		1	526011		0.96		0.73
		2	503029		0.94		0.74
		3	579679		0.98		0.71
		4	575051		0.99		0.70
		5	535357		0.98		0.71
		6	547506		0.99		0.70
		Mean	544438		0.97		0.71

Total Concentration = Sum of the initially measured concentrations of the active ingredient Dichlofluanid and its metabolite DMSA (expressed as Dichlofluanid equivalents)

RE. = Replicate

Table A7_4_1_3-6: Areas under the growth curves ("biomass integrals") and growth rates at different test substance concentrations, and their % deviation from controls (= 100%) after 96 h

Dilution Level	Total Concentration [µg/L]	RE. No.	Biomass Integral	Inhibition of Biomass [%]	Growth Rate	Rate-related Inhibition [%]	Doubling Time [d]
1:4	219	1	101905	93.61	0.46	52.31	1.52
		2	87041	94.54	0.44	53.49	1.56
		3	91115	94.29	0.44	53.71	1.57
		Mean	(+) 93354	94.15	(+) 0.45	53.14	1.55
1:8	109	1	631387	60.43	0.78	17.96	0.88
		2	667634	58.15	0.80	16.60	0.87
		3	675086	57.69	0.80	16.52	0.87
		Mean	(+) 658035	58.76	(+) 0.79	17.03	0.87
1:16	55.0	1	1170306	27.67	0.89	6.37	0.77
		2	1152082	27.79	0.90	5.90	0.77
		3	1246490	21.87	0.91	4.39	0.76
		Mean	(+) 1189626	25.44	(+) 0.90	5.56	0.77
1:32	29.2	1	1403150	12.05	0.97	- 1.07	0.72
		2	1444758	9.45	0.94	2.12	0.74
		3	1521654	4.63	0.94	1.74	0.74
		Mean	(+) 1456520	8.71	(-) 0.95	0.98	0.73
1:64	15.3	1	1596928	- 0.09	0.98	- 2.08	0.71
		2	1542994	3.29	0.96	- 0.60	0.72
		3	1658160	- 3.93	0.98	- 2.25	0.71
		Mean	(-) 1599362	- 0.24	(-) 0.97	- 1.66	0.71
1:128	7.11	1	1625942	- 1.91	0.99	- 3.65	0.70
		2	1581575	0.87	0.97	- 1.21	0.72
		3	1556833	2.42	0.96	- 0.89	0.72
		Mean	(-) 1588118	0.46	(-) 0.97	- 1.90	0.71
Control		1	1549511		0.95		0.73
		2	1514736		0.94		0.74
		3	1618086		0.94		0.73
		4	1719590		0.98		0.71
		5	1597927		0.96		0.72
		6	1573052		0.95		0.73
		Mean	1595483		0.96		0.73

Table A7_4_1_3-7: Effects on algae average biomass and growth rate

Endpoint	Total concentration [µg/l]	Initially measured concentration of the a i. Dichlofluanid [µg/l]	Mean measured concentration of dichlofluanid [µg/l]
Inhibition of biomass growth			
E_bC_{50} (CI 95 %) (0 – 72 h)	**69.3 (63.5 – 78.8)**	**36.9 (33.8 – 40.2)**	-
LOE_bC (0 – 72 h)	29.2	15.7	1.05
NOE_bC (0 – 72 h)	15.3	9.08	0.64
E_bC_{50} (CI 95 %) (0 – 96 h)	**87.1 (78.9 – 96.2)**	**46.1 (41.8 – 50.9)**	n.d.
LOE_bC (0 – 96 h)	29.2	15.7	1.05
NOE_bC (0 – 96 h)	15.3	9.08	0.64
Rate-related inhibition			
E_rC_{50} (CI 95 %) (0 – 72 h)	**158 (140 – 178)**	**83.0 (73.6 – 93.7)**	-
LOE_rC (0 – 72 h)	29.2	15.7	1.05
NOE_rC (0 – 72 h)	15.3	9.08	0.64
E_rC_{50} (CI 95 %) (0 – 96 h)	**218 (190 – >219)**	**114 (99.9 – >115)**	n.d.
E_rC_{10} (CI 95 %) (0 – 96 h)	-	-	0.534 (0.442-0.594)
LOE_rC (0 – 96 h)	55.0	29.3	-
NOE_rC (0 – 96 h)	29.2	15.7	-

Total concentration = Sum of the initially measured concentrations of the active ingredient Dichlofluanid and its metabolite DMSA (expressed as Dichlofluanid equivalents)

n.d.: not determined due to mathematical reasons (inappropriate data) or value is beyond the tested concentrations

Table A7_4_1_3-8: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days (exponential growth)	**X**	
The temperature during the test had to be in the range of 18 ± 2 °C	**X**	
The pH-value of the control replicates should not normally deviate by more than 1.5 units during the test	**X**	
Concentration of test substance ≥ 80% of initial concentration during test (otherwise determination of effect levels based on mean measured concentrations)	**X**	

Criteria for poorly soluble test substances	**X**	

Table A7_4_1_3-9: Concentrations of dichlofluanid (µg/L) over time

Dilution level	0 h	2 h	4 h	24 h	96 h	Mean measured
Control	<LOQ	<LOQ	<LOQ	<LOQ	1.35	-
1:128	5.23	1.08	<LOQ	<LOQ	<LOQ	0.52
1:64	9.08	2.95	2.54	<LOQ	<LOQ	0.64
1:32	15.7	4.05	2.19	<LOQ	1.85	1.05
1:16[1]	NA	NA	NA	<LOQ	<LOQ	-
1:16	29.3	5.2	2.53	<LOQ	<LOQ	0.66
1:8	57.5	10.9	1.97	<LOQ	<LOQ	0.66
1:4	115	25.1	8.29	<LOQ	<LOQ	0.79
1:1	430	NA	NA	NA	NA	-

NA = Not analysed
[1]Sample without algae
LOQ = 1 µg /L

Table A7_4_1_3-10: Concentrations of DMSA (µg/L) over time

Dilution level	0 h	2 h	4 h	24 h	96 h
Control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
1:128	1.13	3.58	3.09	3.94	4.6
1:64	3.73	7.45	7.69	7.8	7.86
1:32	8.11	15	15.5	16.8	14.9
1:16[1]	NA	NA	30.6	NA	29.6
1:16	15.5	30.4	31.8	30.6	30.2
1:8	30.8	63	71.3	77.8	64.7
1:4	62.4	120	122	112	113
1:1	327	NA	NA	NA	NA

NA = Not analysed
[1]Sample without algae
LOQ = 1 µg /L

Table A7_4_1_3-11: Dichlofluanid and DMSA (as equivalent of dichlofluanid) concentrations (µg/L)

Dilution level	0 h	2 h	4 h	24 h	96 h
1:128	7.11	7.02	5.13	6.54	7.64
1:64	15.3	15.4	15.3	12.9	13
1:32	29.2	29	27.9	27.9	26.6
1:16[1]	NA	NA	50.8	NA	49.1
1:16	55	55.7	55.3	50.8	50.1
1:8	109	115	120	129	107

1:4	219	224	211	186	188

NA = Not analysed
[1]Sample without algae