Algae, Growth Inhibition Test (OECD 201-2006/2011): Project 4711

<your company name>
<your adress>
<your adress>
(your adress>

General:

Test identification/project no.	Project 4711
Test item	A4711
Unit of test item concentration	mg/L
Start of experiment on day	
Date and time of the evaluation	13.02.2022; 14:17:29
Algae counts divided by factor	10000
Repeated measurements per replicate	1
Raw data filename:	OECD201 AlgaeGrowthInhibition(Cellcounts) 2006_2011.xls

Test design

Number of treatments (incl. control(s))	6
Duration of the test	96 h
Measurement interval	24 h
Measurement variable	Cellcount
Test system	Desmodesmus subspicatus
Statistical design	Hypothesis testing (NOEC) and regression (ECx)

Find validity information on the next page !

Validity information about the current Algae test (OECD 201)

Tab. 1: Validity information about the current Algae test (OECD 201). The test was performed according to OECD 201 using Desmodesmus subspicatus as test species; please consider below that the user could have chosen settings which deviate from the standard requirements; Required: prescribed settings by the standard; User: settings chosen by the user; Achieved: value computed from current test.; CV: coefficient of variation; 'GR: growth rate; Mean Repl.: mean replicate sectional GR over time; CV Repl.: coefficient of variation of replicate over time; Mean of mean Repl.: mean of 'mean replicate sectional GR over time; Mean CV Repl.: mean the of coefficients of variation of replicate over time.

Criterion	Required	User	Achieved	Valid
Validity time period	72 h	72 h	72 h	yes
BF 0 - 72 h	16,0	16,0	59,5	yes
Mean GR	0,920/ d	0,920/ d	1,360/ d	yes
CV mean GR	7,00	7,00	3,10	yes

Sectional GR Control

Time:	0 - 24 h	24 - 48 h	48 - 72 h
Repl. 1	1,099	1,540	1,488
Mean Repl. 1	1,376		
CV Repl.1	17,55		
Repl. 2	1,099	1,609	1,386
Mean Repl. 2	1,365		
CV Repl.2	18,76		
Repl. 3	0,693	1,946	1,404
Mean Repl. 3	1,348		
CV Repl.3	46,62		
Repl. 4	0,693	1,872	1,578
Mean Repl. 4	1,381		
CV Repl.4	44,43		
Repl. 5	0,693	1,792	1,735
Mean Repl. 5	1,407		
CV Repl.5	43,97		
Repl. 6	1,099	1,466	1,285
Mean Repl. 6	1,283		
CV Repl.6	14,33		

	Required	User	Achieved	
Mean of mean Repl.			1,360	
Mean CV Repl.	35,00	35,00	30,94	yes

Summary of Results for all Endpoints

Tab. 1: Summary of Results for all Endpoints: Critical effect and threshold concentration as observed at end of experimental time; EC: Effective concentration for xx% reduction; 95%-CL: 95% Confidence limits; LOEC: Lowest observed effect concentration; NOEC: No observed effect concentration.

Critical Conc.s [mg/L]		0 - 24 h	0 - 48 h	0 - 72 h	0 - 96 h
Yield					
	EC10	0,013	0,017	0,009	0,005
95%-CL	lower	n.d.	0,007	0,009	0,002
	upper	n.d.	0,046	0,009	0,011
	EC20	0,027	0,027	0,013	0,007
95%-CL	lower	n.d.	0,011	0,013	0,004
	upper	n.d.	0,068	0,014	0,015
	EC50	0,099	0,062	0,028	0,016
95%-CL	lower	n.d.	0,021	0,028	0,007
	upper	n.d.	0,188	0,029	0,038
Yield	LOEC	>0,210	n.d.	<=0,021	<=0,021
	NOEC	>=0,210	n.d.	<0,021	<0,021
Growth rate					
	EC10	0,004	0,052	0,009	0,012
95%-CL	lower	n.d.	0,030	0,003	0,010
	upper	n.d.	0,089	0,023	0,016
	EC20	0,011	0,068	0,023	0,022
95%-CL	lower	n.d.	0,041	0,009	0,018
	upper	n.d.	0,115	0,059	0,029
	EC50	0,088	0,116	0,144	0,070
95%-CL	lower	n.d.	0,061	0,042	0,052
	upper	n.d.	0,217	0,470	0,094
Growth rate	LOEC	>0,210	0,068	<=0,021	<=0,021
	NOEC	>=0,210	0,038	<0,021	<0,021
Sectional growth rate					
	EC10	0,004	0,055	0,000	0,013
95%-CL	lower	n.d.	0,021	n.d.	0,011
	upper	n.d.	0,146	n.d.	0,016

Tab. 1 (continued): Summary of Results for all Endpoints: Critical effect and threshold concentration as observed at end of experimental time; EC: Effective concentration for xx% reduction; 95%-CL: 95% Confidence limits; LOEC: Lowest observed effect concentration; NOEC: No observed effect concentration.

	EC20	0,011	0,070	0,000	0,018
95%-CL	lower	n.d.	0,028	n.d.	0,016
	upper	n.d.	0,177	n.d.	0,022
	EC50	0,088	0,110	0,000	0,034
95%-CL	lower	n.d.	0,036	n.d.	0,028
	upper	n.d.	0,338	n.d.	0,042
Sectional growth rate	LOEC	>0,210	0,120	n.d.	<=0,021
	NOEC	>=0,210	0,068	n.d.	<0,021

n.d.: not determined due to mathematical reasons or inappropriate data

Cell number in Desmodesmus subspicatus as Dependent on Concentration and Time

Tab. 3: Cell number in Desmodesmus subspicatus as dependent on concentration of the test item and time ; Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from sheet InputRawData)

Treatm. [mg/L]	Control	0,021	0,038	0,068	0,120	0,210
0 h	1,0	1,0	1,0	1,0	1,0	1,0
	1,0	1,0	1,0	1,0	1,0	1,0
	1,0	1,0	1,0	1,0	1,0	1,0
	1,0	-	-	-	-	-
	1,0	-	-	-	-	-
	1,0	-	-	-	-	-
Mean:	1,0	1,0	1,0	1,0	1,0	1,0
Std.Dev.:	0,00	0,00	0,00	0,00	0,00	0,00
n:	6	3	3	3	3	3
CV:	0,00	0,00	0,00	0,00	0,00	0,00
24 h	3,0	1,0	1,0	3,0	2,0	1,0
	3,0	4,0	1,0	3,0	1,0	1,0
	2,0	2,0	3,0	1,0	2,0	2,0
	2,0	-	-	-	-	-
	2,0	-	-	-	-	-
	3,0	-	-	-	-	-
Mean:	2,5	2,3	1,7	2,3	1,7	1,3
Std.Dev.:	0,55	1,53	1,15	1,15	0,58	0,58
n:	6	3	3	3	3	3
CV:	21,91	65,47	69,28	49,49	34,64	43,30
48 h	14,0	17,0	11,0	7,0	4,0	1,0
	15,0	8,0	8,0	7,0	2,0	1,0
	14,0	9,0	10,0	6,0	9,0	2,0
	13,0	-	-	-	-	-
	12,0	-	-	-	-	-
	13,0	-	-	-	-	-
Mean:	13,5	11,3	9,7	6,7	5,0	1,3
Std.Dev.:	1,05	4,93	1,53	0,58	3,61	0,58
n:	6	3	3	3	3	3
CV:	7,77	43,53	15,80	8,66	72,11	43,30

72 h	62,0	42,0	19,0	14,0	8,0	7,0
	60,0	44,0	14,0	8,0	11,0	8,0
	57,0	45,0	15,0	13,0	9,0	6,0
	63,0	-	-	-	-	-
	68,0	-	-	-	-	-
	47,0	-	-	-	-	-

Tab. 3 (continued): Cell number in Desmodesmus subspicatus as dependent on concentration of the test item and time ; Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from sheet InputRawData)

Mean:	59,5	43,7	16,0	11,7	9,3	7,0
Std.Dev.:	7,12	1,53	2,65	3,21	1,53	1,00
n:	6	3	3	3	3	3
CV:	11,97	3,50	16,54	27,55	16,37	14,29
96 h	280,0	101,0	45,0	14,0	6,0	4,0
	230,0	81,0	35,0	17,0	7,0	2,0
	218,0	85,0	28,0	16,0	9,0	3,0
	208,0	-	-	-	-	-
	200,0	-	-	-	-	-
	241,0	-	-	-	-	-
Mean:	229,5	89,0	36,0	15,7	7,3	3,0
Std.Dev.:	28,80	10,58	8,54	1,53	1,53	1,00
n:	6	3	3	3	3	3
CV:	12,55	11,89	23,73	9,75	20,83	33,33

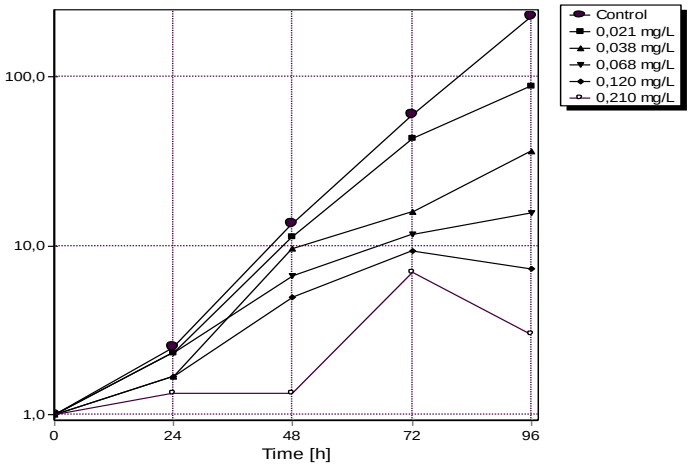

Fig. 1: Cell number in Desmodesmus subspicatus as dependent on test item concentration and time.

Yield of Desmodesmus subspicatus cells as Dependent on Concentration and Time

Tab. 4: Yield of Desmodesmus subspicatus cells as dependent on concentration of the test item and time ; Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (calculated from the cell number variable)

Treatm. [mg/L]	Control	0,021	0,038	0,068	0,120	0,210
0 h	0,0	0,0	0,0	0,0	0,0	0,0
	0,0	0,0	0,0	0,0	0,0	0,0
	0,0	0,0	0,0	0,0	0,0	0,0
	0,0	-	-	-	-	-
	0,0	-	-	-	-	-
	0,0	-	-	-	-	-
Mean:	0,0	0,0	0,0	0,0	0,0	0,0
Std.Dev.:	0,00	0,00	0,00	0,00	0,00	0,00
n:	6	3	3	3	3	3
CV:						
24 h	2,0	0,0	0,0	2,0	1,0	0,0
	2,0	3,0	0,0	2,0	0,0	0,0
	1,0	1,0	2,0	0,0	1,0	1,0
	1,0	-	-	-	-	-
	1,0	-	-	-	-	-
	2,0	-	-	-	-	-
Mean:	1,5	1,3	0,7	1,3	0,7	0,3
Std.Dev.:	0,55	1,53	1,15	1,15	0,58	0,58
n:	6	3	3	3	3	3
CV:	36,51	114,56	173,21	86,60	86,60	173,21
48 h	13,0	16,0	10,0	6,0	3,0	0,0
	14,0	7,0	7,0	6,0	1,0	0,0
	13,0	8,0	9,0	5,0	8,0	1,0
	12,0	-	-	-	-	-
	11,0	-	-	-	-	-
	12,0	-	-	-	-	-
Mean:	12,5	10,3	8,7	5,7	4,0	0,3
Std.Dev.:	1,05	4,93	1,53	0,58	3,61	0,58
n:	6	3	3	3	3	3
CV:	8,39	47,74	17,63	10,19	90,14	173,21

72 h	61,0	41,0	18,0	13,0	7,0	6,0
	59,0	43,0	13,0	7,0	10,0	7,0
	56,0	44,0	14,0	12,0	8,0	5,0
	62,0	-	-	-	-	-
	67,0	-	-	-	-	-
	46,0	-	-	-	-	-

Tab. 4 (continued): Yield of Desmodesmus subspicatus cells as dependent on concentration of the test item and time ; Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (calculated from the cell number variable)

Mean:	58,5	42,7	15,0	10,7	8,3	6,0
Std.Dev.:	7,12	1,53	2,65	3,21	1,53	1,00
n:	6	3	3	3	3	3
CV:	12,17	3,58	17,64	30,14	18,33	16,67
96 h	279,0	100,0	44,0	13,0	5,0	3,0
	229,0	80,0	34,0	16,0	6,0	1,0
	217,0	84,0	27,0	15,0	8,0	2,0
	207,0	-	-	-	-	-
	199,0	-	-	-	-	-
	240,0	-	-	-	-	-
Mean:	228,5	88,0	35,0	14,7	6,3	2,0
Std.Dev.:	28,80	10,58	8,54	1,53	1,53	1,00
n:	6	3	3	3	3	3
CV:	12,60	12,03	24,41	10,41	24,12	50,00

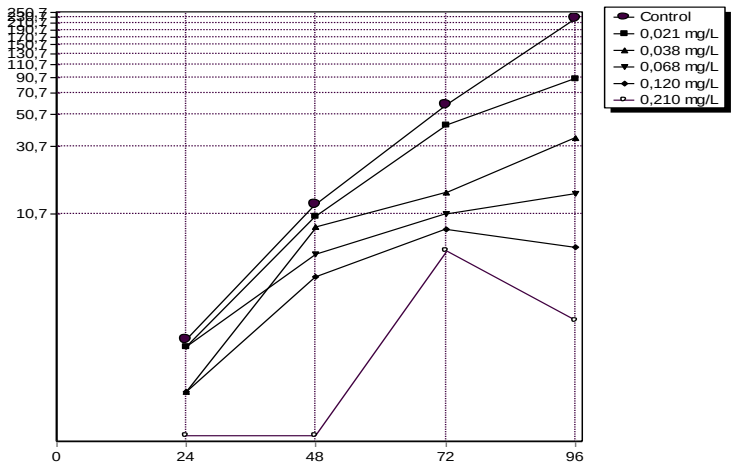

Fig. 2: Yield in Desmodesmus subspicatus as dependent on test item concentration and time.

Effective Concentrations (ECx) for Yield at 24 h

Yield [Cells] of Desmodesmus subspicatus

Tab. 5: %Decrease in yield caused by the test item after 24 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Decrease
Control	1,5	0,55	6	
0,021	1,3	1,53	3	11,11
0,038	0,7	1,15	3	55,56
0,068	1,3	1,15	3	11,11
0,120	0,7	0,58	3	55,56
0,210	0,3	0,58	3	77,78

The 3-param. normal CDF F(x) = b0*[NormalCDF(b1-log10(x)/b2 + zOpt)] was fitted to the data (CDF: cumulative distribution function; b0-b2: parameters; zOpt: adjustment to have the EC10 as parameter b1; x: concentration).
A non-linear regression without weighting was performed.
The optimzation converged thus fitting was successful (Stop Reason = Converged (Optimization method: Levenberg-Marquardt)).

Estimated parameters of the 3-param. normal CDF

Tab. 6: Estimated parameters of the 3-param. normal CDF with yield at 24 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	1,491	0,373	0,708	2,274	4,002	<0.001
b1	-1,874	1,069	-4,119	0,371	-1,754	0,048
b2	0,680	0,747	-0,889	2,249	0,911	0,187

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0,164; adjusted R^2: 0,071.Residual standard error: 0,88321.Akaike Criterion (AIC): 23,047.Shapiro Wilk`s test on normal distribution of residuals: p = 0,645..

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Tab. 7: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield at 24 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	2,79	2	1,39	1,785	0,196
Residuals	14,04	18	0,78		
- Lack of Fit	1,21	3	0,40	0,471	0,707
- Pure Error	12,83	15	0,86		
Total	16,95	20			

Since p(F|Regression) > 0.05, the amount of variance explained by the regression model is NOT significant..Therefore, confidence limits cannot be provided..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit..

Observed and Predicted Results of the 3-param. normal CDF

Tab. 8: Observed values in yield after 24 h as caused by the test item and predicted values as calculated from the function.

Treatm.[mg/L]	Observed	Mean Obs.	Predicted

0,001	2,000	1,501,49
0,001	2,000	1,501,49
0,001	1,000	1,501,49
0,001	1,000	1,501,49
0,001	1,000	1,501,49
0,001	2,000	1,501,49
0,021	0,000	1,331,25
0,021	3,000	1,331,25
0,021	1,000	1,331,25
0,038	0,000	0,671,09
0,038	0,000	0,671,09
0,038	2,000	0,671,09
0,068	2,000	1,330,89
0,068	2,000	1,330,89
0,068	0,000	1,330,89
0,120	1,000	0,670,67
0,120	0,000	0,670,67
0,120	1,000	0,670,67
0,210	0,000	0,330,47
0,210	0,000	0,330,47
0,210	1,000	0,330,47

Point estimates from the 3-param. normal CDF

Tab. 9: Point estimates from the 3-param. normal CDF with yield at 24 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	0,013	0,027	0,099
lower 95%-cl	n.d.	n.d.	n.d.
upper 95%-cl	n.d.	n.d.	n.d.

n.d.: not determined due to mathematical reasons

Since a signifcant lack of fit was found, it is recomended to try other dose/response functions..

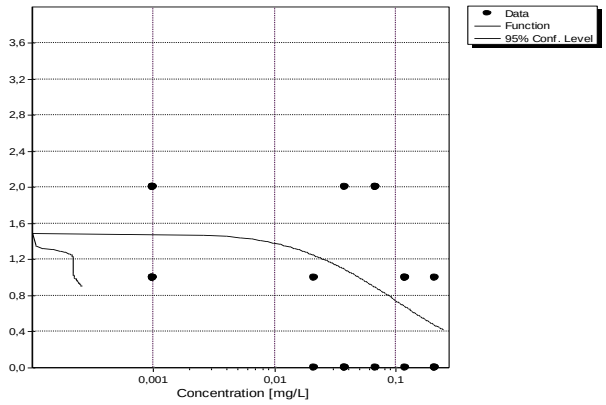

Fig. 3: Concentration-effect curve showing the influence of the test item on yield of the introduced Desmodesmus subspicatus as observed after 24 h

Effective Concentrations (ECx) for Yield at 48 h

Yield [Cells] of Desmodesmus subspicatus

Tab. 10: %Decrease in yield caused by the test item after 48 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Decrease
Control	12,5	1,05	6	
0,021	10,3	4,93	3	17,33
0,038	8,7	1,53	3	30,67
0,068	5,7	0,58	3	54,67
0,120	4,0	3,61	3	68,00
0,210	0,3	0,58	3	97,33

PLEASE NOTE: The non-linear regression procedure was terminated without achieving convergence due to mathematical problems (Stop Reason = Iterations > Max. Iterations (Optimization method: Levenberg-Marquardt)).
Please check whether the fit is nontheless acceptable or try whether an increase in the number of optimization cycles leads to convergence.

The 3-param. normal CDF F(x) = b0*[NormalCDF(b1-log10(x)/b2 + zOpt)] was fitted to the data (CDF: cumulative distribution function; b0-b2: parameters; zOpt: adjustment to have the EC10 as parameter b1; x: concentration).
A non-linear regression without weighting was performed.

Estimated parameters of the 3-param. normal CDF

Tab. 11: Estimated parameters of the 3-param. normal CDF with yield at 48 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	12,386	0,907	10,480	14,291	13,657	<0.001
b1	-1,760	0,202	-2,184	-1,336	-8,719	<0.001
b2	0,432	0,123	0,173	0,691	3,505	0,001

Stop Reason = Iterations > Max. Iterations (Optimization method: Levenberg-Marquardt)

R^2: 0,762; adjusted R^2: 0,736.Residual standard error: 2,26156.Akaike Criterion (AIC): 62,537.Shapiro Wilk`s test on normal distribution of residuals: p = 0,147..

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Tab. 12: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield at 48 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	353,94	2	176,97	34,601	<0.001
Residuals	92,06	18	5,11		
- Lack of Fit	5,90	3	1,97	0,342	0,795
- Pure Error	86,17	15	5,74		
Total	464,29	20			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit..

Observed and Predicted Results of the 3-param. normal CDF

Tab. 13: Observed values in yield after 48 h as caused by the test item and predicted values as calculated from the function.

Treatm.[mg/L]	Observed	Mean Obs.	Predicted
0,001	13,000	12,50	12,39
0,001	14,000	12,50	12,39
0,001	13,000	12,50	12,39
0,001	12,000	12,50	12,39
0,001	11,000	12,50	12,39
0,001	12,000	12,50	12,39
0,021	16,000	10,33	10,68
0,021	7,000	10,33	10,68
0,021	8,000	10,33	10,68
0,038	10,000	8,67	8,55
0,038	7,000	8,67	8,55
0,038	9,000	8,67	8,55
0,068	6,000	5,67	5,75
0,068	6,000	5,67	5,75
0,068	5,000	5,67	5,75
0,120	3,000	4,00	3,15
0,120	1,000	4,00	3,15
0,120	8,000	4,00	3,15
0,210	0,000	0,33	1,37
0,210	0,000	0,33	1,37
0,210	1,000	0,33	1,37

Point estimates from the 3-param. normal CDF

Tab. 14: Point estimates from the 3-param. normal CDF with yield at 48 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	0,017	0,027	0,062
lower 95%-cl	0,007	0,011	0,021
upper 95%-cl	0,046	0,068	0,188

n.d.: not determined due to mathematical reasons

The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs)..

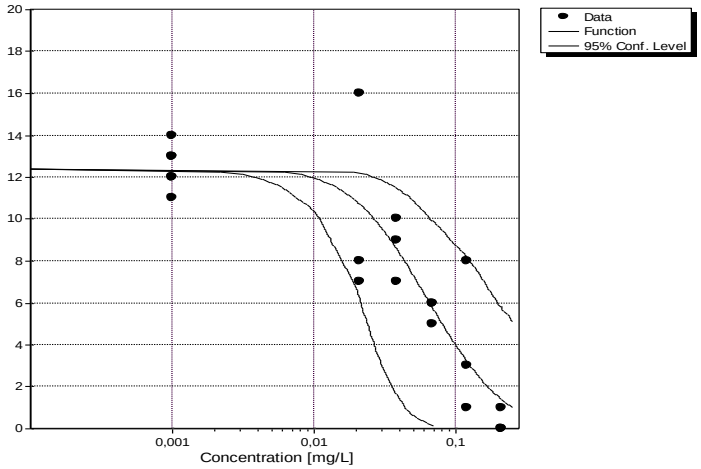

Fig. 4: Concentration-effect curve showing the influence of the test item on yield of the introduced Desmodesmus subspicatus as observed after 48 h

Effective Concentrations (ECx) for Yield at 72 h

Yield [Cells] of Desmodesmus subspicatus

Tab. 15: %Decrease in yield caused by the test item after 72 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Decrease
Control	58,5	7,12	6	
0,021	42,7	1,53	3	27,07
0,038	15,0	2,65	3	74,36
0,068	10,7	3,21	3	81,77
0,120	8,3	1,53	3	85,75
0,210	6,0	1,00	3	89,74

The 3-param. normal CDF F(x) = b0*[NormalCDF(b1-log10(x)/b2 + zOpt)] was fitted to the data (CDF: cumulative distribution function; b0-b2: parameters; zOpt: adjustment to have the EC10 as parameter b1; x:

concentration).
A non-linear regression without weighting was performed.

Estimated parameters of the 3-param. normal CDF

Tab. 16: Estimated parameters of the 3-param. normal CDF with yield at 72 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	59,692	0,218	59,233	60,151	273,202	<0.001
b1	-2,046	0,004	-2,054	-2,038	-524,064	<0.001
b2	0,391	0,002	0,385	0,396	160,228	<0.001

Stop Reason = Iterations > Max. Iterations (Optimization method: Levenberg-Marquardt)

R^2: 0,914; adjusted R^2: 0,904.Residual standard error: 6,24306.Akaike Criterion (AIC): 105,185.Shapiro Wilk`s test on normal distribution of residuals: p = 0,012..

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Tab. 17: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield at 72 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	11451,78	2	5725,89	146,909	<0.001
Residuals	701,56	18	38,98		
- Lack of Fit	402,06	3	134,02	6,712	0,004
- Pure Error	299,50	15	19,97		
Total	10541,24	20			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) <= 0.05, lack of fit is significant. It is recommendes to choose more appropriate regression functions and/or settings..Since a signifcant lack of fit was found, it is recomended to try other dose/response functions..

Observed and Predicted Results of the 3-param. normal CDF

Tab. 18: Observed values in yield after 72 h as caused by the test item and predicted values as calculated from the function.

Treatm.[mg/L]	Observed	Mean Obs.	Predicted
0,001	61,000	58,50	59,69
0,001	59,000	58,50	59,69
0,001	56,000	58,50	59,69
0,001	62,000	58,50	59,69
0,001	67,000	58,50	59,69
0,001	46,000	58,50	59,69
0,021	41,000	42,67	37,77
0,021	43,000	42,67	37,77
0,021	44,000	42,67	37,77
0,038	18,000	15,00	22,35
0,038	13,000	15,00	22,35
0,038	14,000	15,00	22,35

0,068	13,000	10,679,95
0,068	7,000	10,679,95
0,068	12,000	10,679,95
0,120	7,000	8,333,28
0,120	10,000	8,333,28
0,120	8,000	8,333,28
0,210	6,000	6,000,79
0,210	7,000	6,000,79
0,210	5,000	6,000,79

Point estimates from the 3-param. normal CDF

Tab. 19: Point estimates from the 3-param. normal CDF with yield at 72 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	0,009	0,013	0,028
lower 95%-cl	0,009	0,013	0,028
upper 95%-cl	0,009	0,014	0,029

n.d.: not determined due to mathematical reasons

The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs)..

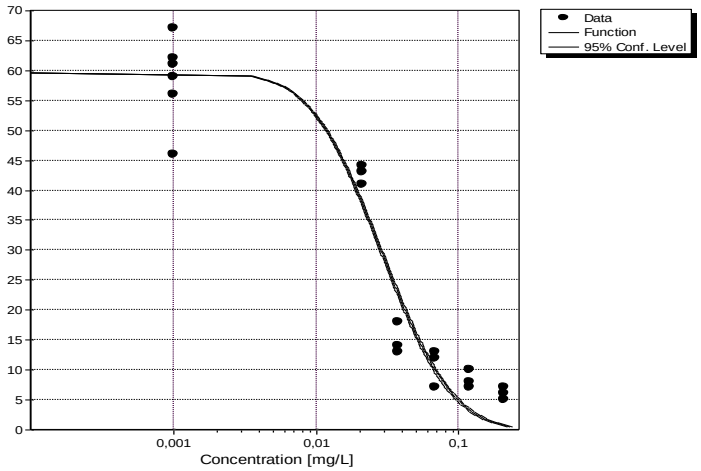

Fig. 5: Concentration-effect curve showing the influence of the test item on yield of the introduced Desmodesmus subspicatus as observed after 72 h

Effective Concentrations (ECx) for Yield at 96 h

Yield [Cells] of Desmodesmus subspicatus

Tab. 20: %Decrease in yield caused by the test item after 96 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Decrease
Control	228,5	28,80	6	
0,021	88,0	10,58	3	61,49
0,038	35,0	8,54	3	84,68
0,068	14,7	1,53	3	93,58
0,120	6,3	1,53	3	97,23
0,210	2,0	1,00	3	99,12

The 3-param. normal CDF F(x) = b0*[NormalCDF(b1-log10(x)/b2 + zOpt)] was fitted to the data (CDF: cumulative distribution function; b0-b2: parameters; zOpt: adjustment to have the EC10 as parameter b1; x: concentration).
A non-linear regression without weighting was performed.
The optimzation converged thus fitting was successful (Stop Reason = Converged (Optimization method: Levenberg-Marquardt)).

Estimated parameters of the 3-param. normal CDF

Tab. 21: Estimated parameters of the 3-param. normal CDF with yield at 96 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	228,892	6,641	214,940	242,844	34,467	<0.001
b1	-2,304	0,163	-2,646	-1,963	-14,173	<0.001
b2	0,394	0,085	0,215	0,573	4,629	<0.001

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0,990; adjusted R^2: 0,989.Residual standard error: 16,03004.Akaike Criterion (AIC): 144,790.Shapiro Wilk`s test on normal distribution of residuals: p = 0,002..

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Tab. 22: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with yield at 96 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	187705,26	2	93852,63	365,239	<0.001
Residuals	4625,32	18	256,96		
- Lack of Fit	96,49	3	32,16	0,107	0,955
- Pure Error	4528,83	15	301,92		
Total	189654,57	20			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit..

Observed and Predicted Results of the 3-param. normal CDF

Tab. 23: Observed values in yield after 96 h as caused by the test item and predicted values as calculated from the function.

Treatm.[mg/L]	Observed	Mean Obs.	Predicted

0,001	279,000	228,50	228,63
0,001	229,000	228,50	228,63
0,001	217,000	228,50	228,63
0,001	207,000	228,50	228,63
0,001	199,000	228,50	228,63
0,001	240,000	228,50	228,63
0,021	100,000	88,00	86,70
0,021	80,000	88,00	86,70
0,021	84,000	88,00	86,70
0,038	44,000	35,00	38,43
0,038	34,000	35,00	38,43
0,038	27,000	35,00	38,43
0,068	13,000	14,67	12,44
0,068	16,000	14,67	12,44
0,068	15,000	14,67	12,44
0,120	5,000	6,33	2,95
0,120	6,000	6,33	2,95
0,120	8,000	6,33	2,95
0,210	3,000	2,00	0,50
0,210	1,000	2,00	0,50
0,210	2,000	2,00	0,50

Point estimates from the 3-param. normal CDF

Tab. 24: Point estimates from the 3-param. normal CDF with yield at 96 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	0,005	0,007	0,016
lower 95%-cl	0,002	0,004	0,007
upper 95%-cl	0,011	0,015	0,038

n.d.: not determined due to mathematical reasons

The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs)..

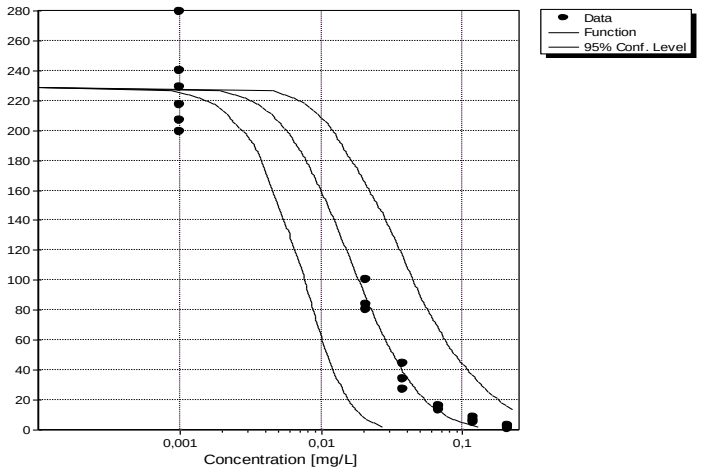

Fig. 6: Concentration-effect curve showing the influence of the test item on yield of the introduced Desmodesmus subspicatus as observed after 96 h

Overview over the ECs of the Test Item on Yield

Effects on Yield

Tab. 25: Yield (Y) and its decrease relative to control (%D) as computed from the raw data for test intervals selected; *nl with yield: nonlinear regression using the 3-param. normal CDF.

Treatment	0-24 h		0-48 h		0-72 h		0-96 h	
[mg/L]	Y	%D	Y	%D	Y	%D	Y	%D
Control	1,5	0,00	12,5	0,00	58,5	0,00	228,5	0,00
0,021	1,3	11,11	10,3	17,33	42,7	27,07	88,0	61,49
0,038	0,7	55,56	8,7	30,67	15,0	74,36	35,0	84,68
0,068	1,3	11,11	5,7	54,67	10,7	81,77	14,7	93,58
0,120	0,7	55,56	4,0	68,00	8,3	85,75	6,3	97,23
0,210	0,3	77,78	0,3	97,33	6,0	89,74	2,0	99,12
EC10	0,013	*nl	0,017	*nl	0,009	*nl	0,005	*nl
lower 95%-cl	n.d.		0,007		0,009		0,002	
upper 95%-cl	n.d.		0,046		0,009		0,011	
EC20	0,027	*nl	0,027	*nl	0,013	*nl	0,007	*nl
lower 95%-cl	n.d.		0,011		0,013		0,004	
upper 95%-cl	n.d.		0,068		0,014		0,015	
EC50	0,099	*nl	0,062	*nl	0,028	*nl	0,016	*nl
lower 95%-cl	n.d.		0,021		0,028		0,007	
upper 95%-cl	n.d.		0,188		0,029		0,038	

Threshold concentrations (NOEC) for Yield at 24 h

Statistical Characteristics of the Samples

Tab. 26: Statistical characteristics with yield at 24 h: Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [mg/L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Control	1,5	1,5	1,0	2,0	6	0,55	36,5	0,22	14,9	0,9	2,1
0,021	1,3	1,0	0,0	3,0	3	1,53	114,6	0,88	66,1	-2,5	5,1
0,038	0,7	0,0	0,0	2,0	3	1,15	173,2	0,67	100,0	-2,2	3,5
0,068	1,3	2,0	0,0	2,0	3	1,15	86,6	0,67	50,0	-1,5	4,2
0,120	0,7	1,0	0,0	1,0	3	0,58	86,6	0,33	50,0	-0,8	2,1
0,210	0,3	0,0	0,0	1,0	3	0,58	173,2	0,33	100,0	-1,1	1,8

Shapiro-Wilk´s Test on Normal Distribution

Tab. 27: Shapiro-Wilk´s Test on Normal Distribution with yield at 24 h: Mean: arithmetic mean; n: sample size; p(ShapiroWilk´s W): probability of the W statistic (i.e. that the observed deviations from the normal distributions are dues to chance). In case p(ShapiroWilk´s W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [mg/L]	Mean	s	n
Control	1,5	0,55	6
0,021	1,3	1,53	3
0,038	0,7	1,15	3
0,068	1,3	1,15	3
0,120	0,7	0,58	3
0,210	0,3	0,58	3

Results:

Number of residuals = 9; Shapiro-Wilk´s W = 0,972; p(W) = 0,744; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Normality check was passed (p > 0,01).

Levene´s Test on Variance Homogeneity (with Residuals)

Tab. 28: Levene´s Test on Variance Homogeneity (with Residuals) with yield at 24 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p: probability that the variance explained by the treatment is due to chance

Source	SS	df	MSS	F	p(F)
Treatment	1,347	5	0,269	2,371	0,089
Residuals	1,704	15	0,114		
Total	3,05	20			

The Levene test indicates variance homogeneity (p > 0,010).

Variance homogeneity check was passed (p > 0,01).

Normal-distribution and variance-homogeneity requirements are fulfilled.
A parametric multiple test is advisable.

To justify the use of Williams test at first a trend analysis by contrasts is performed.

Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 29: Trend analysis by contrasts (monotonicity of concentration/response) with yield at 24 h: Psi: sum of means weighted by contrasts; s(psi): standard error of psi; df: degrees of freedom; t: t-statistic; p(t): probability that the trend is due to chance (Ho: Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	s(psi)	df	t	p(t)
Linear	7,167	4,049	15	1,770	0,049
Quadratic	0,833	4,516	15	0,185	0,428

The linear trend is significant (p <= 0,05) The quadratic trend is not significant (p > 0,05)

The analysis of contrasts revealed a linear trend, thus the selected Williams test was performed.

Williams Multiple Sequential t-test Procedure

Tab. 30: Comparison of treatments with "Control" by the t test procedure after Williams with yield at 24 h: Significance was Alpha = 0,050, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: µ1 = µ2 = ... = µk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [mg/L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	1,5	0,925						
0,021	1,3	0,925	15	1,3	-76,44	-0,25	-1,75	-
0,038	0,7	0,925	15	1,0	-79,58	-0,76	-1,83	-
0,068	1,3	0,925	15	1,0	-80,58	-0,76	-1,85	-
0,120	0,7	0,925	15	0,7	-80,97	-1,27	-1,86	-
0,210	0,3	0,925	15	0,3	-81,36	-1,78	-1,87	-

+: significant; -: non-significant

The NOEC appears to be higher than or equal 0,210 mg/L.

Threshold concentrations (NOEC) for Yield at 48 h

Statistical Characteristics of the Samples

Tab. 31: Statistical characteristics with yield at 48 h: Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [mg/L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Control	12,5	12,5	11,0	14,0	6	1,05	8,4	0,43	3,4	11,4	13,6
0,021	10,3	8,0	7,0	16,0	3	4,93	47,7	2,85	27,6	-1,9	22,6
0,038	8,7	9,0	7,0	10,0	3	1,53	17,6	0,88	10,2	4,9	12,5
0,068	5,7	6,0	5,0	6,0	3	0,58	10,2	0,33	5,9	4,2	7,1
0,120	4,0	3,0	1,0	8,0	3	3,61	90,1	2,08	52,0	-5,0	13,0
0,210	0,3	0,0	0,0	1,0	3	0,58	173,2	0,33	100,0	-1,1	1,8

Shapiro-Wilk´s Test on Normal Distribution

Tab. 32: Shapiro-Wilk´s Test on Normal Distribution with yield at 48 h: Mean: arithmetic mean; n: sample size; p(ShapiroWilk´s W): probability of the W statistic (i.e. that the observed deviations from the normal distributions are dues to chance). In case p(ShapiroWilk´s W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [mg/L]	Mean	s	n
Control	12,5	1,05	6
0,021	10,3	4,93	3
0,038	8,7	1,53	3
0,068	5,7	0,58	3
0,120	4,0	3,61	3
0,210	0,3	0,58	3

Results:

Number of residuals = 16; Shapiro-Wilk´s W = 0,934; p(W) = 0,287; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Normality check was passed (p > 0,01).

Levene´s Test on Variance Homogeneity (with Residuals)

Tab. 33: Levene´s Test on Variance Homogeneity (with Residuals) with yield at 48 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p: probability that the variance explained by the treatment is due to chance

Source	SS	df	MSS	F	p(F)
Treatment	29,389	5	5,878	6,801	0,002
Residuals	12,963	15	0,864		
Total	42,35	20			

The Levene test indicates variance heterogeneity (p <= 0,010)!

Variance homogeneity check failed
However, normal distribution requirements are fulfilled.
The Welch-t-test for non-homogeneous variances with Bonferroni-Holm-adjustment is advisable.

Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm

Tab. 34: Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm with yield at 48 h: Multiple sequentially rejective comparisons of treatments with "Control". Significance was Alpha = 0,050, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; p(t): probability of sample t for Ho: µ1 = µ2; Alpha(i): adjusted significance levels; the differences are significant in case p(t) <= Alpha(i); dfm: modified degrees of freedom due to heteroscedasticity.(Control(c) and treatment(t) variance was applied: $s^2(c)/nc + s^2(t)/nt$, each). Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [mg/L]	Mean	s	df	%MDD	t	p(t)	Alpha(i)	Sign.
Control	12,5	1,05						
0,021	10,3	4,93	2	-67,28	-0,75	0,265	0,050	-
0,038	8,7	1,53	2	-33,75	-3,91	0,030	0,025	-
0,068	5,7	0,58	6	-12,89	-12,59	< 0.001	0,013	+
0,120	4,0	3,61	2	-90,78	-4,00	0,029	0,017	-
0,210	0,3	0,58	6	-13,64	-22,42	< 0.001	0,010	+

+: significant; -: non-significant

The NOEC cannot be determined by the program (expert judgement required).

Threshold concentrations (NOEC) for Yield at 72 h

Statistical Characteristics of the Samples

Tab. 35: Statistical characteristics with yield at 72 h: Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [mg/L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Control	58,5	60,0	46,0	67,0	6	7,12	12,2	2,91	5,0	51,0	66,0
0,021	42,7	43,0	41,0	44,0	3	1,53	3,6	0,88	2,1	38,9	46,5
0,038	15,0	14,0	13,0	18,0	3	2,65	17,6	1,53	10,2	8,4	21,6
0,068	10,7	12,0	7,0	13,0	3	3,21	30,1	1,86	17,4	2,7	18,7
0,120	8,3	8,0	7,0	10,0	3	1,53	18,3	0,88	10,6	4,5	12,1
0,210	6,0	6,0	5,0	7,0	3	1,00	16,7	0,58	9,6	3,5	8,5

Shapiro-Wilk´s Test on Normal Distribution

Tab. 36: Shapiro-Wilk´s Test on Normal Distribution with yield at 72 h: Mean: arithmetic mean; n: sample size; p(ShapiroWilk´s W): probability of the W statistic (i.e. that the observed deviations from the normal distributions are dues to chance). In case p(ShapiroWilk´s W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [mg/L]	Mean	s	n
Control	58,5	7,12	6
0,021	42,7	1,53	3
0,038	15,0	2,65	3
0,068	10,7	3,21	3
0,120	8,3	1,53	3
0,210	6,0	1,00	3

Results:

Number of residuals = 19; Shapiro-Wilk´s W = 0,874; p(W) = 0,017; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Normality check was passed (p > 0,01).

Levene´s Test on Variance Homogeneity (with Residuals)

Tab. 37: Levene´s Test on Variance Homogeneity (with Residuals) with yield at 72 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p: probability that the variance explained by the treatment is due to chance

Source	SS	df	MSS	F	p(F)
Treatment	59,905	5	11,981	1,621	0,215
Residuals	110,833	15	7,389		
Total	170,74	20			

The Levene test indicates variance homogeneity (p > 0,010).

Variance homogeneity check was passed (p > 0,01).

Normal-distribution and variance-homogeneity requirements are fulfilled.
A parametric multiple test is advisable.

To justify the use of Williams test at first a trend analysis by contrasts is performed.

Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 38: Trend analysis by contrasts (monotonicity of concentration/response) with yield at 72 h: Psi: sum of means weighted by contrasts; s(psi): standard error of psi; df: degrees of freedom; t: t-statistic; p(t): probability that the trend is due to chance (Ho: Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	s(psi)	df	t	p(t)
Linear	369,833	19,563	15	18,905	<0.001
Quadratic	-168,833	21,814	15	-7,740	<0.001

The linear trend is significant (p <= 0,05) The quadratic trend is significant (p <= 0,05)

The analysis of contrasts revealed a linear trend, thus the selected Williams test was performed.

Williams Multiple Sequential t-test Procedure

Tab. 39: Comparison of treatments with "Control" by the t test procedure after Williams with yield at 72 h: Significance was Alpha = 0,050, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: µ1 = µ2 = ... = µk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [mg/L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	58,5	4,468						
0,021	42,7	4,468	15	42,7	-9,47	-5,01	-1,75	+
0,038	15,0	4,468	15	15,0	-9,86	-13,77	-1,83	+
0,068	10,7	4,468	15	10,7	-9,98	-15,14	-1,85	+
0,120	8,3	4,468	15	8,3	-10,03	-15,88	-1,86	+
0,210	6,0	4,468	15	6,0	-10,08	-16,62	-1,87	+

+: significant; -: non-significant

The NOEC is lower than 0,021 mg/L.

Threshold concentrations (NOEC) for Yield at 96 h

Statistical Characteristics of the Samples

Tab. 40: Statistical characteristics with yield at 96 h: Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [mg/L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Control	228,5	223,0	199,0	279,0	6	28,80	12,6	11,76	5,1	198,3	258,7
0,021	88,0	84,0	80,0	100,0	3	10,58	12,0	6,11	6,9	61,7	114,3
0,038	35,0	34,0	27,0	44,0	3	8,54	24,4	4,93	14,1	13,8	56,2
0,068	14,7	15,0	13,0	16,0	3	1,53	10,4	0,88	6,0	10,9	18,5
0,120	6,3	6,0	5,0	8,0	3	1,53	24,1	0,88	13,9	2,5	10,1
0,210	2,0	2,0	1,0	3,0	3	1,00	50,0	0,58	28,9	-0,5	4,5

Shapiro-Wilk´s Test on Normal Distribution

Tab. 41: Shapiro-Wilk´s Test on Normal Distribution with yield at 96 h: Mean: arithmetic mean; n: sample size; p(ShapiroWilk´s W): probability of the W statistic (i.e. that the observed deviations from the normal distributions are dues to chance). In case p(ShapiroWilk´s W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [mg/L]	Mean	s	n
Control	228,5	28,80	6
0,021	88,0	10,58	3
0,038	35,0	8,54	3
0,068	14,7	1,53	3
0,120	6,3	1,53	3
0,210	2,0	1,00	3

Results:

Number of residuals = 19; Shapiro-Wilk´s W = 0,816; p(W) = 0,002; p(W) is smaller than or equal to the selected significance level of 0,010; therefore, treatment data significantly differ from normal distribution.

Normality check failed (p <= 0,01).

Levene´s Test on Variance Homogeneity (with Residuals)

Tab. 42: Levene´s Test on Variance Homogeneity (with Residuals) with yield at 96 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p: probability that the variance explained by the treatment is due to chance

Source	SS	df	MSS	F	p(F)
Treatment	1443,442	5	288,688	2,680	0,064
Residuals	1615,926	15	107,728		
Total	3059,37	20			

The Levene test indicates variance homogeneity (p > 0,010).

Variance homogeneity check was passed (p > 0,01).

Normal distribution is poore, but variance homogeneity requirements may be seen as fulfilled.
A parametric multiple test is yet possible.

To justify the use of the Step-down Jonckheere-Terpstra test at first a non-parametric trend analysis by contrasts is performed.

Non-parametric Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 43: Non-parametric trend analysis by contrasts (monotonicity of concentration/response) with yield at 96 h: Psi: total of rank sums weighted by contrasts; Var(psi): variance of psi; df: degrees of freedom; Chi^2: Chi^2-statistic; p(Chi^2): probability that the trend is due to chance. Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	Var(psi)	df	Chi^2	p(Chi^2)
Linear	-112,500	737,917	1	17,151	<0.001
Quadratic	7,500	917,583	1	0,061	0,804

The linear trend is significant (p <= 0,05) The quadratic trend is not significant (p > 0,05)

The analysis of contrasts revealed a linear trend, thus the selected SD Jonckheere-Terpstra test was performed.

Step-down Jonckheere-Terpstra Test Procedure

Tab. 44: Step-down Jonckheere-Terpstra test procedure with yield at 96 h: Step-down test to detect a trend in decreasing median effects on yield (Alpha is 0,050; one-sided smaller); Med: median, n: sample size; J: test statistic; J*: standardized J (in case a value for J* is shown for a treatment, the large-sample approximation was calculated (= sum of all replicates N > 11)); p(J): probability that the observed trend could be due to chance; Ho is accepted, if p(J) > Alpha. Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [mg/L]	Mean	Med	n	J	J*	p(J)	Sign.
Control	228,5	223,0	6				
0,021	88,0	84,0	3	18,0		0,012	+
0,038	35,0	34,0	3	45,0	3,38	<0.001	+
0,068	14,7	15,0	3	81,0	4,22	<0.001	+
0,120	6,3	6,0	3	126,0	4,93	<0.001	+
0,210	2,0	2,0	3	180,0	5,55	<0.001	+

+: significant; -: non-significant

The NOEC is lower than 0,021 mg/L.

Overview over the Effect-Thresholds of the Test Item on Yield

Overview over the LOEC and NOEC Determination

Tab. 45: Overview over the LOEC and NOEC Determination with yield: Arithmetic means and significance marks as computed for yield for all inspection intervals (top); bottom part: obtained LOEC and NOEC with indication of statistical test used; *wl: Williams multiple sequential t-test procedure; *bw: Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm; *jtsd: Step-down Jonckheere-Terpstra test procedure, significance level was 0,050, one-sided smaller.

Treatm. [mg/L]	0-24 h	0-48 h	0-72 h	0-96 h
0,021	1,3 -	10,3 -	42,7+	88,0+
0,038	0,7 -	8,7 -	15,0+	35,0+
0,068	1,3 -	5,7+	10,7+	14,7+
0,120	0,7 -	4,0 -	8,3+	6,3+
0,210	0,3 -	0,3+	6,0+	2,0+
LOEC	>0,210 *wl	n.d. *bw	<=0,021 *wl	<=0,021 *jtsd
NOEC	>=0,210 *wl	n.d. *bw	<0,021 *wl	<0,021 *jtsd

+: Significant difference to control (p <=0,050)

Mean specific growth rate (1/d) in Desmodesmus subspicatus as Dependent on Concentration and Time

Tab. 46: Mean specific growth rate (1/d) in Desmodesmus subspicatus as dependent on concentration of the test item and time ; Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (calculated from the cell number variable)

Treatm. [mg/L]	Control	0,021	0,038	0,068	0,120	0,210
0 - 0 h	0,000	0,000	0,000	0,000	0,000	0,000
	0,000	0,000	0,000	0,000	0,000	0,000
	0,000	0,000	0,000	0,000	0,000	0,000
	0,000	-	-	-	-	-
	0,000	-	-	-	-	-
	0,000	-	-	-	-	-

Tab. 46 (continued): Mean specific growth rate (1/d) in Desmodesmus subspicatus as dependent on concentration of the test item and time ; Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (calculated from the cell number variable)

Mean:	0,000	0,000	0,000	0,000	0,000	0,000
Std.Dev.:	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
n:	6	3	3	3	3	3
CV:						
0 - 24 h	1,099	0,000	0,000	1,099	0,693	0,000
	1,099	1,386	0,000	1,099	0,000	0,000
	0,693	0,693	1,099	0,000	0,693	0,693
	0,693	-	-	-	-	-
	0,693	-	-	-	-	-
	1,099	-	-	-	-	-
Mean:	0,896	0,693	0,366	0,732	0,462	0,231
Std.Dev.:	0,2221	0,6931	0,6343	0,6343	0,4002	0,4002
n:	6	3	3	3	3	3
CV:	24,79	100,00	173,21	86,60	86,60	173,21
0 - 48 h	1,320	1,417	1,199	0,973	0,693	0,000
	1,354	1,040	1,040	0,973	0,347	0,000
	1,320	1,099	1,151	0,896	1,099	0,347
	1,282	-	-	-	-	-
	1,242	-	-	-	-	-
	1,282	-	-	-	-	-
Mean:	1,300	1,185	1,130	0,947	0,713	0,116
Std.Dev.:	0,0390	0,2027	0,0817	0,0445	0,3764	0,2001
n:	6	3	3	3	3	3
CV:	3,00	17,11	7,23	4,70	52,81	173,21
0 - 72 h	1,376	1,246	0,981	0,880	0,693	0,649
	1,365	1,261	0,880	0,693	0,799	0,693
	1,348	1,269	0,903	0,855	0,732	0,597
	1,381	-	-	-	-	-
	1,407	-	-	-	-	-
	1,283	-	-	-	-	-

Tab. 46 (continued): Mean specific growth rate (1/d) in Desmodesmus subspicatus as dependent on concentration of the test item and time ; Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (calculated from the cell number variable)

Mean:	1,360	1,259	0,921	0,809	0,742	0,646
Std.Dev.:	0,0422	0,0117	0,0534	0,1013	0,0537	0,0480
n:	6	3	3	3	3	3
CV:	3,10	0,93	5,79	12,52	7,24	7,42
0 - 96 h	1,409	1,154	0,952	0,660	0,448	0,347
	1,360	1,099	0,889	0,708	0,486	0,173
	1,346	1,111	0,833	0,693	0,549	0,275
	1,334	-	-	-	-	-
	1,325	-	-	-	-	-
	1,371	-	-	-	-	-
Mean:	1,357	1,121	0,891	0,687	0,495	0,265
Std.Dev.:	0,0302	0,0290	0,0593	0,0248	0,0512	0,0871
n:	6	3	3	3	3	3
CV:	2,22	2,59	6,66	3,61	10,35	32,87

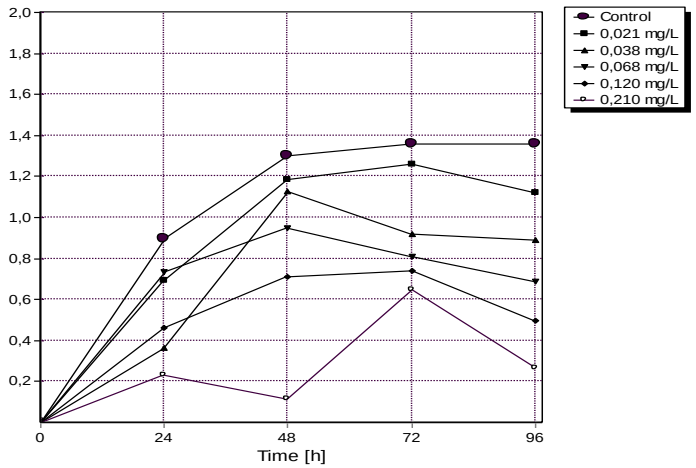

Fig. 7: Growth rate in Desmodesmus subspicatus as dependent on test item concentration and time.

Effective Concentrations (ECx) for Growth Rate at 0 - 24 h

Growth Rate [1/d] of Desmodesmus subspicatus

Tab. 47: %Inhibition of growth rate caused by the test item after 0 - 24 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Inhibition
Control	0,896	0,2221	6	
0,021	0,693	0,6931	3	22,63
0,038	0,366	0,6343	3	59,12
0,068	0,732	0,6343	3	18,25
0,120	0,462	0,4002	3	48,42
0,210	0,231	0,4002	3	74,21

The 3-param. normal CDF F(x) = b0*[NormalCDF(b1-log10(x)/b2 + zOpt)] was fitted to the data (CDF: cumulative distribution function; b0-b2: parameters; zOpt: adjustment to have the EC10 as parameter b1; x: concentration).
A non-linear regression without weighting was performed.
The optimzation converged thus fitting was successful (Stop Reason = Converged (Optimization method: Levenberg-Marquardt)).

Estimated parameters of the 3-param. normal CDF

Tab. 48: Estimated parameters of the 3-param. normal CDF with growth rate at 0 - 24 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	0,925	0,336	0,218	1,632	2,748	0,007
b1	-2,437	2,257	-7,179	2,305	-1,080	0,147
b2	1,077	1,468	-2,007	4,160	0,733	0,236

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0,184; adjusted R^2: 0,094.Residual standard error: 0,45969.Akaike Criterion (AIC): -4,379.Shapiro Wilk`s test on normal distribution of residuals: p = 0,377..

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Tab. 49: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with growth rate at 0 - 24 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	0,864	2	0,432	2,043	0,159
Residuals	3,804	18	0,211		
- Lack of Fit	0,346	3	0,115	0,501	0,687
- Pure Error	3,457	15	0,230		
Total	4,688	20			

Since p(F|Regression) > 0.05, the amount of variance explained by the regression model is NOT significant..Therefore, confidence limits cannot be provided..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit..

Observed and Predicted Results of the 3-param. normal CDF

Tab. 50: Observed values in growth rate after 0 - 24 h as caused by the test item and predicted values as calculated from the function.

Treatm.[mg/L]	Observed	Mean Obs.	Predicted

0,001	1,099	0,89590,8918
0,001	1,099	0,89590,8918
0,001	0,693	0,89590,8918
0,001	0,693	0,89590,8918
0,001	0,693	0,89590,8918
0,001	1,099	0,89590,8918
0,021	0,000	0,69310,6637
0,021	1,386	0,69310,6637
0,021	0,693	0,69310,6637
0,038	0,000	0,36620,5844
0,038	0,000	0,36620,5844
0,038	1,099	0,36620,5844
0,068	1,099	0,73240,5000
0,068	1,099	0,73240,5000
0,068	0,000	0,73240,5000
0,120	0,693	0,46210,4157
0,120	0,000	0,46210,4157
0,120	0,693	0,46210,4157
0,210	0,000	0,23100,3349
0,210	0,000	0,23100,3349
0,210	0,693	0,23100,3349

Point estimates from the 3-param. normal CDF

Tab. 51: Point estimates from the 3-param. normal CDF with growth rate at 0 - 24 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	0,004	0,011	0,088
lower 95%-cl	n.d.	n.d.	n.d.
upper 95%-cl	n.d.	n.d.	n.d.

n.d.: not determined due to mathematical reasons

Since a signifcant lack of fit was found, it is recomended to try other dose/response functions..

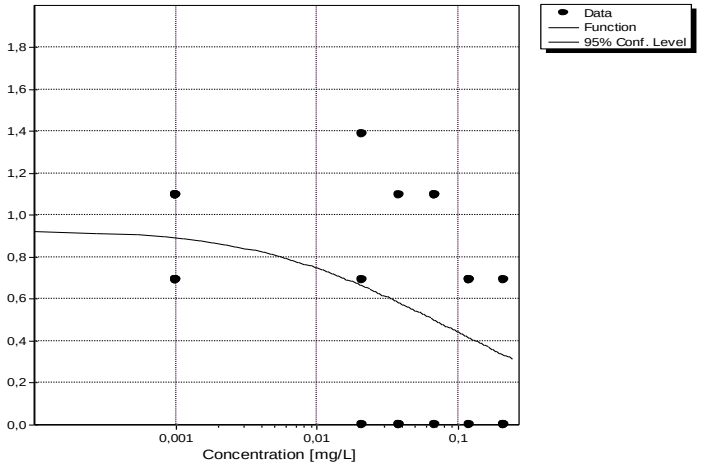

Fig. 8: Concentration-effect curve showing the influence of the test item on growth rate of the introduced Desmodesmus subspicatus as observed after 0 - 24 h

Effective Concentrations (ECx) for Growth Rate at 0 - 48 h

Growth Rate [1/d] of Desmodesmus subspicatus

Tab. 52: %Inhibition of growth rate caused by the test item after 0 - 48 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Inhibition
Control	1,300	0,0390	6	
0,021	1,185	0,2027	3	8,85
0,038	1,130	0,0817	3	13,08
0,068	0,947	0,0445	3	27,14
0,120	0,713	0,3764	3	45,17
0,210	0,116	0,2001	3	91,11

The 3-param. normal CDF F(x) = b0*[NormalCDF(b1-log10(x)/b2 + zOpt)] was fitted to the data (CDF: cumulative distribution function; b0-b2: parameters; zOpt: adjustment to have the EC10 as parameter b1; x: concentration).
A non-linear regression without weighting was performed.

Estimated parameters of the 3-param. normal CDF

Tab. 53: Estimated parameters of the 3-param. normal CDF with growth rate at 0 - 48 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	1,242	0,059	1,118	1,366	21,002	<0.001
b1	-1,286	0,113	-1,524	-1,049	-11,391	<0.001
b2	0,275	0,073	0,122	0,428	3,773	<0.001

Stop Reason = Iterations > Max. Iterations (Optimization method: Levenberg-Marquardt)

R^2: 0,785; adjusted R^2: 0,761.Residual standard error: 0,18111.Akaike Criterion (AIC): -43,501.Shapiro Wilk`s test on normal distribution of residuals: p = 0,159..

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Tab. 54: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with growth rate at 0 - 48 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	2,923	2	1,462	44,563	<0.001
Residuals	0,590	18	0,033		
- Lack of Fit	0,120	3	0,040	1,273	0,319
- Pure Error	0,471	15	0,031		
Total	3,726	20			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit..

Observed and Predicted Results of the 3-param. normal CDF

Tab. 55: Observed values in growth rate after 0 - 48 h as caused by the test item and predicted values as calculated from the function.

Treatm.[mg/L]	Observed	Mean Obs.	Predicted
0,001	1,320	1,3001	1,2420
0,001	1,354	1,3001	1,2420
0,001	1,320	1,3001	1,2420
0,001	1,282	1,3001	1,2420
0,001	1,242	1,3001	1,2420
0,001	1,282	1,3001	1,2420
0,021	1,417	1,1850	1,2378
0,021	1,040	1,1850	1,2378
0,021	1,099	1,1850	1,2378
0,038	1,199	1,1300	1,1942
0,038	1,040	1,1300	1,1942
0,038	1,151	1,1300	1,1942
0,068	0,973	0,9473	0,9962
0,068	0,973	0,9473	0,9962
0,068	0,896	0,9473	0,9962
0,120	0,693	0,7128	0,5972
0,120	0,347	0,7128	0,5972
0,120	1,099	0,7128	0,5972
0,210	0,000	0,1155	0,2182
0,210	0,000	0,1155	0,2182
0,210	0,347	0,1155	0,2182

Point estimates from the 3-param. normal CDF

Tab. 56: Point estimates from the 3-param. normal CDF with growth rate at 0 - 48 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	0,052	0,068	0,116
lower 95%-cl	0,030	0,041	0,061
upper 95%-cl	0,089	0,115	0,217

n.d.: not determined due to mathematical reasons

The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs)..

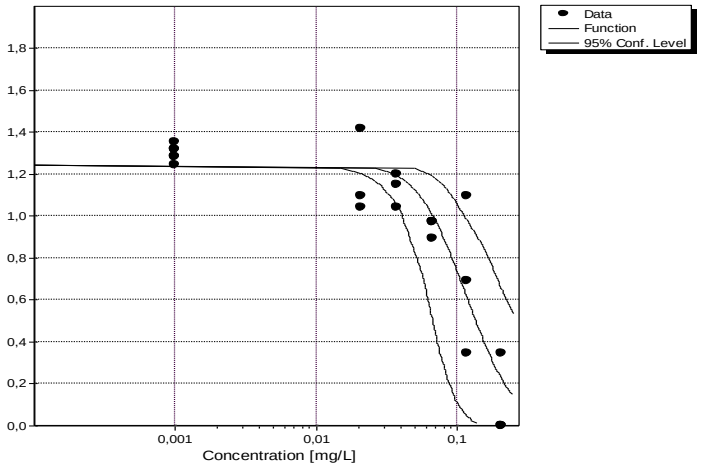

Fig. 9: Concentration-effect curve showing the influence of the test item on growth rate of the introduced Desmodesmus subspicatus as observed after 0 - 48 h

Effective Concentrations (ECx) for Growth Rate at 0 - 72 h

Growth Rate [1/d] of Desmodesmus subspicatus

Tab. 57: %Inhibition of growth rate caused by the test item after 0 - 72 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Inhibition
Control	1,360	0,0422	6	
0,021	1,259	0,0117	3	7,44
0,038	0,921	0,0534	3	32,25
0,068	0,809	0,1013	3	40,49
0,120	0,742	0,0537	3	45,46
0,210	0,646	0,0480	3	52,47

The 3-param. normal CDF F(x) = b0*[NormalCDF(b1-log10(x)/b2 + zOpt)] was fitted to the data (CDF: cumulative distribution function; b0-b2: parameters; zOpt: adjustment to have the EC10 as parameter b1; x: concentration).
A non-linear regression without weighting was performed.

The optimzation converged thus fitting was successful (Stop Reason = Converged (Optimization method: Levenberg-Marquardt)).

Estimated parameters of the 3-param. normal CDF

Tab. 58: Estimated parameters of the 3-param. normal CDF with growth rate at 0 - 72 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	1,386	0,046	1,290	1,483	30,278	<0.001
b1	-2,055	0,199	-2,473	-1,637	-10,326	<0.001
b2	0,946	0,153	0,625	1,266	6,190	<0.001

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0,931; adjusted R^2: 0,923.Residual standard error: 0,09020.Akaike Criterion (AIC): -72,777.Shapiro Wilk`s test on normal distribution of residuals: p = 0,678..

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Tab. 59: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with growth rate at 0 - 72 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	1,604	2	0,802	98,559	<0.001
Residuals	0,146	18	0,008		
- Lack of Fit	0,101	3	0,034	10,998	<0.001
- Pure Error	0,046	15	0,003		
Total	1,723	20			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) <= 0.05, lack of fit is significant. It is recommendes to choose more appropriate regression functions and/or settings..Since a signifcant lack of fit was found, it is recomended to try other dose/response functions..

Observed and Predicted Results of the 3-param. normal CDF

Tab. 60: Observed values in growth rate after 0 - 72 h as caused by the test item and predicted values as calculated from the function.

Treatm.[mg/L]	Observed	Mean Obs.	Predicted
0,001	1,376	1,3599	1,3707
0,001	1,365	1,3599	1,3707
0,001	1,348	1,3599	1,3707
0,001	1,381	1,3599	1,3707
0,001	1,407	1,3599	1,3707
0,001	1,283	1,3599	1,3707
0,021	1,246	1,2587	1,1248
0,021	1,261	1,2587	1,1248
0,021	1,269	1,2587	1,1248
0,038	0,981	0,9213	1,0110
0,038	0,880	0,9213	1,0110
0,038	0,903	0,9213	1,0110

0,068	0,880	0,80930,8794
0,068	0,693	0,80930,8794
0,068	0,855	0,80930,8794
0,120	0,693	0,74160,7387
0,120	0,799	0,74160,7387
0,120	0,732	0,74160,7387
0,210	0,649	0,64630,5971
0,210	0,693	0,64630,5971
0,210	0,597	0,64630,5971

Point estimates from the 3-param. normal CDF

Tab. 61: Point estimates from the 3-param. normal CDF with growth rate at 0 - 72 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	0,009	0,023	0,144
lower 95%-cl	0,003	0,009	0,042
upper 95%-cl	0,023	0,059	0,470

n.d.: not determined due to mathematical reasons

The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs)..

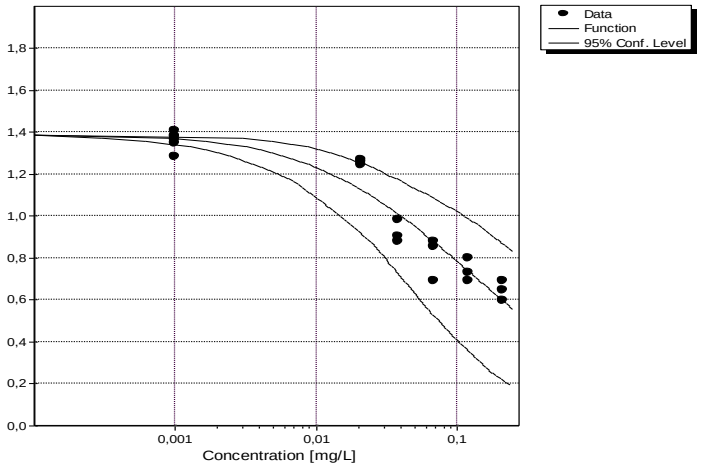

Fig. 10: Concentration-effect curve showing the influence of the test item on growth rate of the introduced Desmodesmus subspicatus as observed after 0 - 72 h

Effective Concentrations (ECx) for Growth Rate at 0 - 96 h

Growth Rate [1/d] of Desmodesmus subspicatus

Tab. 62: %Inhibition of growth rate caused by the test item after 0 - 96 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Inhibition
Control	1,357	0,0302	6	
0,021	1,121	0,0290	3	17,42
0,038	0,891	0,0593	3	34,35
0,068	0,687	0,0248	3	49,38
0,120	0,495	0,0512	3	63,57
0,210	0,265	0,0871	3	80,49

The 3-param. normal CDF F(x) = b0*[NormalCDF(b1-log10(x)/b2 + zOpt)] was fitted to the data (CDF: cumulative distribution function; b0-b2: parameters; zOpt: adjustment to have the EC10 as parameter b1; x: concentration).
A non-linear regression without weighting was performed.

Estimated parameters of the 3-param. normal CDF

Tab. 63: Estimated parameters of the 3-param. normal CDF with growth rate at 0 - 96 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	1,359	0,020	1,318	1,400	69,180	<0.001
b1	-1,909	0,053	-2,020	-1,798	-36,186	<0.001
b2	0,587	0,034	0,516	0,658	17,344	<0.001

Stop Reason = Iterations > Max. Iterations (Optimization method: Levenberg-Marquardt)

R^2: 0,984; adjusted R^2: 0,982.Residual standard error: 0,04745.Akaike Criterion (AIC): -99,754.Shapiro Wilk`s test on normal distribution of residuals: p = 0,606..

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Tab. 64: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with growth rate at 0 - 96 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	3,218	2	1,609	714,579	<0.001
Residuals	0,041	18	0,002		
- Lack of Fit	0,006	3	0,002	0,804	0,511
- Pure Error	0,035	15	0,002		
Total	3,270	20			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit..

Observed and Predicted Results of the 3-param. normal CDF

Tab. 65: Observed values in growth rate after 0 - 96 h as caused by the test item and predicted values as calculated from the function.

Treatm.[mg/L]	Observed	Mean Obs.	Predicted
0,001	1,409	1,3574	1,3579
0,001	1,360	1,3574	1,3579
0,001	1,346	1,3574	1,3579

0,001	1,334	1,35741,3579
0,001	1,325	1,35741,3579
0,001	1,371	1,35741,3579
0,021	1,154	1,12101,1045
0,021	1,099	1,12101,1045
0,021	1,111	1,12101,1045
0,038	0,952	0,89120,9150
0,038	0,889	0,89120,9150
0,038	0,833	0,89120,9150
0,068	0,660	0,68710,6896
0,068	0,708	0,68710,6896
0,068	0,693	0,68710,6896
0,120	0,448	0,49460,4674
0,120	0,486	0,49460,4674
0,120	0,549	0,49460,4674
0,210	0,347	0,26480,2818
0,210	0,173	0,26480,2818
0,210	0,275	0,26480,2818

Point estimates from the 3-param. normal CDF

Tab. 66: Point estimates from the 3-param. normal CDF with growth rate at 0 - 96 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	0,012	0,022	0,070
lower 95%-cl	0,010	0,018	0,052
upper 95%-cl	0,016	0,029	0,094

n.d.: not determined due to mathematical reasons

The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs)..

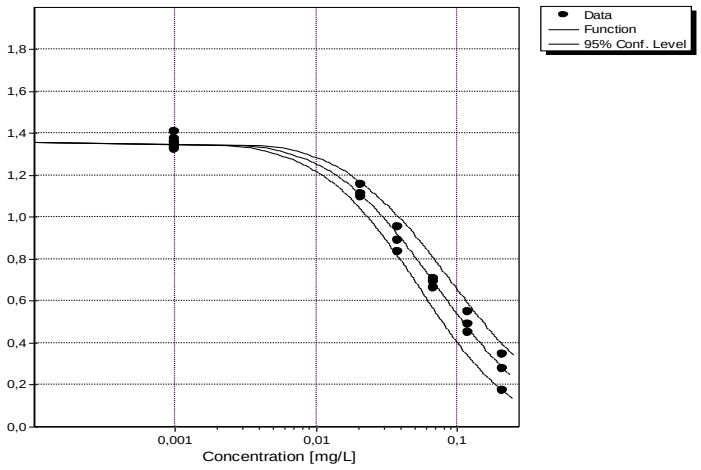

Fig. 11: Concentration-effect curve showing the influence of the test item on growth rate of the introduced Desmodesmus subspicatus as observed after 0 - 96 h

Overview over the ECs of the Test Item on Growth Rate

Effects on Growth Rate

Tab. 67: Growth rate (G) and its inhibition relative to control (%I) as computed from the raw data for test intervals selected; *nl with growth rate: nonlinear regression using the 3-param. normal CDF.

Treatment	0-24 h		0-48 h		0-72 h		0-96 h	
[mg/L]	G	%I	G	%I	G	%I	G	%I
Control	0,896	0,00	1,300	0,00	1,360	0,00	1,357	0,00
0,021	0,693	22,63	1,185	8,85	1,259	7,44	1,121	17,42
0,038	0,366	59,12	1,130	13,08	0,921	32,25	0,891	34,35
0,068	0,732	18,25	0,947	27,14	0,809	40,49	0,687	49,38
0,120	0,462	48,42	0,713	45,17	0,742	45,46	0,495	63,57
0,210	0,231	74,21	0,116	91,11	0,646	52,47	0,265	80,49
EC10	0,004	*nl	0,052	*nl	0,009	*nl	0,012	*nl
lower 95%-cl	n.d.		0,030		0,003		0,010	
upper 95%-cl	n.d.		0,089		0,023		0,016	
EC20	0,011	*nl	0,068	*nl	0,023	*nl	0,022	*nl
lower 95%-cl	n.d.		0,041		0,009		0,018	
upper 95%-cl	n.d.		0,115		0,059		0,029	
EC50	0,088	*nl	0,116	*nl	0,144	*nl	0,070	*nl
lower 95%-cl	n.d.		0,061		0,042		0,052	
upper 95%-cl	n.d.		0,217		0,470		0,094	

Threshold concentrations (NOEC) for Growth Rate at 0 - 24 h

Statistical Characteristics of the Samples

Tab. 68: Statistical characteristics with growth rate at 0 - 24 h: Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [mg/L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Control	0,896	0,896	0,693	1,099	6	0,2221	24,8	0,0907	10,1	0,663	1,129
0,021	0,693	0,693	0,000	1,386	3	0,6931	100,0	0,4002	57,7	-1,029	2,415
0,038	0,366	0,000	0,000	1,099	3	0,6343	173,2	0,3662	100,0	-1,209	1,942
0,068	0,732	1,099	0,000	1,099	3	0,6343	86,6	0,3662	50,0	-0,843	2,308
0,120	0,462	0,693	0,000	0,693	3	0,4002	86,6	0,2310	50,0	-0,532	1,456
0,210	0,231	0,000	0,000	0,693	3	0,4002	173,2	0,2310	100,0	-0,763	1,225

Shapiro-Wilk´s Test on Normal Distribution

Tab. 69: Shapiro-Wilk´s Test on Normal Distribution with growth rate at 0 - 24 h: Mean: arithmetic mean; n: sample size; p(ShapiroWilk´s W): probability of the W statistic (i.e. that the observed deviations from the normal distributions are dues to chance). In case p(ShapiroWilk´s W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [mg/L]	Mean	s	n
Control	0,896	0,2221	6
0,021	0,693	0,6931	3
0,038	0,366	0,6343	3
0,068	0,732	0,6343	3
0,120	0,462	0,4002	3
0,210	0,231	0,4002	3

Results:

Number of residuals = 13; Shapiro-Wilk´s W = 0,952; p(W) = 0,626; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Normality check was passed (p > 0,01).

Levene´s Test on Variance Homogeneity (with Residuals)

Tab. 70: Levene´s Test on Variance Homogeneity (with Residuals) with growth rate at 0 - 24 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p: probability that the variance explained by the treatment is due to chance

Source	SS	df	MSS	F	p(F)
Treatment	0,29305	5	0,05861	1,542	0,236
Residuals	0,57029	15	0,03802		
Total	0,8633	20			

The Levene test indicates variance homogeneity (p > 0,010).

Variance homogeneity check was passed (p > 0,01).

Normal-distribution and variance-homogeneity requirements are fulfilled.
A parametric multiple test is advisable.

To justify the use of Williams test at first a trend analysis by contrasts is performed.

Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 71: Trend analysis by contrasts (monotonicity of concentration/response) with growth rate at 0 - 24 h: Psi: sum of means weighted by contrasts; s(psi): standard error of psi; df: degrees of freedom; t: t-statistic; p(t): probability that the trend is due to chance (Ho: Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	s(psi)	df	t	p(t)
Linear	3,65110	2,10185	15	1,737	0,051
Quadratic	-0,08495	2,34380	15	-0,036	0,486

The linear trend is not significant (p > 0,05) The quadratic trend is not significant (p > 0,05)

The analysis of contrasts did not reveal a linear trend, thus the selected Williams test was replaced by Dunnett test.

Dunnett`s Multiple t-test Procedure

Tab. 72: Dunnett´s multiple t-test procedure with growth rate at 0 - 24 h: Comparison of treatments with "Control". Significance was Alpha = 0,050, one-sided smaller (multiple level); Mean: arithmetic mean; n: sample size; s: standard deviation; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; t*: critical t for Ho: µ1 = µ2 = ... = µk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments).

Treatm. [mg/L]	Mean	s	df	%MDD	t	t*	Sign.
Control	0,896	0,48010					
0,021	0,693	0,48010	15	-95,26	-0,60	-2,51	-
0,038	0,366	0,48010	15	-95,26	-1,56	-2,51	-
0,068	0,732	0,48010	15	-95,26	-0,48	-2,51	-
0,120	0,462	0,48010	15	-95,26	-1,28	-2,51	-
0,210	0,231	0,48010	15	-95,26	-1,96	-2,51	-

+: significant; -: non-significant

The NOEC appears to be higher than or equal 0,210 mg/L.

Threshold concentrations (NOEC) for Growth Rate at 0 - 48 h

Statistical Characteristics of the Samples

Tab. 73: Statistical characteristics with growth rate at 0 - 48 h: Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [mg/L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Control	1,300	1,301	1,242	1,354	6	0,0390	3,0	0,0159	1,2	1,259	1,341
0,021	1,185	1,099	1,040	1,417	3	0,2027	17,1	0,1171	9,9	0,681	1,689
0,038	1,130	1,151	1,040	1,199	3	0,0817	7,2	0,0472	4,2	0,927	1,333
0,068	0,947	0,973	0,896	0,973	3	0,0445	4,7	0,0257	2,7	0,837	1,058
0,120	0,713	0,693	0,347	1,099	3	0,3764	52,8	0,2173	30,5	-0,222	1,648

0,210	0,116	0,000	0,000	0,347	3	0,2001	173,2	0,1155	100,0	-0,382	0,613

Shapiro-Wilk´s Test on Normal Distribution

Tab. 74: Shapiro-Wilk´s Test on Normal Distribution with growth rate at 0 - 48 h: Mean: arithmetic mean; n: sample size; p(ShapiroWilk´s W): probability of the W statistic (i.e. that the observed deviations from the normal distributions are dues to chance). In case p(ShapiroWilk´s W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [mg/L]	Mean	s	n
Control	1,300	0,0390	6
0,021	1,185	0,2027	3
0,038	1,130	0,0817	3
0,068	0,947	0,0445	3
0,120	0,713	0,3764	3
0,210	0,116	0,2001	3

Results:

Number of residuals = 17; Shapiro-Wilk´s W = 0,939; p(W) = 0,304; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Normality check was passed (p > 0,01).

Levene´s Test on Variance Homogeneity (with Residuals)

Tab. 75: Levene´s Test on Variance Homogeneity (with Residuals) with growth rate at 0 - 48 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p: probability that the variance explained by the treatment is due to chance

Source	SS	df	MSS	F	p(F)
Treatment	0,13793	5	0,02759	3,788	0,020
Residuals	0,10924	15	0,00728		
Total	0,2472	20			

The Levene test indicates variance homogeneity (p > 0,010).

Variance homogeneity check was passed (p > 0,01).

Normal-distribution and variance-homogeneity requirements are fulfilled.
A parametric multiple test is advisable.

To justify the use of Williams test at first a trend analysis by contrasts is performed.

Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 76: Trend analysis by contrasts (monotonicity of concentration/response) with growth rate at 0 - 48 h: Psi: sum of means weighted by contrasts; s(psi): standard error of psi; df: degrees of freedom; t: t-statistic; p(t): probability that the trend is due to chance (Ho: Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	s(psi)	df	t	p(t)
Linear	7,52211	0,77543	15	9,701	<0.001
Quadratic	3,12873	0,86469	15	3,618	0,001

The linear trend is significant (p <= 0,05) The quadratic trend is significant (p <= 0,05)

The analysis of contrasts revealed a linear trend, thus the selected Williams test was performed.

Williams Multiple Sequential t-test Procedure

Tab. 77: Comparison of treatments with "Control" by the t test procedure after Williams with growth rate at 0 - 48 h: Significance was Alpha = 0,050, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [mg/L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	1,300	0,17712						
0,021	1,185	0,17712	15	1,185	-16,89	-0,92	-1,75	-
0,038	1,130	0,17712	15	1,130	-17,58	-1,36	-1,83	-
0,068	0,947	0,17712	15	0,947	-17,80	-2,82	-1,85	+
0,120	0,713	0,17712	15	0,713	-17,89	-4,69	-1,86	+
0,210	0,116	0,17712	15	0,116	-17,98	-9,46	-1,87	+

+: significant; -: non-significant

A NOEC of 0,038 mg/L is suggested by the program.

Threshold concentrations (NOEC) for Growth Rate at 0 - 72 h

Statistical Characteristics of the Samples

Tab. 78: Statistical characteristics with growth rate at 0 - 72 h: Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [mg/L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Control	1,360	1,370	1,283	1,407	6	0,0422	3,1	0,0172	1,3	1,316	1,404
0,021	1,259	1,261	1,246	1,269	3	0,0117	0,9	0,0068	0,5	1,230	1,288
0,038	0,921	0,903	0,880	0,981	3	0,0534	5,8	0,0308	3,3	0,789	1,054
0,068	0,809	0,855	0,693	0,880	3	0,1013	12,5	0,0585	7,2	0,558	1,061
0,120	0,742	0,732	0,693	0,799	3	0,0537	7,2	0,0310	4,2	0,608	0,875
0,210	0,646	0,649	0,597	0,693	3	0,0480	7,4	0,0277	4,3	0,527	0,766

Shapiro-Wilk´s Test on Normal Distribution

Tab. 79: Shapiro-Wilk´s Test on Normal Distribution with growth rate at 0 - 72 h: Mean: arithmetic mean; n: sample size; p(ShapiroWilk´s W): probability of the W statistic (i.e. that the observed deviations from the normal distributions are dues to chance). In case p(ShapiroWilk´s W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [mg/L]	Mean	s	n
Control	1,360	0,0422	6
0,021	1,259	0,0117	3
0,038	0,921	0,0534	3
0,068	0,809	0,1013	3
0,120	0,742	0,0537	3

0,210	0,646	0,0480	3

Results:

Number of residuals = 21; Shapiro-Wilk´s W = 0,956; p(W) = 0,443; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Normality check was passed (p > 0,01).

Levene´s Test on Variance Homogeneity (with Residuals)

Tab. 80: Levene´s Test on Variance Homogeneity (with Residuals) with growth rate at 0 - 72 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p: probability that the variance explained by the treatment is due to chance

Source	SS	df	MSS	F	p(F)
Treatment	0,00775	5	0,00155	2,359	0,091
Residuals	0,00985	15	0,00066		
Total	0,0176	20			

The Levene test indicates variance homogeneity (p > 0,010).

Variance homogeneity check was passed (p > 0,01).

Normal-distribution and variance-homogeneity requirements are fulfilled.
A parametric multiple test is advisable.

To justify the use of Williams test at first a trend analysis by contrasts is performed.

Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 81: Trend analysis by contrasts (monotonicity of concentration/response) with growth rate at 0 - 72 h: Psi: sum of means weighted by contrasts; s(psi): standard error of psi; df: degrees of freedom; t: t-statistic; p(t): probability that the trend is due to chance (Ho: Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	s(psi)	df	t	p(t)
Linear	5,23086	0,24184	15	21,630	<0.001
Quadratic	-1,10842	0,26968	15	-4,110	<0.001

The linear trend is significant (p <= 0,05) The quadratic trend is significant (p <= 0,05)

The analysis of contrasts revealed a linear trend, thus the selected Williams test was performed.

Williams Multiple Sequential t-test Procedure

Tab. 82: Comparison of treatments with "Control" by the t test procedure after Williams with growth rate at 0 - 72 h: Significance was Alpha = 0,050, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [mg/L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	1,360	0,05524						
0,021	1,259	0,05524	15	1,259	-5,04	-2,59	-1,75	+
0,038	0,921	0,05524	15	0,921	-5,24	-11,23	-1,83	+

0,068	0,809	0,05524	15	0,809	-5,31	-14,10	-1,85	+
0,120	0,742	0,05524	15	0,742	-5,33	-15,83	-1,86	+
0,210	0,646	0,05524	15	0,646	-5,36	-18,27	-1,87	+

+: significant; -: non-significant

The NOEC is lower than 0,021 mg/L.

Threshold concentrations (NOEC) for Growth Rate at 0 - 96 h

Statistical Characteristics of the Samples

Tab. 83: Statistical characteristics with growth rate at 0 - 96 h: Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [mg/L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Control	1,357	1,353	1,325	1,409	6	0,0302	2,2	0,0123	0,9	1,326	1,389
0,021	1,121	1,111	1,099	1,154	3	0,0290	2,6	0,0167	1,5	1,049	1,193
0,038	0,891	0,889	0,833	0,952	3	0,0593	6,7	0,0343	3,8	0,744	1,039
0,068	0,687	0,693	0,660	0,708	3	0,0248	3,6	0,0143	2,1	0,625	0,749
0,120	0,495	0,486	0,448	0,549	3	0,0512	10,3	0,0295	6,0	0,367	0,622
0,210	0,265	0,275	0,173	0,347	3	0,0871	32,9	0,0503	19,0	0,049	0,481

Shapiro-Wilk´s Test on Normal Distribution

Tab. 84: Shapiro-Wilk´s Test on Normal Distribution with growth rate at 0 - 96 h: Mean: arithmetic mean; n: sample size; p(ShapiroWilk´s W): probability of the W statistic (i.e. that the observed deviations from the normal distributions are dues to chance). In case p(ShapiroWilk´s W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [mg/L]	Mean	s	n
Control	1,357	0,0302	6
0,021	1,121	0,0290	3
0,038	0,891	0,0593	3
0,068	0,687	0,0248	3
0,120	0,495	0,0512	3
0,210	0,265	0,0871	3

Results:

Number of residuals = 21; Shapiro-Wilk´s W = 0,987; p(W) = 0,988; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Normality check was passed (p > 0,01).

Levene´s Test on Variance Homogeneity (with Residuals)

Tab. 85: Levene´s Test on Variance Homogeneity (with Residuals) with growth rate at 0 - 96 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p: probability that the variance explained by the treatment is due to chance

Source	SS	df	MSS	F	p(F)
Treatment	0,00423	5	0,00085	1,345	0,299
Residuals	0,00943	15	0,00063		
Total	0,0137	20			

The Levene test indicates variance homogeneity (p > 0,010).

Variance homogeneity check was passed (p > 0,01).

Normal-distribution and variance-homogeneity requirements are fulfilled.
A parametric multiple test is advisable.

To justify the use of Williams test at first a trend analysis by contrasts is performed.

Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 86: Trend analysis by contrasts (monotonicity of concentration/response) with growth rate at 0 - 96 h: Psi: sum of means weighted by contrasts; s(psi): standard error of psi; df: degrees of freedom; t: t-statistic; p(t): probability that the trend is due to chance (Ho: Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	s(psi)	df	t	p(t)
Linear	7,54634	0,21122	15	35,728	<0.001
Quadratic	-0,18266	0,23553	15	-0,776	0,225

The linear trend is significant (p <= 0,05) The quadratic trend is not significant (p > 0,05)

The analysis of contrasts revealed a linear trend, thus the selected Williams test was performed.

Williams Multiple Sequential t-test Procedure

Tab. 87: Comparison of treatments with "Control" by the t test procedure after Williams with growth rate at 0 - 96 h: Significance was Alpha = 0,050, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [mg/L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	1,357	0,04825						
0,021	1,121	0,04825	15	1,121	-4,41	-6,93	-1,75	+
0,038	0,891	0,04825	15	0,891	-4,59	-13,67	-1,83	+
0,068	0,687	0,04825	15	0,687	-4,64	-19,65	-1,85	+
0,120	0,495	0,04825	15	0,495	-4,67	-25,29	-1,86	+
0,210	0,265	0,04825	15	0,265	-4,69	-32,03	-1,87	+

+: significant; -: non-significant

The NOEC is lower than 0,021 mg/L.

Overview over the Effect-Thresholds of the Test Item on Growth Rate

Overview over the LOEC and NOEC Determination

Tab. 88: Overview over the LOEC and NOEC Determination with growth rate: Arithmetic means and significance marks as

computed for growth rate for all inspection intervals (top); bottom part: obtained LOEC and NOEC with indication of statistical test used; *dt: Dunnett´s multiple t-test procedure; *wl: Williams multiple sequential t-test procedure, significance level was 0,050, one-sided smaller.

Treatm. [mg/L]	0-0 - 24 h	0-0 - 48 h	0-0 - 72 h	0-0 - 96 h
0,021	0,693 -	1,185 -	1,259+	1,121+
0,038	0,366 -	1,130 -	0,921+	0,891+
0,068	0,732 -	0,947+	0,809+	0,687+
0,120	0,462 -	0,713+	0,742+	0,495+
0,210	0,231 -	0,116+	0,646+	0,265+

Tab. 88 (continued): Overview over the LOEC and NOEC Determination with growth rate: Arithmetic means and significance marks as computed for growth rate for all inspection intervals (top); bottom part: obtained LOEC and NOEC with indication of statistical test used; *dt: Dunnett´s multiple t-test procedure; *wl: Williams multiple sequential t-test procedure, significance level was 0,050, one-sided smaller.

LOEC	>0,210 *dt	0,068 *wl	<=0,021 *wl	<=0,021 *wl
NOEC	>=0,210 *dt	0,038 *wl	<0,021 *wl	<0,021 *wl

+: Significant difference to control (p <=0,050)

Section-by-section growth rate (1/d) in Desmodesmus subspicatus as Dependent on Concentration and Time

Tab. 89: Section-by-section growth rate (1/d) in Desmodesmus subspicatus as dependent on concentration of the test item and time ; Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (calculated from the cell number variable)

Treatm. [mg/L]	**Control**	**0,021**	**0,038**	**0,068**	**0,120**	**0,210**
0 - 0 h	0,000	0,000	0,000	0,000	0,000	0,000
	0,000	0,000	0,000	0,000	0,000	0,000
	0,000	0,000	0,000	0,000	0,000	0,000
	0,000	-	-	-	-	-
	0,000	-	-	-	-	-
	0,000	-	-	-	-	-
Mean:	0,000	0,000	0,000	0,000	0,000	0,000
Std.Dev.:	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
n:	6	3	3	3	3	3
CV:						
0 - 24 h	1,099	0,000	0,000	1,099	0,693	0,000
	1,099	1,386	0,000	1,099	0,000	0,000
	0,693	0,693	1,099	0,000	0,693	0,693
	0,693	-	-	-	-	-
	0,693	-	-	-	-	-
	1,099	-	-	-	-	-
Mean:	0,896	0,693	0,366	0,732	0,462	0,231
Std.Dev.:	0,2221	0,6931	0,6343	0,6343	0,4002	0,4002
n:	6	3	3	3	3	3
CV:	24,79	100,00	173,21	86,60	86,60	173,21

Tab. 89 (continued): Section-by-section growth rate (1/d) in Desmodesmus subspicatus as dependent on concentration of the test item and time ; Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (calculated from the cell number variable)

24 - 48 h	1,540	2,833	2,398	0,847	0,693	0,000
	1,609	0,693	2,079	0,847	0,693	0,000
	1,946	1,504	1,204	1,792	1,504	0,000
	1,872	-	-	-	-	-
	1,792	-	-	-	-	-
	1,466	-	-	-	-	-
Mean:	1,704	1,677	1,894	1,162	0,963	0,000
Std.Dev.:	0,1932	1,0804	0,6182	0,5453	0,4682	0,0000
n:	6	3	3	3	3	3
CV:	11,33	64,43	32,65	46,92	48,59	173,21
48 - 72 h	1,488	0,904	0,547	0,693	0,693	1,946
	1,386	1,705	0,560	0,134	1,705	2,079
	1,404	1,609	0,405	0,773	0,000	1,099
	1,578	-	-	-	-	-
	1,735	-	-	-	-	-
	1,285	-	-	-	-	-
Mean:	1,479	1,406	0,504	0,533	0,799	1,708
Std.Dev.:	0,1594	0,4371	0,0855	0,3485	0,8573	0,5319
n:	6	3	3	3	3	3
CV:	10,77	31,09	16,96	65,35	107,26	31,14
72 - 96 h	1,508	0,877	0,862	0,000	-0,288	-0,560
	1,344	0,610	0,916	0,754	-0,452	-1,386
	1,341	0,636	0,624	0,208	0,000	-0,693
	1,194	-	-	-	-	-
	1,079	-	-	-	-	-
	1,635	-	-	-	-	-

Tab. 89 (continued): Section-by-section growth rate (1/d) in Desmodesmus subspicatus as dependent on concentration of the test item and time ; Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (calculated from the cell number variable)

Mean:	1,350	0,708	0,801	0,320	-0,247	-0,880
Std.Dev.:	0,2019	0,1474	0,1554	0,3893	0,2288	0,4438
n:	6	3	3	3	3	3
CV:	14,95	20,82	19,41	121,49	92,79	50,45

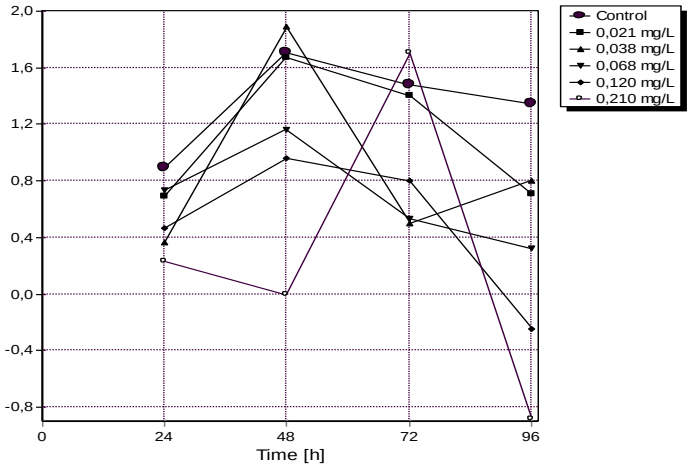

Fig. 12: Sectional growth rate in Desmodesmus subspicatus as dependent on test item concentration and time.

Effective Concentrations (ECx) for Sectional Growth Rate at 0 - 24 h

Sectional Growth Rate [1/d] of Desmodesmus subspicatus

Tab. 90: %Inhibition of sectional growth rate caused by the test item after 0 - 24 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Inhibition
Control	0,896	0,2221	6	
0,021	0,693	0,6931	3	22,63
0,038	0,366	0,6343	3	59,12
0,068	0,732	0,6343	3	18,25
0,120	0,462	0,4002	3	48,42
0,210	0,231	0,4002	3	74,21

The 3-param. normal CDF F(x) = b0*[NormalCDF(b1-log10(x)/b2 + zOpt)] was fitted to the data (CDF: cumulative distribution function; b0-b2: parameters; zOpt: adjustment to have the EC10 as parameter b1; x:

concentration).
A non-linear regression without weighting was performed.
The optimzation converged thus fitting was successful (Stop Reason = Converged (Optimization method: Levenberg-Marquardt)).

Estimated parameters of the 3-param. normal CDF

Tab. 91: Estimated parameters of the 3-param. normal CDF with sectional growth rate at 0 - 24 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	0,925	0,336	0,218	1,632	2,748	0,007
b1	-2,437	2,257	-7,179	2,305	-1,080	0,147
b2	1,077	1,468	-2,007	4,160	0,733	0,236

Stop Reason = Converged (Optimization method: Levenberg-Marquardt)

R^2: 0,184; adjusted R^2: 0,094.Residual standard error: 0,45969.Akaike Criterion (AIC): -4,379.Shapiro Wilk`s test on normal distribution of residuals: p = 0,377..

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Tab. 92: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with sectional growth rate at 0 - 24 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	0,864	2	0,432	2,043	0,159
Residuals	3,804	18	0,211		
- Lack of Fit	0,346	3	0,115	0,501	0,687
- Pure Error	3,457	15	0,230		
Total	4,688	20			

Since p(F|Regression) > 0.05, the amount of variance explained by the regression model is NOT significant..Therefore, confidence limits cannot be provided..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit..

Observed and Predicted Results of the 3-param. normal CDF

Tab. 93: Observed values in sectional growth rate after 0 - 24 h as caused by the test item and predicted values as calculated from the function.

Treatm.[mg/L]	Observed	Mean Obs.	Predicted
0,001	1,099	0,8959	0,8918
0,001	1,099	0,8959	0,8918
0,001	0,693	0,8959	0,8918
0,001	0,693	0,8959	0,8918
0,001	0,693	0,8959	0,8918
0,001	1,099	0,8959	0,8918
0,021	0,000	0,6931	0,6637
0,021	1,386	0,6931	0,6637
0,021	0,693	0,6931	0,6637
0,038	0,000	0,3662	0,5844
0,038	0,000	0,3662	0,5844
0,038	1,099	0,3662	0,5844

0,068	1,099	0,73240,5000
0,068	1,099	0,73240,5000
0,068	0,000	0,73240,5000
0,120	0,693	0,46210,4157
0,120	0,000	0,46210,4157
0,120	0,693	0,46210,4157
0,210	0,000	0,23100,3349
0,210	0,000	0,23100,3349
0,210	0,693	0,23100,3349

Point estimates from the 3-param. normal CDF

Tab. 94: Point estimates from the 3-param. normal CDF with sectional growth rate at 0 - 24 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	0,004	0,011	0,088
lower 95%-cl	n.d.	n.d.	n.d.
upper 95%-cl	n.d.	n.d.	n.d.

n.d.: not determined due to mathematical reasons

Since a signifcant lack of fit was found, it is recomended to try other dose/response functions..

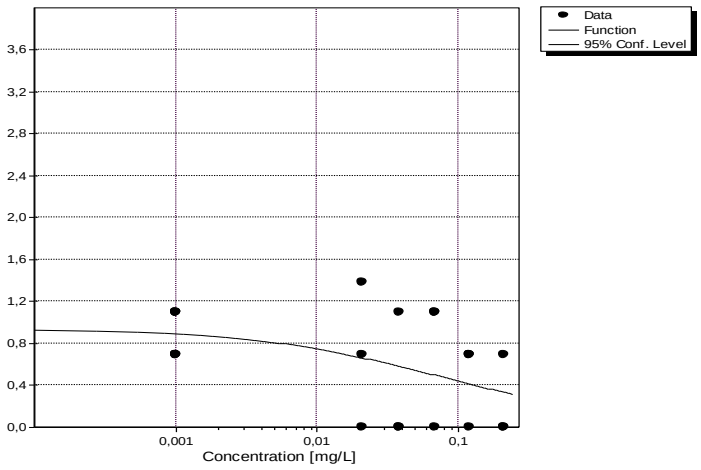

Fig. 13: Concentration-effect curve showing the influence of the test item on sectional growth rate of the introduced Desmodesmus subspicatus as observed after 0 - 24 h

Effective Concentrations (ECx) for Sectional Growth Rate at 24 - 48 h

Sectional Growth Rate [1/d] of Desmodesmus subspicatus

Tab. 95: %Inhibition of sectional growth rate caused by the test item after 24 - 48 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Inhibition
Control	1,704	0,1932	6	
0,021	1,677	1,0804	3	1,61
0,038	1,894	0,6182	3	-11,12
0,068	1,162	0,5453	3	31,81
0,120	0,963	0,4682	3	43,47
0,210	0,000	0,0000	3	100,00

The 3-param. normal CDF F(x) = b0*[NormalCDF(b1-log10(x)/b2 + zOpt)] was fitted to the data (CDF: cumulative distribution function; b0-b2: parameters; zOpt: adjustment to have the EC10 as parameter b1; x: concentration).
A non-linear regression without weighting was performed.

Estimated parameters of the 3-param. normal CDF

Tab. 96: Estimated parameters of the 3-param. normal CDF with sectional growth rate at 24 - 48 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	1,711	0,162	1,371	2,051	10,572	<0.001
b1	-1,258	0,200	-1,679	-0,837	-6,276	<0.001
b2	0,233	0,129	-0,038	0,504	1,804	0,044

Stop Reason = Iterations > Max. Iterations (Optimization method: Levenberg-Marquardt)

R^2: 0,535; adjusted R^2: 0,483.Residual standard error: 0,52127.Akaike Criterion (AIC): 0,901.Shapiro Wilk`s test on normal distribution of residuals: p = 0,971..

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Tab. 97: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with sectional growth rate at 24 - 48 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	6,55	2	3,28	12,055	<0.001
Residuals	4,89	18	0,27		
- Lack of Fit	0,57	3	0,19	0,663	0,588
- Pure Error	4,32	15	0,29		
Total	12,25	20			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) > 0.05, there is no significant lack of fit..

Observed and Predicted Results of the 3-param. normal CDF

Tab. 98: Observed values in sectional growth rate after 24 - 48 h as caused by the test item and predicted values as calculated from the function.

Treatm.[mg/L]	Observed	Mean Obs.	Predicted
0,001	1,540	1,7043	1,7108
0,001	1,609	1,7043	1,7108
0,001	1,946	1,7043	1,7108
0,001	1,872	1,7043	1,7108

0,001	1,792	1,7043 1,7108
0,001	1,466	1,7043 1,7108
0,021	2,833	1,6768 1,7091
0,021	0,693	1,6768 1,7091
0,021	1,504	1,6768 1,7091
0,038	2,398	1,8938 1,6700
0,038	2,079	1,8938 1,6700
0,038	1,204	1,8938 1,6700
0,068	0,847	1,1621 1,3934
0,068	0,847	1,1621 1,3934
0,068	1,792	1,1621 1,3934
0,120	0,693	0,9635 0,7433
0,120	0,693	0,9635 0,7433
0,120	1,504	0,9635 0,7433
0,210	0,000	0,0000 0,1939
0,210	0,000	0,0000 0,1939
0,210	0,000	0,0000 0,1939

Point estimates from the 3-param. normal CDF

Tab. 99: Point estimates from the 3-param. normal CDF with sectional growth rate at 24 - 48 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	0,055	0,070	0,110
lower 95%-cl	0,021	0,028	0,036
upper 95%-cl	0,146	0,177	0,338

n.d.: not determined due to mathematical reasons

The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs)..

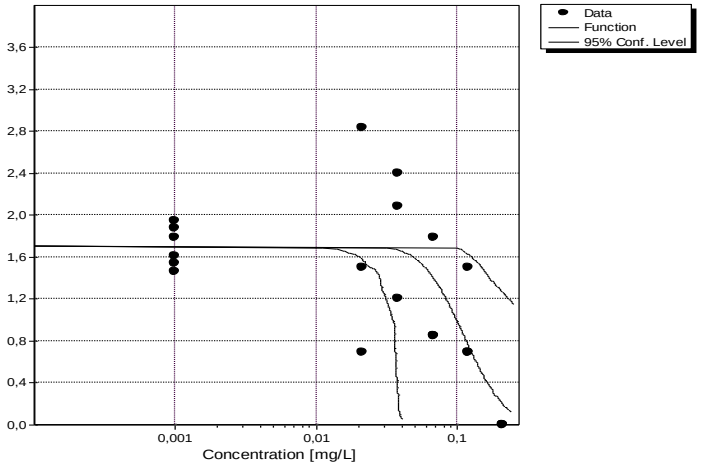

Fig. 14: Concentration-effect curve showing the influence of the test item on sectional growth rate of the introduced Desmodesmus subspicatus as observed after 24 - 48 h

Effective Concentrations (ECx) for Sectional Growth Rate at 48 - 72 h

Sectional Growth Rate [1/d] of Desmodesmus subspicatus

Tab. 100: %Inhibition of sectional growth rate caused by the test item after 48 - 72 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Inhibition
Control	1,479	0,1594	6	
0,021	1,406	0,4371	3	4,95
0,038	0,504	0,0855	3	65,94
0,068	0,533	0,3485	3	63,95
0,120	0,799	0,8573	3	45,97
0,210	1,708	0,5319	3	-15,45

The 3-param. normal CDF F(x) = b0*[NormalCDF(b1-log10(x)/b2 + zOpt)] was fitted to the data (CDF: cumulative distribution function; b0-b2: parameters; zOpt: adjustment to have the EC10 as parameter b1; x: concentration).
A non-linear regression without weighting was performed.

Estimated parameters of the 3-param. normal CDF

Tab. 101: Estimated parameters of the 3-param. normal CDF with sectional growth rate at 48 - 72 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	3,914	0,759	2,319	5,509	5,155	<0.001
b1	-11,312	1,339	-14,126	-8,498	-8,445	<0.001
b2	5,185	0,735	3,640	6,730	7,050	<0.001

Stop Reason = Iterations > Max. Iterations (Optimization method: Levenberg-Marquardt)

R^2: 0,151; adjusted R^2: 0,057.Residual standard error: 0,61058.Akaike Criterion (AIC): 7,542.Shapiro Wilk`s test on normal distribution of residuals: p = 0,582..

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Tab. 102: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with sectional growth rate at 48 - 72 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	1,108	2	0,554	1,485	0,253
Residuals	6,710	18	0,373		
- Lack of Fit	3,908	3	1,303	6,972	0,004
- Pure Error	2,803	15	0,187		
Total	7,339	20			

Since p(F|Regression) > 0.05, the amount of variance explained by the regression model is NOT significant..Therefore, confidence limits cannot be provided..Since p(F|Lack of Fit) <= 0.05, lack of fit is significant. It is recommendes to choose more appropriate regression functions and/or settings..Since a signifcant lack of fit was found, it is recomended to try other dose/response functions..

Observed and Predicted Results of the 3-param. normal CDF

Tab. 103: Observed values in sectional growth rate after 48 - 72 h as caused by the test item and predicted values as calculated from the function.

Treatm.[mg/L]	Observed	Mean Obs.	Predicted
0,001	1,488	1,4794	1,4631
0,001	1,386	1,4794	1,4631
0,001	1,404	1,4794	1,4631
0,001	1,578	1,4794	1,4631
0,001	1,735	1,4794	1,4631
0,001	1,285	1,4794	1,4631
0,021	0,904	1,4062	1,1039
0,021	1,705	1,4062	1,1039
0,021	1,609	1,4062	1,1039
0,038	0,547	0,5039	1,0392
0,038	0,560	0,5039	1,0392
0,038	0,405	0,5039	1,0392
0,068	0,693	0,5333	0,9776
0,068	0,134	0,5333	0,9776
0,068	0,773	0,5333	0,9776
0,120	0,693	0,7993	0,9194
0,120	1,705	0,7993	0,9194
0,120	0,000	0,7993	0,9194
0,210	1,946	1,7080	0,8640
0,210	2,079	1,7080	0,8640
0,210	1,099	1,7080	0,8640

Point estimates from the 3-param. normal CDF

Tab. 104: Point estimates from the 3-param. normal CDF with sectional growth rate at 48 - 72 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	0,000	0,000	0,000
lower 95%-cl	n.d.	n.d.	n.d.
upper 95%-cl	n.d.	n.d.	n.d.

n.d.: not determined due to mathematical reasons

Since a signifcant lack of fit was found, it is recomended to try other dose/response functions..

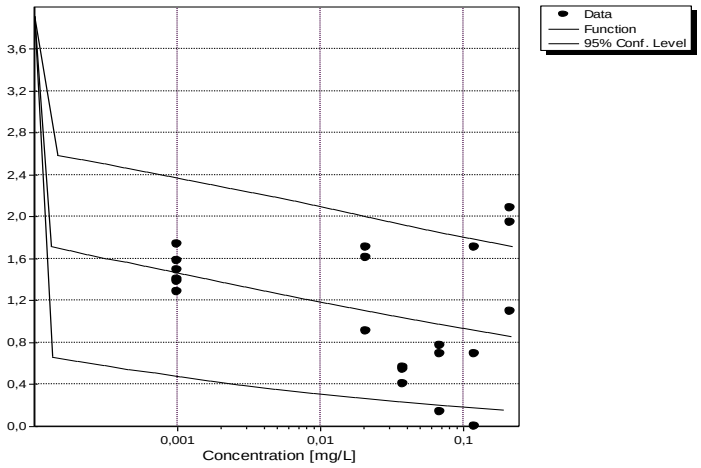

Fig. 15: Concentration-effect curve showing the influence of the test item on sectional growth rate of the introduced Desmodesmus subspicatus as observed after 48 - 72 h

Effective Concentrations (ECx) for Sectional Growth Rate at 72 - 96 h

Sectional Growth Rate [1/d] of Desmodesmus subspicatus

Tab. 105: %Inhibition of sectional growth rate caused by the test item after 72 - 96 h.

Treatm.[mg/L]	Mean	Std. Dev.	n	%Inhibition
Control	1,350	0,2019	6	
0,021	0,708	0,1474	3	47,57
0,038	0,801	0,1554	3	40,68
0,068	0,320	0,3893	3	76,26
0,120	-0,247	0,2288	3	118,26
0,210	-0,880	0,4438	3	165,16

The 3-param. normal CDF F(x) = b0*[NormalCDF(b1-log10(x)/b2 + zOpt)] was fitted to the data (CDF: cumulative distribution function; b0-b2: parameters; zOpt: adjustment to have the EC10 as parameter b1; x: concentration).
A non-linear regression without weighting was performed.

Estimated parameters of the 3-param. normal CDF

Tab. 106: Estimated parameters of the 3-param. normal CDF with sectional growth rate at 72 - 96 h: Results of the non-linear regression analysis; b0 - b2: parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic (Ho: b0|b1|b2 = 0); p(t): probability that the deviation from zero is due to chance (b1 = log EC10)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b0	1,272	0,048	1,172	1,373	26,628	<0.001
b1	-1,877	0,036	-1,953	-1,800	-51,455	<0.001
b2	0,319	0,024	0,269	0,370	13,263	<0.001

Stop Reason = Iterations > Max. Iterations (Optimization method: Levenberg-Marquardt)

R^2: 0,437; adjusted R^2: 0,374.Residual standard error: 0,48005.Akaike Criterion (AIC): -2,559.Shapiro Wilk`s test on normal distribution of residuals: p = 0,155..

Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF

Tab. 107: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with sectional growth rate at 72 - 96 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p(F): probability that the variance explained by the regression is due to chance; Pure error: residual SS|MSS of an one-way ANOVA with the original data (CDF: cumulative distribution function)

Source	SS	df	MSS	F	p(F)
Regression	5,82	2	2,91	12,619	<0.001
Residuals	4,15	18	0,23		
- Lack of Fit	3,05	3	1,02	13,902	<0.001
- Pure Error	1,10	15	0,07		
Total	13,31	20			

Since p(F|Regression) <= 0.05, a significant amount of variance is explained by the regression model..Since p(F|Lack of Fit) <= 0.05, lack of fit is significant. It is recommendes to choose more appropriate regression functions and/or settings..Since a signifcant lack of fit was found, it is recomended to try other dose/response functions..

Observed and Predicted Results of the 3-param. normal CDF

Tab. 108: Observed values in sectional growth rate after 72 - 96 h as caused by the test item and predicted values as calculated from the function.

Treatm.[mg/L]	Observed	Mean Obs.	Predicted
0,001	1,508	1,3501	1,2723
0,001	1,344	1,3501	1,2723
0,001	1,341	1,3501	1,2723
0,001	1,194	1,3501	1,2723
0,001	1,079	1,3501	1,2723
0,001	1,635	1,3501	1,2723
0,021	0,877	0,7079	0,9478
0,021	0,610	0,7079	0,9478
0,021	0,636	0,7079	0,9478
0,038	0,862	0,8009	0,5615
0,038	0,916	0,8009	0,5615
0,038	0,624	0,8009	0,5615
0,068	0,000	0,3205	0,2213

0,068	0,754	0,32050,2213
0,068	0,208	0,32050,2213
0,120	-0,288	-0,24660,0554
0,120	-0,452	-0,24660,0554
0,120	0,000	-0,24660,0554
0,210	-0,560	-0,87970,0086
0,210	-1,386	-0,87970,0086
0,210	-0,693	-0,87970,0086

Point estimates from the 3-param. normal CDF

Tab. 109: Point estimates from the 3-param. normal CDF with sectional growth rate at 72 - 96 h: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [mg/L]	0,013	0,018	0,034
lower 95%-cl	0,011	0,016	0,028
upper 95%-cl	0,016	0,022	0,042

n.d.: not determined due to mathematical reasons

The confidence limits of the EC10 used as a parameter were computed by means of the standard error of parameter b1; confidence limits for the remaining ECx were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix (1000 runs)..

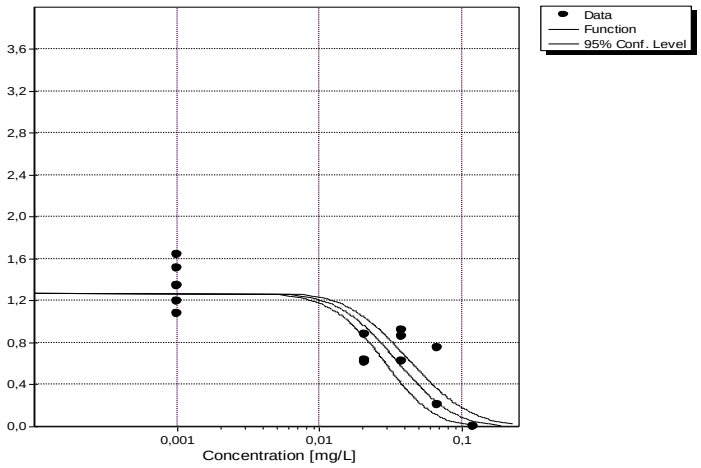

Fig. 16: Concentration-effect curve showing the influence of the test item on sectional growth rate of the introduced Desmodesmus subspicatus as observed after 72 - 96 h

Overview over the ECs of the Test Item on Sectional Growth Rate

Effects on Sectional Growth Rate

Tab. 110: Sectional growth rate (SG) and its inhibition relative to control (%I) as computed from the raw data for test intervals selected; *nl with sectional growth rate: nonlinear regression using the 3-param. normal CDF.

Treatment	0 - 24 h		24 - 48 h		48 - 72 h		72 - 96 h	
[mg/L]	SG	%I	SG	%I	SG	%I	SG	%I
Control	0,896	0,00	1,704	0,00	1,479	0,00	1,350	0,00
0,021	0,693	22,63	1,677	1,61	1,406	4,95	0,708	47,57
0,038	0,366	59,12	1,894	-11,12	0,504	65,94	0,801	40,68
0,068	0,732	18,25	1,162	31,81	0,533	63,95	0,320	76,26
0,120	0,462	48,42	0,963	43,47	0,799	45,97	-0,247	118,26
0,210	0,231	74,21	0,000	100,00	1,708	-15,45	-0,880	165,16

Tab. 110 (continued): Sectional growth rate (SG) and its inhibition relative to control (%I) as computed from the raw data for test intervals selected; *nl with sectional growth rate: nonlinear regression using the 3-param. normal CDF.

EC10	0,004	*nl	0,055	*nl	0,000	*nl	0,013	*nl
lower 95%-cl	n.d.		0,021		n.d.		0,011	
upper 95%-cl	n.d.		0,146		n.d.		0,016	
EC20	0,011	*nl	0,070	*nl	0,000	*nl	0,018	*nl
lower 95%-cl	n.d.		0,028		n.d.		0,016	
upper 95%-cl	n.d.		0,177		n.d.		0,022	
EC50	0,088	*nl	0,110	*nl	0,000	*nl	0,034	*nl
lower 95%-cl	n.d.		0,036		n.d.		0,028	
upper 95%-cl	n.d.		0,338		n.d.		0,042	

Statistical Characteristics of the Samples

Tab. 111: Statistical characteristics with sectional growth rate at 0 - 24 h: Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [mg/L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Control	0,896	0,896	0,693	1,099	6	0,2221	24,8	0,0907	10,1	0,663	1,129
0,021	0,693	0,693	0,000	1,386	3	0,6931	100,0	0,4002	57,7	-1,029	2,415
0,038	0,366	0,000	0,000	1,099	3	0,6343	173,2	0,3662	100,0	-1,209	1,942
0,068	0,732	1,099	0,000	1,099	3	0,6343	86,6	0,3662	50,0	-0,843	2,308
0,120	0,462	0,693	0,000	0,693	3	0,4002	86,6	0,2310	50,0	-0,532	1,456
0,210	0,231	0,000	0,000	0,693	3	0,4002	173,2	0,2310	100,0	-0,763	1,225

Shapiro-Wilk´s Test on Normal Distribution

Tab. 112: Shapiro-Wilk´s Test on Normal Distribution with sectional growth rate at 0 - 24 h: Mean: arithmetic mean; n: sample size; p(ShapiroWilk´s W): probability of the W statistic (i.e. that the observed deviations from the normal distributions are dues to chance). In case p(ShapiroWilk´s W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [mg/L]	Mean	s	n
Control	0,896	0,2221	6
0,021	0,693	0,6931	3
0,038	0,366	0,6343	3
0,068	0,732	0,6343	3
0,120	0,462	0,4002	3
0,210	0,231	0,4002	3

Results:

Number of residuals = 13; Shapiro-Wilk´s W = 0,952; p(W) = 0,626; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Normality check was passed (p > 0,01).

Levene´s Test on Variance Homogeneity (with Residuals)

Tab. 113: Levene´s Test on Variance Homogeneity (with Residuals) with sectional growth rate at 0 - 24 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p: probability that the variance explained by the treatment is due to chance

Source	SS	df	MSS	F	p(F)
Treatment	0,29305	5	0,05861	1,542	0,236
Residuals	0,57029	15	0,03802		
Total	0,8633	20			

The Levene test indicates variance homogeneity (p > 0,010).

Variance homogeneity check was passed (p > 0,01).

Normal-distribution and variance-homogeneity requirements are fulfilled.
A parametric multiple test is advisable.

To justify the use of Williams test at first a trend analysis by contrasts is performed.

Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 114: Trend analysis by contrasts (monotonicity of concentration/response) with sectional growth rate at 0 - 24 h: Psi: sum of means weighted by contrasts; s(psi): standard error of psi; df: degrees of freedom; t: t-statistic; p(t): probability that the trend is due to chance (Ho: Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	s(psi)	df	t	p(t)
Linear	3,65110	2,10185	15	1,737	0,051
Quadratic	-0,08495	2,34380	15	-0,036	0,486

The linear trend is not significant (p > 0,05) The quadratic trend is not significant (p > 0,05)

The analysis of contrasts did not reveal a linear trend, thus the selected Williams test was replaced by Dunnett test.

Dunnett`s Multiple t-test Procedure

Tab. 115: Dunnett´s multiple t-test procedure with sectional growth rate at 0 - 24 h: Comparison of treatments with "Control". Significance was Alpha = 0,050, one-sided smaller (multiple level); Mean: arithmetic mean; n: sample size; s: standard deviation; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; t*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments).

Treatm. [mg/L]	Mean	s	df	%MDD	t	t*	Sign.
Control	0,896	0,48010					
0,021	0,693	0,48010	15	-95,26	-0,60	-2,51	-
0,038	0,366	0,48010	15	-95,26	-1,56	-2,51	-
0,068	0,732	0,48010	15	-95,26	-0,48	-2,51	-
0,120	0,462	0,48010	15	-95,26	-1,28	-2,51	-
0,210	0,231	0,48010	15	-95,26	-1,96	-2,51	-

+: significant; -: non-significant

The NOEC appears to be higher than or equal 0,210 mg/L.

Threshold concentrations (NOEC) for Sectional Growth Rate at 24 - 48 h

Statistical Characteristics of the Samples

Tab. 116: Statistical characteristics with sectional growth rate at 24 - 48 h: Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [mg/L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Control	1,704	1,701	1,466	1,946	6	0,1932	11,3	0,0789	4,6	1,502	1,907
0,021	1,677	1,504	0,693	2,833	3	1,0804	64,4	0,6238	37,2	-1,007	4,361
0,038	1,894	2,079	1,204	2,398	3	0,6182	32,6	0,3569	18,8	0,358	3,430
0,068	1,162	0,847	0,847	1,792	3	0,5453	46,9	0,3148	27,1	-0,192	2,517
0,120	0,963	0,693	0,693	1,504	3	0,4682	48,6	0,2703	28,1	-0,200	2,127
0,210	0,000	0,000	0,000	0,000	3	0,0000	-173,2	0,0000	-100,0	0,000	0,000

Shapiro-Wilk´s Test on Normal Distribution

Tab. 117: Shapiro-Wilk´s Test on Normal Distribution with sectional growth rate at 24 - 48 h: Mean: arithmetic mean; n: sample size; p(ShapiroWilk´s W): probability of the W statistic (i.e. that the observed deviations from the normal distributions are dues to chance). In case p(ShapiroWilk´s W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [mg/L]	Mean	s	n
Control	1,704	0,1932	6
0,021	1,677	1,0804	3
0,038	1,894	0,6182	3
0,068	1,162	0,5453	3
0,120	0,963	0,4682	3
0,210	0,000	0,0000	3

Results:

Number of residuals = 17; Shapiro-Wilk´s W = 0,979; p(W) = 0,945; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Normality check was passed (p > 0,01).

Levene´s Test on Variance Homogeneity (with Residuals)

Tab. 118: Levene´s Test on Variance Homogeneity (with Residuals) with sectional growth rate at 24 - 48 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p: probability that the variance explained by the treatment is due to chance

Source	SS	df	MSS	F	p(F)
Treatment	1,14935	5	0,22987	4,212	0,014
Residuals	0,81861	15	0,05457		
Total	1,9680	20			

The Levene test indicates variance homogeneity (p > 0,010).

Variance homogeneity check was passed (p > 0,01).

Normal-distribution and variance-homogeneity requirements are fulfilled.
A parametric multiple test is advisable.

To justify the use of Williams test at first a trend analysis by contrasts is performed.

Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 119: Trend analysis by contrasts (monotonicity of concentration/response) with sectional growth rate at 24 - 48 h: Psi: sum of means weighted by contrasts; s(psi): standard error of psi; df: degrees of freedom; t: t-statistic; p(t): probability that the trend is due to chance (Ho: Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	s(psi)	df	t	p(t)
Linear	11,39313	2,34913	15	4,850	<0.001
Quadratic	6,34241	2,61955	15	2,421	0,014

The linear trend is significant (p <= 0,05) The quadratic trend is significant (p <= 0,05)

The analysis of contrasts revealed a linear trend, thus the selected Williams test was performed.

Williams Multiple Sequential t-test Procedure

Tab. 120: Comparison of treatments with "Control" by the t test procedure after Williams with sectional growth rate at 24 - 48 h: Significance was Alpha = 0,050, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [mg/L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	1,704	0,53658						
0,021	1,677	0,53658	15	1,785	-39,03	0,21	-1,75	-
0,038	1,894	0,53658	15	1,785	-40,63	0,21	-1,83	-
0,068	1,162	0,53658	15	1,162	-41,14	-1,43	-1,85	-
0,120	0,963	0,53658	15	0,963	-41,34	-1,95	-1,86	+
0,210	0,000	0,53658	15	0,000	-41,54	-4,49	-1,87	+

+: significant; -: non-significant

A NOEC of 0,068 mg/L is suggested by the program.

Threshold concentrations (NOEC) for Sectional Growth Rate at 48 - 72 h

Statistical Characteristics of the Samples

Tab. 121: Statistical characteristics with sectional growth rate at 48 - 72 h: Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [mg/L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Control	1,479	1,446	1,285	1,735	6	0,1594	10,8	0,0651	4,4	1,312	1,647
0,021	1,406	1,609	0,904	1,705	3	0,4371	31,1	0,2524	17,9	0,320	2,492
0,038	0,504	0,547	0,405	0,560	3	0,0855	17,0	0,0493	9,8	0,292	0,716

0,068	0,533	0,693	0,134	0,773	3	0,3485	65,4	0,2012	37,7	-0,332	1,399
0,120	0,799	0,693	0,000	1,705	3	0,8573	107,3	0,4950	61,9	-1,330	2,929
0,210	1,708	1,946	1,099	2,079	3	0,5319	31,1	0,3071	18,0	0,387	3,029

Shapiro-Wilk´s Test on Normal Distribution

Tab. 122: Shapiro-Wilk´s Test on Normal Distribution with sectional growth rate at 48 - 72 h: Mean: arithmetic mean; n: sample size; p(ShapiroWilk´s W): probability of the W statistic (i.e. that the observed deviations from the normal distributions are dues to chance). In case p(ShapiroWilk´s W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [mg/L]	Mean	s	n
Control	1,479	0,1594	6
0,021	1,406	0,4371	3
0,038	0,504	0,0855	3
0,068	0,533	0,3485	3
0,120	0,799	0,8573	3
0,210	1,708	0,5319	3

Results:

Number of residuals = 21; Shapiro-Wilk´s W = 0,956; p(W) = 0,443; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Normality check was passed (p > 0,01).

Levene´s Test on Variance Homogeneity (with Residuals)

Tab. 123: Levene´s Test on Variance Homogeneity (with Residuals) with sectional growth rate at 48 - 72 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p: probability that the variance explained by the treatment is due to chance

Source	SS	df	MSS	F	p(F)
Treatment	0,66052	5	0,13210	3,507	0,027
Residuals	0,56505	15	0,03767		
Total	1,2256	20			

The Levene test indicates variance homogeneity (p > 0,010).

Variance homogeneity check was passed (p > 0,01).

Normal-distribution and variance-homogeneity requirements are fulfilled.
A parametric multiple test is advisable.

To justify the use of Williams test at first a trend analysis by contrasts is performed.

Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 124: Trend analysis by contrasts (monotonicity of concentration/response) with sectional growth rate at 48 - 72 h: Psi: sum of means weighted by contrasts; s(psi): standard error of psi; df: degrees of freedom; t: t-statistic; p(t): probability that the trend is due to chance (Ho: Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	s(psi)	df	t	p(t)
Linear	0,64835	1,89240	15	0,343	0,368

Quadratic	-9,58273	2,11024	15	-4,541	<0.001

The linear trend is not significant (p > 0,05) The quadratic trend is significant (p <= 0,05)

The analysis of contrasts did not reveal a linear trend, thus the selected Williams test was replaced by Dunnett test.

Dunnett`s Multiple t-test Procedure

Tab. 125: Dunnett´s multiple t-test procedure with sectional growth rate at 48 - 72 h: Comparison of treatments with "Control". Significance was Alpha = 0,050, one-sided smaller (multiple level); Mean: arithmetic mean; n: sample size; s: standard deviation; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; t*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments).

Treatm. [mg/L]	Mean	s	df	%MDD	t	t*	Sign.
Control	1,479	0,43225					
0,021	1,406	0,43225	15	-51,94	-0,24	-2,51	-
0,038	0,504	0,43225	15	-51,94	-3,19	-2,51	+
0,068	0,533	0,43225	15	-51,94	-3,10	-2,51	+
0,120	0,799	0,43225	15	-51,94	-2,23	-2,51	-
0,210	1,708	0,43225	15	-51,94	0,75	-2,51	-

+: significant; -: non-significant

The NOEC cannot be determined by the program (expert judgement required).

Threshold concentrations (NOEC) for Sectional Growth Rate at 72 - 96 h

Statistical Characteristics of the Samples

Tab. 126: Statistical characteristics with sectional growth rate at 72 - 96 h: Mean: arithmetic mean (X); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; s%: coefficient of variation; s(X): standard error; %s(X): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [mg/L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Control	1,350	1,343	1,079	1,635	6	0,2019	15,0	0,0824	6,1	1,138	1,562
0,021	0,708	0,636	0,610	0,877	3	0,1474	20,8	0,0851	12,0	0,342	1,074
0,038	0,801	0,862	0,624	0,916	3	0,1554	19,4	0,0897	11,2	0,415	1,187
0,068	0,320	0,208	0,000	0,754	3	0,3893	121,5	0,2248	70,1	-0,647	1,288
0,120	-0,247	-0,288	-0,452	0,000	3	0,2288	-92,8	0,1321	-53,6	-0,815	0,322
0,210	-0,880	-0,693	-1,386	-0,560	3	0,4438	-50,4	0,2562	-29,1	-1,982	0,223

Shapiro-Wilk´s Test on Normal Distribution

Tab. 127: Shapiro-Wilk´s Test on Normal Distribution with sectional growth rate at 72 - 96 h: Mean: arithmetic mean; n: sample size; p(ShapiroWilk´s W): probability of the W statistic (i.e. that the observed deviations from the normal distributions are dues to chance). In case p(ShapiroWilk´s W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [mg/L]	Mean	s	n
Control	1,350	0,2019	6
0,021	0,708	0,1474	3
0,038	0,801	0,1554	3

0,068	0,320	0,3893	3
0,120	-0,247	0,2288	3
0,210	-0,880	0,4438	3

Results:

Number of residuals = 21; Shapiro-Wilk´s W = 0,990; p(W) = 0,998; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Normality check was passed (p > 0,01).

Levene´s Test on Variance Homogeneity (with Residuals)

Tab. 128: Levene´s Test on Variance Homogeneity (with Residuals) with sectional growth rate at 72 - 96 h: Source: source of variance; SS: sum of squares; df: degrees of freedom; MSS: mean sum of squares; F: test statistic: p: probability that the variance explained by the treatment is due to chance

Source	SS	df	MSS	F	p(F)
Treatment	0,14101	5	0,02820	1,982	0,140
Residuals	0,21342	15	0,01423		
Total	0,3544	20			

The Levene test indicates variance homogeneity (p > 0,010).

Variance homogeneity check was passed (p > 0,01).

Normal-distribution and variance-homogeneity requirements are fulfilled.
A parametric multiple test is advisable.

To justify the use of Williams test at first a trend analysis by contrasts is performed.

Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 129: Trend analysis by contrasts (monotonicity of concentration/response) with sectional growth rate at 72 - 96 h: Psi: sum of means weighted by contrasts; s(psi): standard error of psi; df: degrees of freedom; t: t-statistic; p(t): probability that the trend is due to chance (Ho: Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	s(psi)	df	t	p(t)
Linear	14,49279	1,18409	15	12,240	<0.001
Quadratic	2,59463	1,32040	15	1,965	0,034

The linear trend is significant (p <= 0,05) The quadratic trend is significant (p <= 0,05)

The analysis of contrasts revealed a linear trend, thus the selected Williams test was performed.

Williams Multiple Sequential t-test Procedure

Tab. 130: Comparison of treatments with "Control" by the t test procedure after Williams with sectional growth rate at 72 - 96 h: Significance was Alpha = 0,050, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: µ1 = µ2 = ... = µk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [mg/L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Control	1,350	0,27047						

0,021	0,708	0,27047	15	0,754	-24,83	-3,11	-1,75	+
0,038	0,801	0,27047	15	0,754	-25,85	-3,11	-1,83	+
0,068	0,320	0,27047	15	0,320	-26,18	-5,38	-1,85	+
0,120	-0,247	0,27047	15	-0,247	-26,30	-8,35	-1,86	+
0,210	-0,880	0,27047	15	-0,880	-26,43	-11,66	-1,87	+

+: significant; -: non-significant

The NOEC is lower than 0,021 mg/L.

Overview over the Effect-Thresholds of the Test Item on Sectional Growth Rate

Overview over the LOEC and NOEC Determination

Tab. 131: Overview over the LOEC and NOEC Determination with sectional growth rate: Arithmetic means and significance marks as computed for sectional growth rate for all inspection intervals (top); bottom part: obtained LOEC and NOEC with indication of statistical test used; *dt: Dunnett´s multiple t-test procedure; *wl: Williams multiple sequential t-test procedure, significance level was 0,050, one-sided smaller.

Treatm. [mg/L]	0 - 24 h	24 - 48 h	48 - 72 h	72 - 96 h
0,021	0,693 -	1,677 -	1,406 -	0,708+
0,038	0,366 -	1,894 -	0,504+	0,801+
0,068	0,732 -	1,162 -	0,533+	0,320+
0,120	0,462 -	0,963+	0,799 -	-0,247+
0,210	0,231 -	0,000+	1,708 -	-0,880+
LOEC	>0,210 *dt	0,120 *wl	n.d. *dt	<=0,021 *wl
NOEC	>=0,210 *dt	0,068 *wl	n.d. *dt	<0,021 *wl

+: Significant difference to control (p <=0,050)

Settings Table

Tab. 132:

Area	Item	Default Settings	User Settings
Global			
	Type of Exposure	Concentration	Concentration
	Extrapolation of ECx	By program	By program
	Show non-significant ECx	YES	YES
	Statistical design		NOEC/ECx
Variables			
Yield	State	Selected for analysis	Selected for analysis
	Data transformation	none	none
	Decimals data	1	1
	Statistical pre-testing		
	Normal distribution	Shapiro-Wilk´s	Shapiro-Wilk´s
	Significance level	0,01	0,01
	Variance homogeneity	Levene	Levene
	Significance level	0,01	0,01
	Who selected pre-tests	Program	Program
	Additional tests	None	None
	Monotonicity	Contrast Analysis	Contrast Analysis
	Sig. Level	0,05	0,05
	Final testíng (NOEC)		
	Test procedure	Williams	Williams
	Who selected final test	Program	Program
	Variance used	Residual from ANOVA	Residual from ANOVA
	Significance level	0,05	0,05
	Test direction	one-sided smaller	one-sided smaller
	ECx computation		
	Selected ECx values	EC10, EC20, EC50	EC10, EC20, EC50
	Selected method	Non-linear Regr.	Non-linear Regr.
	Optimization	Levenberg-Marquardt (IRLS)	Levenberg-Marquardt (IRLS)
	Dose/resp. function metric	3-param Normal	3-param Normal
	Weights	Not used	Not used
	Calculation of confid. limits		Monte-Carlo simulation Not changeable
Growth Rate	State	Selected for analysis	Selected for analysis
	Data transformation	none	none
	Decimals data	3	3
	Statistical pre-testing		
	Normal distribution	Shapiro-Wilk´s	Shapiro-Wilk´s

	Significance level	0,01	0,01
	Variance homogeneity	Levene	Levene
	Significance level	0,01	0,01
	Who selected pre-tests	Program	Program
	Additional tests	None	None
	Final testíng (NOEC)		
	Test procedure	Williams	Dunnett
	Who selected final test	Program	Program
	Variance used	Residual from ANOVA	Residual from ANOVA
	Significance level	0,05	0,05
	Test direction	one-sided smaller	one-sided smaller
	ECx computation		
	Selected ECx values	EC10, EC20, EC50	EC10, EC20, EC50
	Selected method	Non-linear Regr.	Non-linear Regr.
	Optimization	Levenberg-Marquardt (IRLS)	Levenberg-Marquardt (IRLS)
	Dose/resp. function metric	3-param Normal	3-param Normal
	Weights	Not used	Not used
	Calculation of confid. limits		Monte-Carlo simulation Not changeable
Sectional Growth Rate	State	Selected for analysis	Selected for analysis
	Data transformation	none	none
	Decimals data	3	3
	Statistical pre-testing		
	Normal distribution	Shapiro-Wilk´s	Shapiro-Wilk´s
	Significance level	0,01	0,01
	Variance homogeneity	Levene	Levene
	Significance level	0,01	0,01
	Who selected pre-tests	Program	Program
	Additional tests	None	None
	Final testíng (NOEC)		
	Test procedure	Williams	Dunnett
	Who selected final test	Program	Program
	Variance used	Residual from ANOVA	Residual from ANOVA
	Significance level	0,05	0,05
	Test direction	one-sided smaller	one-sided smaller
	ECx computation		
	Selected ECx values	EC10, EC20, EC50	EC10, EC20, EC50
	Selected method	Non-linear Regr.	Non-linear Regr.
	Optimization	Levenberg-Marquardt (IRLS)	Levenberg-Marquardt (IRLS)

	Dose/resp. function metric	3-param Normal	3-param Normal
	Weights	Not used	Not used
	Calculation of confid. limits		Monte-Carlo simulation Not changeable
Biomass Integral	State	Selected for analysis	Deselected from analysis