

Fish, Juvenile Growth Test (OECD 215-2000): 535A-105

General:

Test identification/project no.	535A-105
Test item	1,4 dimethylnaphthalene
Unit of test item concentration	µg a.s./L
Start of experiment on day	24-Mar-2006
Date and time of the evaluation	23.05.2019; 15:04:39
Raw data filename:	1,4 dimethylnaphthalene_ToxRat_Fish_JGT.xls

Test design

Number of treatments (incl. control(s))	7
Duration of the test	28 d
Measurement interval	28 d
Measurement variable	Growth and Mortality
Test system	Oncorhynchusmykiss
Statistical design	Hypothesis testing (NOEC) and regression (ECx)

Comments:

Controls pooled for NOEC/ECx evaluations of weight and growth (not for mortality)

Validity of the test

The test requires a mortality not exceeding 10% and a minimum factor for weight increase of 1,5 in the control to be valid.

In the present test mortality was 0,0% and the weightfactor was 2,6; thus the test is valid.

Relation of *Oncorhynchus mykiss* Endpoints on Concentration

Summary of Results for all Endpoints at the End of Exposure Period

Tab. 1: Summary of Results for all Endpoints at the End of Exposure Period: 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 [µg a.s./L]

Weight		
	EC10	212,685
95%-CL	lower	164,240
	upper	275,418
	EC20	288,154
95%-CL	lower	219,428
	upper	378,864
	EC50	515,154
95%-CL	lower	352,856
	upper	738,635
Weight	LOEC	205,000
	NOEC	90,000
Specific growth rate		
	EC10	199,564
95%-CL	lower	158,912
	upper	250,614
	EC20	245,674
95%-CL	lower	196,789
	upper	308,903
	EC50	365,651
95%-CL	lower	279,187
	upper	482,004

Tab. 1 (continued): Summary of Results for all Endpoints at the End of Exposure Period: 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.

Specific growth rate	LOEC	205,000
	NOEC	90,000

Specific interval growth rate

	EC10	199,564
95%-CL	lower	158,912
	upper	250,614

	EC20	245,674
95%-CL	lower	196,789
	upper	308,903

	EC50	365,651
95%-CL	lower	279,187
	upper	482,004

Specific interval growth rate	LOEC	205,000
	NOEC	90,000

0-28 d

Mortality

	LC10	n.d.
95%-CL	lower	n.d.
	upper	n.d.

	LC20	n.d.
95%-CL	lower	n.d.
	upper	n.d.

	LC50	n.d.
95%-CL	lower	n.d.
	upper	n.d.

Tab. 1 (continued): Summary of Results for all Endpoints at the End of Exposure Period: 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.

Mortality	LOEC	n.d.
	NOEC	n.d.

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

Weight (Data)

Weight of *Oncorhynchus mykiss* as Dependent on Concentration and Time

Tab. 2: Weight of *Oncorhynchus mykiss* 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 RawDataWeight)

Treatm. [µg a.s./L]	Control	Solv. Contr.	18,000	40,000	90,000	205,000	488,000
0 d	1,00	1,00	1,00	1,20	1,20	1,20	1,00
	1,20	1,20	1,10	1,20	1,20	1,00	1,00
	1,00	1,10	1,10	1,30	1,00	1,00	1,10
	1,00	1,00	1,10	1,20	1,00	1,00	1,00
	1,10	1,00	1,30	1,00	1,00	1,00	1,00
	1,00	1,00	1,30	1,20	1,00	1,20	1,10
	1,00	1,00	1,00	1,20	1,00	1,10	1,10
	1,00	1,00	1,00	1,00	1,00	1,20	1,00
	1,00	1,10	1,10	1,10	1,10	1,10	1,00
	1,10	1,00	1,00	1,10	1,10	1,00	1,00
	1,10	1,00	1,20	1,00	1,10	1,20	1,20
	1,20	1,20	1,10	1,00	1,10	1,30	1,10
	1,00	1,20	1,00	1,10	1,00	1,10	1,00
	1,00	1,00	1,00	1,00	1,10	1,00	1,00
	1,30	1,00	1,00	1,00	1,00	1,00	1,00
	1,00	1,00	1,00	1,10	1,00	1,00	1,10
	1,00	1,30	1,00	1,20	1,10	1,00	1,30
	1,20	1,10	1,20	1,00	1,10	1,10	1,00
	1,00	1,30	1,00	1,10	1,30	1,00	1,00
	1,00	1,10	1,20	1,10	1,00	1,30	1,10
	1,20	1,00	1,00	1,20	1,10	1,20	1,00
	1,20	1,00	1,00	1,00	1,00	1,00	1,00
	1,00	1,00	1,00	1,10	1,00	1,00	1,20
	1,10	1,00	1,10	1,20	1,20	1,00	1,00
	1,00	1,00	1,00	1,00	1,00	1,20	1,00
	1,00	1,00	1,00	1,20	1,00	1,00	1,00
	1,00	1,10	1,10	1,30	1,20	1,00	1,20
	1,10	1,10	1,00	1,10	1,00	1,00	1,20
	1,00	1,00	1,10	1,10	1,00	1,30	1,20
	1,20	1,00	1,20	1,20	1,00	1,00	1,00
	1,10	1,00	1,00	1,00	1,00	1,10	1,20

	1,00	1,00	1,00	1,00	1,00	1,00	1,00
	1,00	1,00	1,00	1,00	1,00	1,00	1,00
	1,00	1,00	1,00	1,00	1,00	1,00	1,00
	1,00	1,00	1,10	1,20	1,00	1,00	1,00
	1,00	1,10	1,00	1,10	1,00	1,10	1,00
	1,10	1,00	1,30	1,00	1,10	1,00	1,10
	1,00	1,00	1,10	1,30	1,10	1,00	1,20
	1,20	1,00	1,20	1,10	1,10	1,10	1,20
Mean:	1,06	1,05	1,07	1,11	1,06	1,07	1,07
Std.Dev.:	0,087	0,085	0,096	0,099	0,078	0,099	0,089
n:	40	40	40	40	40	40	40
CV:	8,2	8,1	9,0	8,9	7,4	9,3	8,4
28 d	2,10	3,10	3,50	3,40	2,70	2,80	2,20
	3,00	2,30	2,20	3,00	3,50	2,80	2,00
	2,20	2,60	3,70	2,00	3,00	2,40	1,60
	2,30	2,30	3,40	2,60	3,30	2,50	0,70
	2,30	3,20	3,10	3,80	2,80	2,20	1,30
	2,70	2,30	2,60	2,70	2,20	2,70	1,40
	2,90	2,30	1,90	3,20	2,60	1,50	1,50
	2,80	2,60	2,70	2,30	2,20	2,20	1,00
	3,90	3,80	2,50	3,50	3,10	2,60	0,60
	2,40	3,00	2,50	2,60	2,50	2,70	1,60
	2,80	2,70	3,50	2,70	2,60	3,10	2,20
	3,50	2,70	2,70	2,50	3,50	2,70	2,60
	3,50	2,60	3,10	3,50	2,40	2,40	2,00
	2,20	3,90	2,20	2,50	2,10	2,70	1,00
	2,70	2,50	3,20	2,90	3,10	2,80	2,90
	2,90	2,80	2,70	2,50	2,40	3,90	1,70
	2,80	2,60	2,70	2,70	2,80	2,80	1,50
	2,20	2,80	2,40	2,90	3,90	2,20	1,40
	2,40	3,50	2,80	2,70	3,20	2,30	1,00
	3,40	2,70	3,40	2,40	1,60	1,70	1,30
	2,30	2,60	2,70	3,50	4,00	1,70	2,10
	3,30	2,90	1,70	3,00	2,70	3,40	1,70
	2,80	3,00	2,60	2,30	2,60	2,70	2,10
	3,20	2,20	2,50	2,80	3,40	2,50	1,20
	2,90	2,30	3,10	2,50	2,70	3,80	1,80
	3,00	2,60	2,80	3,50	3,30	1,70	1,80
	2,70	3,10	2,70	2,10	3,30	2,70	1,00
	3,30	2,40	2,50	2,60	2,00	2,10	1,30
	2,70	3,30	2,00	2,50	3,00	2,70	0,70
	2,70	2,70	2,50	4,10	2,20	3,10	0,50
	2,50	3,00	3,50	2,60	3,70	2,80	1,50

	4,50	2,50	2,50	2,70	2,60	2,40	1,10
	1,90	2,80	2,50	3,30	2,30	2,50	1,70
	2,80	2,20	3,70	2,50	2,50	2,50	0,60
	2,70	2,30	3,60	2,50	2,20	2,20	2,20
	1,80	3,10	2,90	2,60	2,70	2,70	1,70
	2,70	2,10	2,50	3,30	2,80	1,80	1,10
	2,50	1,90	2,60	3,10	2,80	1,90	0,70
	2,50	2,40	2,50	3,10	3,60	2,50	-
	2,50	2,60	2,70	3,20	3,00	2,10	-
Mean:	2,76	2,71	2,77	2,85	2,82	2,52	1,48
Std.Dev.:	0,532	0,439	0,491	0,471	0,545	0,518	0,577
n:	40	40	40	40	40	40	38
CV:	19,3	16,2	17,7	16,5	19,3	20,6	38,9

Comparison between Control and Solv. Contr. for Weight at 28 d

Shapiro-Wilk's Test on Normal Distribution

Tab. 3: Shapiro-Wilk's Test on Normal Distribution with weight at 28 d: 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 due to chance). In case p(ShapiroWilk's W) is greater than the chosen significance level, the normality hypothesis(Ho) is accepted.

Treatm. [µg a.s./L]	Mean	s	n
Control	2,76	0,532	40
Solv. Contr.	2,71	0,439	40

Results:

Number of residuals = 120; Shapiro-Wilk's W = 0,984; p(W) = 0,179; 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 (Shapiro-Wilk's; p > 0,01).

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

STUDENT-t test for Homogeneous Variances

Tab. 4: STUDENT-t test for Homogeneous Variances with weight at 28 d: Two-sample comparison of the two controls. Significance was Alpha = 0,050, two-sided; 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: $\mu_1 = \mu_2$; the differences are significant in case p(t) <= Alpha ; p(F): two-sided probability of F computed by the F-test (Ho: var1 = var2 (homogeneity)); p(F) > 0,010 is the criterion of variance homogeneity. (Control(c) and treatment(t) variance was applied: $s^2(c)/nc + s^2(t)/nt$, each).

Treatm. [µg a.s./L]	Mean	s	df	%MDD	t	p(t)	Sign.	p(F)
Control	2,76	0,532						
Solv. Contr.	2,71	0,439	78	7,9	-0,46	0,648	-	0,235

+: significant; -: non-significant

There is no statistically significant difference between control and solv. contr..

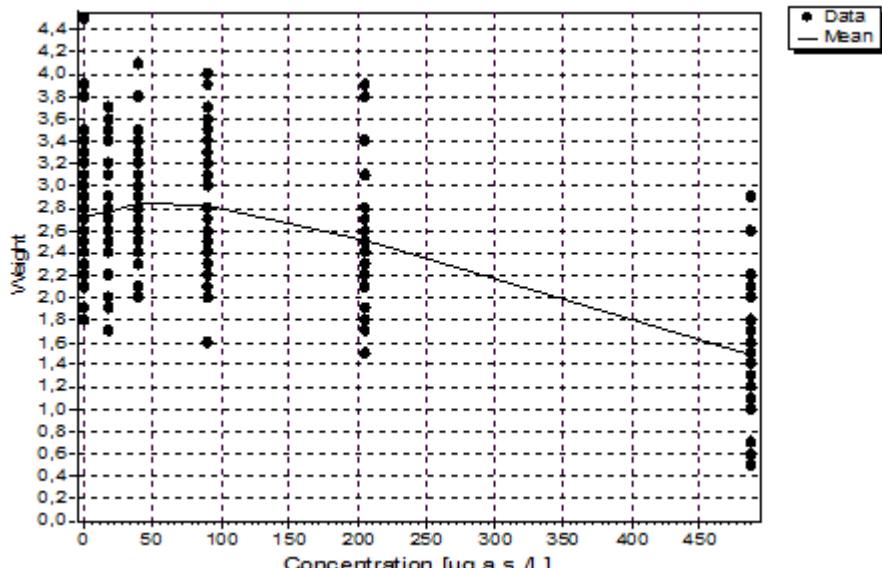


Fig. 1: Weight of *Oncorhynchus mykiss* as observed under presence of the test item after 28 d.

Effective Concentrations (ECx) for Weight at 28 d

Weight in *Oncorhynchus mykiss* after 28 d.

Tab. 5: %Reduction of weight caused by the test item after 28 d.

Treatm.[µg a.s./L]	Mean	Std. Dev.	n	%Reduction
Pooled Control	2,73	0,485	80	
18,000	2,77	0,491	40	-1,5
40,000	2,85	0,471	40	-4,5
90,000	2,82	0,545	40	-3,3
205,000	2,52	0,518	40	7,8
488,000	1,48	0,577	38	45,8

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 nonetheless acceptable or try whether an increase in the number of optimization cycles leads to convergence.

The 3-param. normal CDF $F(x) = b_0 * [\text{NormalCDF}(b_1 - \log_{10}(x)/b_2 + z_{\text{Opt}})]$ was fitted to the data (CDF: cumulative distribution function; b_0 - b_2 : parameters; z_{Opt} : adjustment to have the EC10 as parameter b_1 ; x : concentration). A non-linear regression without weighting was performed.

Estimated parameters of the 3-param. normal CDF

Tab. 6: Estimated parameters of the 3-param. normal CDF with weight at 28 d: Results of the non-linear regression analysis; b_0 - b_2 : parameters; Std. Err.: standard error; 95%LCL|UCL: 95%-lower|upper confidence limits; t: t-statistic ($H_0: b_0|b_1|b_2 = 0$); $p(t)$: probability that the deviation from zero is due to chance ($b_1 = \log \text{EC}10$)

Parameter	Value	Std. Err.	95%LCL	95%UCL	t	p(t)
b_0	2,784	0,037	2,711	2,858	74,421	<0.001
b_1	2,328	0,057	2,215	2,440	40,821	<0.001
b_2	0,300	0,049	0,204	0,395	6,179	<0.001

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

R²: 0,431; adjusted R²: 0,427. Residual standard error: 0,51001. Akaike Criterion (AIC): -91,241. Shapiro-Wilk's test on normal distribution of residuals: p = 0,195..

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 weight at 28 d: 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	54,19	2	27,09	104,167	<0.001
Residuals	71,53	275	0,26		
- Lack of Fit	0,54	3	0,18	0,695	0,556
- Pure Error	70,99	272	0,26		
Total	125,65	277			

Since $p(F|Regression) \leq 0.05$, 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. 8: Observed values in weight after 28 d as caused by the test item and predicted values as calculated from the function.

Treatm.[μ g a.s./L]	Observed	Mean Obs.	Predicted
0,001	2,100	2,732	2,784
0,001	3,000	2,732	2,784
0,001	2,200	2,732	2,784
0,001	2,300	2,732	2,784
0,001	2,300	2,732	2,784
0,001	2,700	2,732	2,784
0,001	2,900	2,732	2,784
0,001	2,800	2,732	2,784
0,001	3,900	2,732	2,784
0,001	2,400	2,732	2,784
0,001	2,800	2,732	2,784
0,001	3,500	2,732	2,784
0,001	3,500	2,732	2,784
0,001	2,200	2,732	2,784
0,001	2,700	2,732	2,784
0,001	2,900	2,732	2,784
0,001	2,800	2,732	2,784
0,001	2,200	2,732	2,784
0,001	2,400	2,732	2,784
0,001	3,400	2,732	2,784
0,001	2,300	2,732	2,784
0,001	3,300	2,732	2,784
0,001	2,800	2,732	2,784
0,001	3,200	2,732	2,784
0,001	2,900	2,732	2,784
0,001	3,000	2,732	2,784
0,001	2,700	2,732	2,784
0,001	3,300	2,732	2,784
0,001	2,700	2,732	2,784
0,001	2,700	2,732	2,784

0,001	2,500	2,732	2,784
0,001	4,500	2,732	2,784
0,001	1,900	2,732	2,784
0,001	2,800	2,732	2,784
0,001	2,700	2,732	2,784
0,001	1,800	2,732	2,784
0,001	2,700	2,732	2,784
0,001	2,500	2,732	2,784
0,001	2,500	2,732	2,784
0,001	2,500	2,732	2,784
0,001	3,100	2,732	2,784
0,001	2,300	2,732	2,784
0,001	2,600	2,732	2,784
0,001	2,300	2,732	2,784
0,001	3,200	2,732	2,784
0,001	2,300	2,732	2,784
0,001	2,300	2,732	2,784
0,001	2,600	2,732	2,784
0,001	3,800	2,732	2,784
0,001	3,000	2,732	2,784
0,001	2,700	2,732	2,784
0,001	2,700	2,732	2,784
0,001	2,600	2,732	2,784
0,001	3,900	2,732	2,784
0,001	2,500	2,732	2,784
0,001	2,800	2,732	2,784
0,001	2,600	2,732	2,784
0,001	2,800	2,732	2,784
0,001	3,500	2,732	2,784
0,001	2,700	2,732	2,784
0,001	2,600	2,732	2,784
0,001	2,900	2,732	2,784
0,001	3,000	2,732	2,784
0,001	2,200	2,732	2,784
0,001	2,300	2,732	2,784
0,001	2,600	2,732	2,784
0,001	3,100	2,732	2,784
0,001	2,400	2,732	2,784
0,001	3,300	2,732	2,784
0,001	2,700	2,732	2,784
0,001	3,000	2,732	2,784
0,001	2,500	2,732	2,784
0,001	2,800	2,732	2,784
0,001	2,200	2,732	2,784
0,001	2,300	2,732	2,784

0,001	3,100	2,732	2,784
0,001	2,100	2,732	2,784
0,001	1,900	2,732	2,784
0,001	2,400	2,732	2,784
0,001	2,600	2,732	2,784
18,000	3,500	2,773	2,784
18,000	2,200	2,773	2,784
18,000	3,700	2,773	2,784
18,000	3,400	2,773	2,784
18,000	3,100	2,773	2,784
18,000	2,600	2,773	2,784
18,000	1,900	2,773	2,784
18,000	2,700	2,773	2,784
18,000	2,500	2,773	2,784
18,000	2,500	2,773	2,784
18,000	3,500	2,773	2,784
18,000	2,700	2,773	2,784
18,000	3,100	2,773	2,784
18,000	2,200	2,773	2,784
18,000	3,200	2,773	2,784
18,000	2,700	2,773	2,784
18,000	2,700	2,773	2,784
18,000	2,400	2,773	2,784
18,000	2,800	2,773	2,784
18,000	3,400	2,773	2,784
18,000	2,700	2,773	2,784
18,000	1,700	2,773	2,784
18,000	2,600	2,773	2,784
18,000	2,500	2,773	2,784
18,000	3,100	2,773	2,784
18,000	2,800	2,773	2,784
18,000	2,700	2,773	2,784
18,000	2,500	2,773	2,784
18,000	2,000	2,773	2,784
18,000	2,500	2,773	2,784
18,000	3,500	2,773	2,784
18,000	2,500	2,773	2,784
18,000	2,500	2,773	2,784
18,000	3,700	2,773	2,784
18,000	3,600	2,773	2,784
18,000	2,900	2,773	2,784
18,000	2,500	2,773	2,784
18,000	2,600	2,773	2,784
18,000	2,500	2,773	2,784
18,000	2,700	2,773	2,784

40,000	3,400	2,855	2,784
40,000	3,000	2,855	2,784
40,000	2,000	2,855	2,784
40,000	2,600	2,855	2,784
40,000	3,800	2,855	2,784
40,000	2,700	2,855	2,784
40,000	3,200	2,855	2,784
40,000	2,300	2,855	2,784
40,000	3,500	2,855	2,784
40,000	2,600	2,855	2,784
40,000	2,700	2,855	2,784
40,000	2,500	2,855	2,784
40,000	3,500	2,855	2,784
40,000	2,500	2,855	2,784
40,000	2,900	2,855	2,784
40,000	2,500	2,855	2,784
40,000	2,700	2,855	2,784
40,000	2,900	2,855	2,784
40,000	2,700	2,855	2,784
40,000	2,400	2,855	2,784
40,000	3,500	2,855	2,784
40,000	3,000	2,855	2,784
40,000	2,300	2,855	2,784
40,000	2,800	2,855	2,784
40,000	2,500	2,855	2,784
40,000	3,500	2,855	2,784
40,000	2,100	2,855	2,784
40,000	2,600	2,855	2,784
40,000	2,500	2,855	2,784
40,000	4,100	2,855	2,784
40,000	2,600	2,855	2,784
40,000	2,700	2,855	2,784
40,000	3,300	2,855	2,784
40,000	2,500	2,855	2,784
40,000	2,500	2,855	2,784
40,000	2,600	2,855	2,784
40,000	3,300	2,855	2,784
40,000	3,100	2,855	2,784
40,000	3,100	2,855	2,784
40,000	3,200	2,855	2,784
90,000	2,700	2,822	2,768
90,000	3,500	2,822	2,768
90,000	3,000	2,822	2,768
90,000	3,300	2,822	2,768
90,000	2,800	2,822	2,768

90,000	2,200	2,822	2,768
90,000	2,600	2,822	2,768
90,000	2,200	2,822	2,768
90,000	3,100	2,822	2,768
90,000	2,500	2,822	2,768
90,000	2,600	2,822	2,768
90,000	3,500	2,822	2,768
90,000	2,400	2,822	2,768
90,000	2,100	2,822	2,768
90,000	3,100	2,822	2,768
90,000	2,400	2,822	2,768
90,000	2,800	2,822	2,768
90,000	3,900	2,822	2,768
90,000	3,200	2,822	2,768
90,000	1,600	2,822	2,768
90,000	4,000	2,822	2,768
90,000	2,700	2,822	2,768
90,000	2,600	2,822	2,768
90,000	3,400	2,822	2,768
90,000	2,700	2,822	2,768
90,000	3,300	2,822	2,768
90,000	3,300	2,822	2,768
90,000	2,000	2,822	2,768
90,000	3,000	2,822	2,768
90,000	2,200	2,822	2,768
90,000	3,700	2,822	2,768
90,000	2,600	2,822	2,768
90,000	2,300	2,822	2,768
90,000	2,500	2,822	2,768
90,000	2,200	2,822	2,768
90,000	2,700	2,822	2,768
90,000	2,800	2,822	2,768
90,000	2,800	2,822	2,768
90,000	3,600	2,822	2,768
90,000	3,000	2,822	2,768
205,000	2,800	2,520	2,531
205,000	2,800	2,520	2,531
205,000	2,400	2,520	2,531
205,000	2,500	2,520	2,531
205,000	2,200	2,520	2,531
205,000	2,700	2,520	2,531
205,000	1,500	2,520	2,531
205,000	2,200	2,520	2,531
205,000	2,600	2,520	2,531
205,000	2,700	2,520	2,531

205,000	3,100	2,520	2,531
205,000	2,700	2,520	2,531
205,000	2,400	2,520	2,531
205,000	2,700	2,520	2,531
205,000	2,800	2,520	2,531
205,000	3,900	2,520	2,531
205,000	2,800	2,520	2,531
205,000	2,200	2,520	2,531
205,000	2,300	2,520	2,531
205,000	1,700	2,520	2,531
205,000	1,700	2,520	2,531
205,000	3,400	2,520	2,531
205,000	2,700	2,520	2,531
205,000	2,500	2,520	2,531
205,000	3,800	2,520	2,531
205,000	1,700	2,520	2,531
205,000	2,700	2,520	2,531
205,000	2,100	2,520	2,531
205,000	2,700	2,520	2,531
205,000	3,100	2,520	2,531
205,000	2,800	2,520	2,531
205,000	2,400	2,520	2,531
205,000	2,500	2,520	2,531
205,000	2,500	2,520	2,531
205,000	2,200	2,520	2,531
205,000	2,700	2,520	2,531
205,000	1,800	2,520	2,531
205,000	1,900	2,520	2,531
205,000	2,500	2,520	2,531
205,000	2,100	2,520	2,531
488,000	2,200	1,482	1,479
488,000	2,000	1,482	1,479
488,000	1,600	1,482	1,479
488,000	0,700	1,482	1,479
488,000	1,300	1,482	1,479
488,000	1,400	1,482	1,479
488,000	1,500	1,482	1,479
488,000	1,000	1,482	1,479
488,000	0,600	1,482	1,479
488,000	1,600	1,482	1,479
488,000	2,200	1,482	1,479
488,000	2,600	1,482	1,479
488,000	2,000	1,482	1,479
488,000	1,000	1,482	1,479
488,000	2,900	1,482	1,479

488,000	1,700	1,482	1,479
488,000	1,500	1,482	1,479
488,000	1,400	1,482	1,479
488,000	1,000	1,482	1,479
488,000	1,300	1,482	1,479
488,000	2,100	1,482	1,479
488,000	1,700	1,482	1,479
488,000	2,100	1,482	1,479
488,000	1,200	1,482	1,479
488,000	1,800	1,482	1,479
488,000	1,800	1,482	1,479
488,000	1,000	1,482	1,479
488,000	1,300	1,482	1,479
488,000	0,700	1,482	1,479
488,000	0,500	1,482	1,479
488,000	1,500	1,482	1,479
488,000	1,100	1,482	1,479
488,000	1,700	1,482	1,479
488,000	0,600	1,482	1,479
488,000	2,200	1,482	1,479
488,000	1,700	1,482	1,479
488,000	1,100	1,482	1,479
488,000	0,700	1,482	1,479

Point estimates from the 3-param. normal CDF

Tab. 9: Point estimates from the 3-param. normal CDF with weight at 28 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [µg a.s./L]	212,685	288,154	515,154
lower 95%-cl	164,240	219,428	352,856
upper 95%-cl	275,418	378,864	738,635

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)..

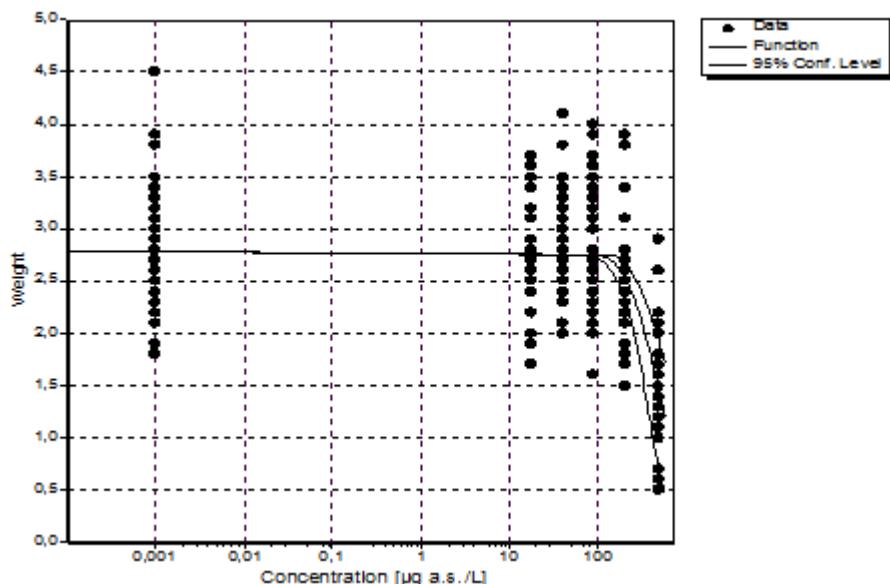


Fig. 2: Concentration-effect curve showing the influence of the test item on weight of the introduced *Oncorhynchus mykiss* as observed after 28 d

Threshold concentrations (NOEC) for Weight at 28 d

Statistical Characteristics of the Samples

Tab. 10: Statistical characteristics with weight at 28 d: Mean: arithmetic mean (\bar{X}); Med: median; Min: minimum value, Max: maximum value; n: sample size; s: standard deviation; %s: coefficient of variation; s(\bar{X}): standard error; %s(\bar{X}): %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [µg a.s./L]	Mean	Med	Min	Max	n	s	%s	s(\bar{X})	%s(\bar{X})	95%l	95%u
Pooled Control	2,73	2,70	1,80	4,50	80	0,485	17,7	0,054	2,0	2,62	2,84
18,000	2,77	2,70	1,70	3,70	40	0,491	17,7	0,078	2,8	2,62	2,93
40,000	2,85	2,70	2,00	4,10	40	0,471	16,5	0,075	2,6	2,70	3,01
90,000	2,82	2,75	1,60	4,00	40	0,545	19,3	0,086	3,1	2,65	3,00
205,000	2,52	2,50	1,50	3,90	40	0,518	20,6	0,082	3,3	2,35	2,69
488,000	1,48	1,50	0,50	2,90	38	0,577	38,9	0,094	6,3	1,29	1,67

Initially the results of the additionally included One-way ANOVA are shown

Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 11: Trend analysis by contrasts (monotonicity of concentration/response) with weight at 28 d: 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 (H_0 : Slope = 0). Hypothesis of monotonicity is accepted if at least the linear contrast is significant.

Trend	Psi	s(psi)	df	t	p(t)
Linear	7,0446	0,6195	272	11,372	<0,001
Quadratic	6,9321	0,6893	272	10,057	<0,001

The linear trend is significant ($p \leq 0,05$) The quadratic trend is significant ($p \leq 0,05$)

The first analysis by the included test revealed significant results: Pretesting is continued.

Shapiro-Wilk's Test on Normal Distribution

Tab. 12: Shapiro-Wilk's Test on Normal Distribution with weight at 28 d: 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 due to chance). In case p(ShapiroWilk's W) is greater than the chosen significance level, the normality hypothesis(H_0) is accepted.

Treatm. [µg a.s./L]	Mean	s	n
Pooled Control	2,73	0,485	80
18,000	2,77	0,491	40
40,000	2,85	0,471	40
90,000	2,82	0,545	40
205,000	2,52	0,518	40
488,000	1,48	0,577	38

Results:

Number of residuals = 106; Shapiro-Wilk's W = 0,984; p(W) = 0,255; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Levene's Test on Variance Homogeneity (with Residuals)

Tab. 13: Levene's Test on Variance Homogeneity (with Residuals) with weight at 28 d: 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	0,3457	5	0,0691	0,695	0,628
Residuals	27,0613	272	0,0995		
Total	27,407	277			

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

Variance homogeneitycheck 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.

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

Williams Multiple Sequential t-test Procedure

Tab. 14: Comparison of treatments with "Pooled Control" by the ttest procedure after Williams with weight at 28 d: 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 Pooled Control (in percent of Pooled Control); t: sample t; 't*': critical t for Ho: $\mu_1 = \mu_2 = \dots = \mu_k$; 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. [µg a.s./L]	Mean	s	df	LhM	%MDD	t	t*	Sign.
Pooled Control	2,73	0,5109						
18,000	2,77	0,5109	272	2,82	-6,0	0,85	-1,65	-
40,000	2,85	0,5109	272	2,82	-6,2	0,85	-1,71	-
90,000	2,82	0,5109	272	2,82	-6,2	0,85	-1,72	-
205,000	2,52	0,5109	272	2,52	-6,3	-2,15	-1,73	+
488,000	1,48	0,5109	272	1,48	-6,4	-12,43	-1,74	+

+: significant; -: non-significant

A NOEC of 90,000 µg a.s./L is suggested by the program.

Specific Growth Rate (Data)

Specific Growth Rate of Oncorhynchus mykiss as Dependent on Concentration and Time

Tab. 15: Specific growth rate of Oncorhynchus mykiss 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 RawDataWeight)

Tab. 15 (continued): Specific growth rate of *Oncorhynchus mykiss* 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 RawDataWeight)

Mean:	0,000	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	0,0000
n:	40	40	40	40	40	40	40
CV:							
28 d	0,026	0,040	0,045	0,037	0,029	0,030	0,028
	0,033	0,023	0,025	0,033	0,038	0,037	0,025
	0,028	0,031	0,043	0,015	0,039	0,031	0,013
	0,030	0,030	0,040	0,028	0,043	0,033	-0,013
	0,026	0,042	0,031	0,048	0,037	0,028	0,009
	0,035	0,030	0,025	0,029	0,028	0,029	0,009
	0,038	0,030	0,023	0,035	0,034	0,011	0,011
	0,037	0,034	0,035	0,030	0,028	0,022	0,000
	0,049	0,044	0,029	0,041	0,037	0,031	-0,018
	0,028	0,039	0,033	0,031	0,029	0,035	0,017
	0,033	0,035	0,038	0,035	0,031	0,034	0,022
	0,038	0,029	0,032	0,033	0,041	0,026	0,031
	0,045	0,028	0,040	0,041	0,031	0,028	0,025
	0,028	0,049	0,028	0,033	0,023	0,035	0,000
	0,026	0,033	0,042	0,038	0,040	0,037	0,038
	0,038	0,037	0,035	0,029	0,031	0,049	0,016
	0,037	0,025	0,035	0,029	0,033	0,037	0,005
	0,022	0,033	0,025	0,038	0,045	0,025	0,012
	0,031	0,035	0,037	0,032	0,032	0,030	0,000
	0,044	0,032	0,037	0,028	0,017	0,010	0,006
	0,023	0,034	0,035	0,038	0,046	0,012	0,026
	0,036	0,038	0,019	0,039	0,035	0,044	0,019
	0,037	0,039	0,034	0,026	0,034	0,035	0,020
	0,038	0,028	0,029	0,030	0,037	0,033	0,007
	0,038	0,030	0,040	0,033	0,035	0,041	0,021
	0,039	0,034	0,037	0,038	0,043	0,019	0,021
	0,035	0,040	0,035	0,026	0,043	0,035	0,000
	0,039	0,028	0,029	0,025	0,018	0,026	0,003
	0,032	0,039	0,025	0,029	0,039	0,035	-0,019
	0,035	0,035	0,029	0,047	0,028	0,031	-0,031
	0,026	0,039	0,038	0,028	0,047	0,037	0,014
	0,050	0,033	0,033	0,035	0,034	0,028	-0,003
	0,023	0,037	0,033	0,043	0,030	0,033	0,019
	0,037	0,028	0,047	0,033	0,033	0,033	-0,018
	0,035	0,030	0,046	0,033	0,028	0,028	0,028
	0,021	0,040	0,035	0,028	0,035	0,035	0,019

	0,035	0,023	0,033	0,039	0,037	0,018	0,003
	0,029	0,023	0,025	0,040	0,033	0,023	-0,016
	0,033	0,031	0,029	0,031	0,042	0,033	-
	0,026	0,034	0,029	0,038	0,036	0,023	-
Mean:	0,034	0,034	0,033	0,034	0,035	0,030	0,009
Std.Dev.:	0,0070	0,0059	0,0066	0,0064	0,0068	0,0083	0,0160
n:	40	40	40	40	40	40	38
CV:	20,7	17,7	19,8	19,0	19,8	27,6	174,8

Shapiro-Wilk's Test on Normal Distribution

Tab. 16: Shapiro-Wilk's Test on Normal Distribution with specific growth rate at 28 d: 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 due to chance). In case p(ShapiroWilk's W) is greater than the chosen significance level, the normality hypothesis(H₀) is accepted.

Treatm. [µg a.s./L]	Mean	s	n
Control	0,034	0,0070	40
Solv. Contr.	0,034	0,0059	40

Results:

Number of residuals = 101; Shapiro-Wilk's W = 0,987; p(W) = 0,448; 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 (Shapiro-Wilk's; p > 0,01).

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

STUDENT-t test for Homogeneous Variances

Tab. 17: STUDENT-t test for Homogeneous Variances with specific growth rate at 28 d: Two-sample comparison of the two controls. Significance was Alpha = 0,050, two-sided; 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 H₀: µ₁ = µ₂; the differences are significant in case p(t) <= Alpha ; p(F): two-sided probability of F computed by the F-test (H₀: var1 = var2 (homogeneity); p(F) > 0,010 is the criterion of variance homogeneity. (Control(c) and treatment(t) variance was applied: s²(c)/nc + s²(t)/nt, each).

Treatm. [µg a.s./L]	Mean	s	df	%MDD	t	p(t)	Sign.	p(F)
Control	0,034	0,0070						
Solv. Contr.	0,034	0,0059	78	8,6	0,02	0,987	-	0,323

+: significant; -: non-significant

There is no statistically significant difference between control and solv. contr..

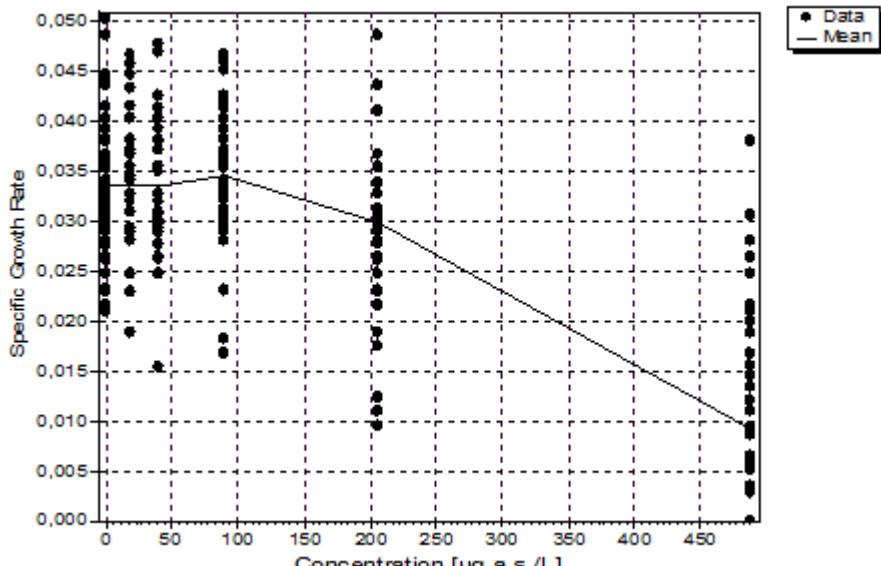


Fig. 3: Specific growth rate of *Oncorhynchus mykiss* as observed under presence of the test item after 28 d.

Effective Concentrations (ECx) for Specific Growth Rate at 28 d

Specific Growth Rate in *Oncorhynchus mykiss* after 28 d.

Tab. 18: %Inhibition of specific growth rate caused by the test item after 28 d.

Treatm.[µg a.s./L]	Mean	Std. Dev.	n	%Inhibition
Pooled Control	0,034	0,0064	80	
18,000	0,033	0,0066	40	0,2
40,000	0,034	0,0064	40	0,0
90,000	0,035	0,0068	40	-3,0
205,000	0,030	0,0083	40	10,7
488,000	0,009	0,0160	38	72,7

The 3-param. normal CDF $F(x) = b0 * [\text{NormalCDF}(b1 - \log_{10}(x)/b2 + zOpt)]$ 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. 19: Estimated parameters of the 3-param. normal CDF with specific growth rate at 28 d: 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 ($H_0: b0|b1|b2 = 0$); 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	0,034	0,001	0,033	0,035	54,659	<0,001
b1	2,300	0,050	2,201	2,399	45,772	<0,001
b2	0,205	0,030	0,146	0,264	6,829	<0,001

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

R²: 0,485; adjusted R²: 0,481. Residual standard error: 0,00865. Akaike Criterion (AIC): -2358,252. ShapiroWilk's test on normal distribution of residuals: p = 0,354..

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

Tab. 20: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with specific growth rate at 28 d:
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	0,019	2	0,010	129,403	<0.001
Residuals	0,021	275	0,000		
- Lack of Fit	0,000	3	0,000	0,162	0,922
- Pure Error	0,021	272	0,000		
Total	0,040	277			

Since $p(F|Regression) \leq 0.05$, 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. 21: Observed values in specific growth rate after 28 d as caused by the test item and predicted values as calculated from the function.

Treatm.[μ g a.s./L]	Observed	Mean Obs.	Predicted
0,001	0,026	0,0336	0,0338
0,001	0,033	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,030	0,0336	0,0338
0,001	0,026	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,038	0,0336	0,0338
0,001	0,037	0,0336	0,0338
0,001	0,049	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,033	0,0336	0,0338
0,001	0,038	0,0336	0,0338
0,001	0,045	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,026	0,0336	0,0338
0,001	0,038	0,0336	0,0338
0,001	0,037	0,0336	0,0338
0,001	0,022	0,0336	0,0338
0,001	0,031	0,0336	0,0338
0,001	0,044	0,0336	0,0338
0,001	0,023	0,0336	0,0338
0,001	0,036	0,0336	0,0338
0,001	0,037	0,0336	0,0338
0,001	0,038	0,0336	0,0338
0,001	0,038	0,0336	0,0338
0,001	0,039	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,039	0,0336	0,0338
0,001	0,032	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,026	0,0336	0,0338
0,001	0,050	0,0336	0,0338
0,001	0,023	0,0336	0,0338
0,001	0,037	0,0336	0,0338

0,001	0,035	0,0336	0,0338
0,001	0,021	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,029	0,0336	0,0338
0,001	0,033	0,0336	0,0338
0,001	0,026	0,0336	0,0338
0,001	0,040	0,0336	0,0338
0,001	0,023	0,0336	0,0338
0,001	0,031	0,0336	0,0338
0,001	0,030	0,0336	0,0338
0,001	0,042	0,0336	0,0338
0,001	0,030	0,0336	0,0338
0,001	0,030	0,0336	0,0338
0,001	0,034	0,0336	0,0338
0,001	0,044	0,0336	0,0338
0,001	0,039	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,029	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,049	0,0336	0,0338
0,001	0,033	0,0336	0,0338
0,001	0,037	0,0336	0,0338
0,001	0,025	0,0336	0,0338
0,001	0,033	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,032	0,0336	0,0338
0,001	0,034	0,0336	0,0338
0,001	0,038	0,0336	0,0338
0,001	0,039	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,030	0,0336	0,0338
0,001	0,034	0,0336	0,0338
0,001	0,040	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,039	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,039	0,0336	0,0338
0,001	0,033	0,0336	0,0338
0,001	0,037	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,030	0,0336	0,0338
0,001	0,040	0,0336	0,0338
0,001	0,023	0,0336	0,0338
0,001	0,023	0,0336	0,0338
0,001	0,031	0,0336	0,0338

0,001	0,034	0,0336	0,0338
18,000	0,045	0,0335	0,0338
18,000	0,025	0,0335	0,0338
18,000	0,043	0,0335	0,0338
18,000	0,040	0,0335	0,0338
18,000	0,031	0,0335	0,0338
18,000	0,025	0,0335	0,0338
18,000	0,023	0,0335	0,0338
18,000	0,035	0,0335	0,0338
18,000	0,029	0,0335	0,0338
18,000	0,033	0,0335	0,0338
18,000	0,038	0,0335	0,0338
18,000	0,032	0,0335	0,0338
18,000	0,040	0,0335	0,0338
18,000	0,028	0,0335	0,0338
18,000	0,042	0,0335	0,0338
18,000	0,035	0,0335	0,0338
18,000	0,035	0,0335	0,0338
18,000	0,025	0,0335	0,0338
18,000	0,037	0,0335	0,0338
18,000	0,037	0,0335	0,0338
18,000	0,035	0,0335	0,0338
18,000	0,019	0,0335	0,0338
18,000	0,034	0,0335	0,0338
18,000	0,029	0,0335	0,0338
18,000	0,040	0,0335	0,0338
18,000	0,037	0,0335	0,0338
18,000	0,035	0,0335	0,0338
18,000	0,029	0,0335	0,0338
18,000	0,025	0,0335	0,0338
18,000	0,029	0,0335	0,0338
18,000	0,038	0,0335	0,0338
18,000	0,033	0,0335	0,0338
18,000	0,033	0,0335	0,0338
18,000	0,047	0,0335	0,0338
18,000	0,046	0,0335	0,0338
18,000	0,035	0,0335	0,0338
18,000	0,033	0,0335	0,0338
18,000	0,025	0,0335	0,0338
18,000	0,029	0,0335	0,0338
18,000	0,029	0,0335	0,0338
40,000	0,037	0,0336	0,0338
40,000	0,033	0,0336	0,0338
40,000	0,015	0,0336	0,0338
40,000	0,028	0,0336	0,0338

40,000	0,048	0,0336	0,0338
40,000	0,029	0,0336	0,0338
40,000	0,035	0,0336	0,0338
40,000	0,030	0,0336	0,0338
40,000	0,041	0,0336	0,0338
40,000	0,031	0,0336	0,0338
40,000	0,035	0,0336	0,0338
40,000	0,033	0,0336	0,0338
40,000	0,041	0,0336	0,0338
40,000	0,033	0,0336	0,0338
40,000	0,038	0,0336	0,0338
40,000	0,029	0,0336	0,0338
40,000	0,029	0,0336	0,0338
40,000	0,038	0,0336	0,0338
40,000	0,032	0,0336	0,0338
40,000	0,028	0,0336	0,0338
40,000	0,038	0,0336	0,0338
40,000	0,039	0,0336	0,0338
40,000	0,026	0,0336	0,0338
40,000	0,030	0,0336	0,0338
40,000	0,033	0,0336	0,0338
40,000	0,038	0,0336	0,0338
40,000	0,026	0,0336	0,0338
40,000	0,025	0,0336	0,0338
40,000	0,029	0,0336	0,0338
40,000	0,047	0,0336	0,0338
40,000	0,028	0,0336	0,0338
40,000	0,035	0,0336	0,0338
40,000	0,043	0,0336	0,0338
40,000	0,033	0,0336	0,0338
40,000	0,033	0,0336	0,0338
40,000	0,028	0,0336	0,0338
40,000	0,039	0,0336	0,0338
40,000	0,040	0,0336	0,0338
40,000	0,031	0,0336	0,0338
40,000	0,038	0,0336	0,0338
90,000	0,029	0,0346	0,0337
90,000	0,038	0,0346	0,0337
90,000	0,039	0,0346	0,0337
90,000	0,043	0,0346	0,0337
90,000	0,037	0,0346	0,0337
90,000	0,028	0,0346	0,0337
90,000	0,034	0,0346	0,0337
90,000	0,028	0,0346	0,0337
90,000	0,037	0,0346	0,0337

90,000	0,029	0,0346	0,0337
90,000	0,031	0,0346	0,0337
90,000	0,041	0,0346	0,0337
90,000	0,031	0,0346	0,0337
90,000	0,023	0,0346	0,0337
90,000	0,040	0,0346	0,0337
90,000	0,031	0,0346	0,0337
90,000	0,033	0,0346	0,0337
90,000	0,045	0,0346	0,0337
90,000	0,032	0,0346	0,0337
90,000	0,017	0,0346	0,0337
90,000	0,046	0,0346	0,0337
90,000	0,035	0,0346	0,0337
90,000	0,034	0,0346	0,0337
90,000	0,037	0,0346	0,0337
90,000	0,035	0,0346	0,0337
90,000	0,043	0,0346	0,0337
90,000	0,043	0,0346	0,0337
90,000	0,018	0,0346	0,0337
90,000	0,039	0,0346	0,0337
90,000	0,028	0,0346	0,0337
90,000	0,047	0,0346	0,0337
90,000	0,034	0,0346	0,0337
90,000	0,030	0,0346	0,0337
90,000	0,033	0,0346	0,0337
90,000	0,028	0,0346	0,0337
90,000	0,035	0,0346	0,0337
90,000	0,037	0,0346	0,0337
90,000	0,033	0,0346	0,0337
90,000	0,042	0,0346	0,0337
90,000	0,036	0,0346	0,0337
205,000	0,030	0,0300	0,0300
205,000	0,037	0,0300	0,0300
205,000	0,031	0,0300	0,0300
205,000	0,033	0,0300	0,0300
205,000	0,028	0,0300	0,0300
205,000	0,029	0,0300	0,0300
205,000	0,011	0,0300	0,0300
205,000	0,022	0,0300	0,0300
205,000	0,031	0,0300	0,0300
205,000	0,035	0,0300	0,0300
205,000	0,034	0,0300	0,0300
205,000	0,026	0,0300	0,0300
205,000	0,028	0,0300	0,0300
205,000	0,035	0,0300	0,0300

205,000	0,037	0,0300	0,0300
205,000	0,049	0,0300	0,0300
205,000	0,037	0,0300	0,0300
205,000	0,025	0,0300	0,0300
205,000	0,030	0,0300	0,0300
205,000	0,010	0,0300	0,0300
205,000	0,012	0,0300	0,0300
205,000	0,044	0,0300	0,0300
205,000	0,035	0,0300	0,0300
205,000	0,033	0,0300	0,0300
205,000	0,041	0,0300	0,0300
205,000	0,019	0,0300	0,0300
205,000	0,035	0,0300	0,0300
205,000	0,026	0,0300	0,0300
205,000	0,035	0,0300	0,0300
205,000	0,031	0,0300	0,0300
205,000	0,037	0,0300	0,0300
205,000	0,028	0,0300	0,0300
205,000	0,033	0,0300	0,0300
205,000	0,028	0,0300	0,0300
205,000	0,035	0,0300	0,0300
205,000	0,018	0,0300	0,0300
205,000	0,023	0,0300	0,0300
205,000	0,033	0,0300	0,0300
205,000	0,023	0,0300	0,0300
488,000	0,028	0,0091	0,0091
488,000	0,025	0,0091	0,0091
488,000	0,013	0,0091	0,0091
488,000	-0,013	0,0091	0,0091
488,000	0,009	0,0091	0,0091
488,000	0,009	0,0091	0,0091
488,000	0,011	0,0091	0,0091
488,000	0,000	0,0091	0,0091
488,000	-0,018	0,0091	0,0091
488,000	0,017	0,0091	0,0091
488,000	0,022	0,0091	0,0091
488,000	0,031	0,0091	0,0091
488,000	0,025	0,0091	0,0091
488,000	0,000	0,0091	0,0091
488,000	0,038	0,0091	0,0091
488,000	0,016	0,0091	0,0091
488,000	0,005	0,0091	0,0091
488,000	0,012	0,0091	0,0091
488,000	0,000	0,0091	0,0091

488,000	0,006	0,0091	0,0091
488,000	0,026	0,0091	0,0091
488,000	0,019	0,0091	0,0091
488,000	0,020	0,0091	0,0091
488,000	0,007	0,0091	0,0091
488,000	0,021	0,0091	0,0091
488,000	0,021	0,0091	0,0091
488,000	0,000	0,0091	0,0091
488,000	0,003	0,0091	0,0091
488,000	-0,019	0,0091	0,0091
488,000	-0,031	0,0091	0,0091
488,000	0,014	0,0091	0,0091
488,000	-0,003	0,0091	0,0091
488,000	0,019	0,0091	0,0091
488,000	-0,018	0,0091	0,0091
488,000	0,028	0,0091	0,0091
488,000	0,019	0,0091	0,0091
488,000	0,003	0,0091	0,0091
488,000	-0,016	0,0091	0,0091

Point estimates from the 3-param. normal CDF

Tab. 22: Point estimates from the 3-param. normal CDF with specific growth rate at 28 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [µg a.s./L]	199,564	245,674	365,651
lower 95%-cl	158,912	196,789	279,187
upper 95%-cl	250,614	308,903	482,004

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)..

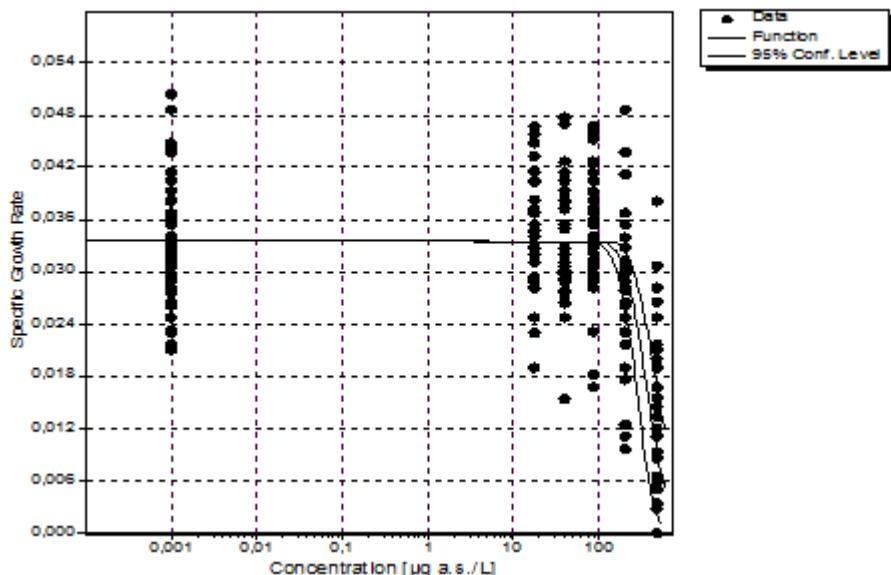


Fig. 4: Concentration-effect curve showing the influence of the test item on specific growth rate of the introduced *Oncorhynchus mykiss* as observed after 28 d

Statistical Characteristics of the Samples

Tab. 23: Statistical characteristics with specific growth rate at 28 d: 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. [µg a.s./L]	Mean	Med	Min	Max	n	s	%s	s(X)	%s(X)	95%l	95%u
Pooled Control	0,034	0,034	0,021	0,050	80	0,0064	19,1	0,0007	2,1	0,032	0,035
18,000	0,033	0,033	0,019	0,047	40	0,0066	19,8	0,0010	3,1	0,031	0,036
40,000	0,034	0,033	0,015	0,048	40	0,0064	19,0	0,0010	3,0	0,032	0,036
90,000	0,035	0,035	0,017	0,047	40	0,0068	19,8	0,0011	3,1	0,032	0,037
205,000	0,030	0,031	0,010	0,049	40	0,0083	27,6	0,0013	4,4	0,027	0,033
488,000	0,009	0,012	-0,031	0,038	38	0,0160	174,8	0,0026	28,4	0,004	0,014

Shapiro-Wilk's Test on Normal Distribution

Tab. 24: Shapiro-Wilk's Test on Normal Distribution with specific growth rate at 28 d: 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 due to chance). In case p(ShapiroWilk's W) is greater than the chosen significance level, the normality hypothesis(H₀) is accepted.

Treatm. [µg a.s./L]	Mean	s	n
Pooled Control	0,034	0,0064	80
18,000	0,033	0,0066	40
40,000	0,034	0,0064	40
90,000	0,035	0,0068	40
205,000	0,030	0,0083	40
488,000	0,009	0,0160	38

Results:

Number of residuals = 102; Shapiro-Wilk's W = 0,987; p(W) = 0,457; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Levene's Test on Variance Homogeneity (with Residuals)

Tab. 25: Levene's Test on Variance Homogeneity (with Residuals) with specific growth rate at 28 d: 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	0,00181	5	0,00036	13,380	< 0.001
Residuals	0,00737	272	0,00003		
Total	0,0092	277			

The Levene test indicates variance heterogeneity($p \leq 0,010$)!

Variance homogeneitycheck failed

However, normal distribution requirementsare fulfilled.

The Welch-t-test for non-homogeneousvariances with Bonferroni-Holm-adjustmentis advisable.

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

Tab. 26: Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm with specific growth rate at 28 d: Multiple sequentially rejective comparisons of treatments with "Pooled Control". Significance was Alpha = 0,050, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; MDD: minimum detectable difference to Pooled Control (in percent of Pooled Control); t: sample t; p(t): probability of sample t for Ho: $\mu_1 = \mu_2$; Alpha(i): adjusted significance levels; the differences are significant in case p(t) \leq 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. [µg a.s./L]	Mean	s	df	%MDD	t	p(t)	Alpha(i)	Sign.
Pooled Control	0,034	0,0064						
18,000	0,033	0,0066	76	-8,2	-0,06	0,476	0,017	-
40,000	0,034	0,0064	78	-7,4	0,00	0,502	0,025	-
90,000	0,035	0,0068	73	-6,4	0,77	0,778	0,050	-
205,000	0,030	0,0083	63	-10,2	-2,40	0,010	0,013	+
488,000	0,009	0,0160	42	-19,4	-9,07	< 0,001	0,010	+

+: significant; -: non-significant

A NOEC of 90,000 µg a.s./L is suggested by the program.

Specific Interval Growth Rate (Data)

Specific Interval Growth Rate of Oncorhynchus mykiss as Dependent on Concentration and Time

Tab. 27: Specific interval growth rate of Oncorhynchus mykiss 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 RawDataWeight)

Treatm. [µg a.s./L]	Control	Solv. Contr.	18,000	40,000	90,000	205,000	488,000
0 d	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
	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
	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
	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
	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
	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

0,045	0,028	0,040	0,041	0,031	0,028	0,025
0,028	0,049	0,028	0,033	0,023	0,035	0,000
0,026	0,033	0,042	0,038	0,040	0,037	0,038
0,038	0,037	0,035	0,029	0,031	0,049	0,016
0,037	0,025	0,035	0,029	0,033	0,037	0,005
0,022	0,033	0,025	0,038	0,045	0,025	0,012
0,031	0,035	0,037	0,032	0,032	0,030	0,000
0,044	0,032	0,037	0,028	0,017	0,010	0,006
0,023	0,034	0,035	0,038	0,046	0,012	0,026
0,036	0,038	0,019	0,039	0,035	0,044	0,019
0,037	0,039	0,034	0,026	0,034	0,035	0,020
0,038	0,028	0,029	0,030	0,037	0,033	0,007
0,038	0,030	0,040	0,033	0,035	0,041	0,021
0,039	0,034	0,037	0,038	0,043	0,019	0,021
0,035	0,040	0,035	0,026	0,043	0,035	0,000
0,039	0,028	0,029	0,025	0,018	0,026	0,003
0,032	0,039	0,025	0,029	0,039	0,035	-0,019
0,035	0,035	0,029	0,047	0,028	0,031	-0,031
0,026	0,039	0,038	0,028	0,047	0,037	0,014
0,050	0,033	0,033	0,035	0,034	0,028	-0,003
0,023	0,037	0,033	0,043	0,030	0,033	0,019
0,037	0,028	0,047	0,033	0,033	0,033	-0,018
0,035	0,030	0,046	0,033	0,028	0,028	0,028
0,021	0,040	0,035	0,028	0,035	0,035	0,019
0,035	0,023	0,033	0,039	0,037	0,018	0,003
0,029	0,023	0,025	0,040	0,033	0,023	-0,016
0,033	0,031	0,029	0,031	0,042	0,033	-
0,026	0,034	0,029	0,038	0,036	0,023	-
Mean:	0,034	0,034	0,033	0,034	0,035	0,030
Std.Dev.:	0,0070	0,0059	0,0066	0,0064	0,0068	0,0083
n:	40	40	40	40	40	40
CV:	20,7	17,7	19,8	19,0	19,8	27,6
						174,8

Shapiro-Wilk's Test on Normal Distribution

Tab. 28: Shapiro-Wilk's Test on Normal Distribution with specific interval growth rate at 28 d; 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 due to chance). In case p(ShapiroWilk's W) is greater than the chosen significance level, the normality hypothesis(H₀) is accepted.

Treatm. [µg a.s./L]	Mean	s	n
Control	0,034	0,0070	40
Solv. Contr.	0,034	0,0059	40

Results:

Number of residuals = 101; Shapiro-Wilk's W = 0,987; p(W) = 0,448; 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 (Shapiro-Wilk's; p > 0,01).

Variance homogeneityckeck (F-test) was passed ($p > 0,01$).

STUDENT-t test for Homogeneous Variances

Tab. 29: STUDENT-t test for Homogeneous Variances with specific interval growth rate at 28 d: Two-sample comparison of the two controls. Significance was Alpha = 0,050, two-sided; 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: $\mu_1 = \mu_2$; the differences are significant in case $p(t) \leq \text{Alpha}$; p(F): two-sided probability of F computed by the F-test (Ho: var1 = var2 (homogeneity); $p(F) > 0,010$ is the criterion of variance homogeneity. (Control(c) and treatment(t) variance was applied: $s^2(c)/nc + s^2(t)/nt$, each).

Treatm. [µg a.s./L]	Mean	s	df	%MDD	t	p(t)	Sign.	p(F)
Control	0,034	0,0070						
Solv. Contr.	0,034	0,0059	78	8,6	0,02	0,987	-	0,323

+: significant; -: non-significant

There is no statistically significant difference between control and solv. contr..

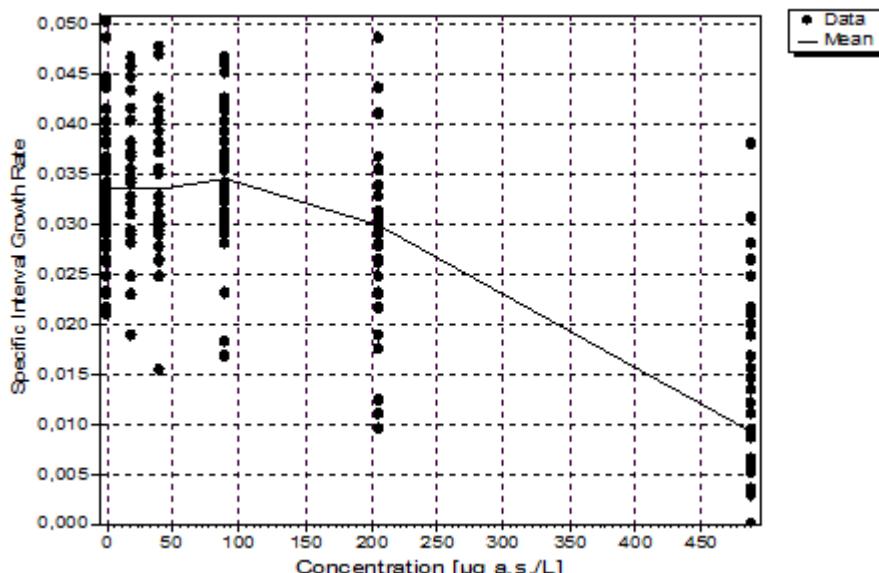


Fig. 5: Specific interval growth rate of *Oncorhynchus mykiss* as observed under presence of the test item after 28 d.

Effective Concentrations (ECx) for Specific Interval Growth Rate at 28 d

Specific Interval Growth Rate in *Oncorhynchus mykiss* after 28 d.

Tab. 30: %Inhibition of specific interval growth rate caused by the test item after 28 d.

Treatm.[µg a.s./L]	Mean	Std. Dev.	n	%Inhibition
Pooled Control	0,034	0,0064	80	
18,000	0,033	0,0066	40	0,2
40,000	0,034	0,0064	40	0,0
90,000	0,035	0,0068	40	-3,0
205,000	0,030	0,0083	40	10,7
488,000	0,009	0,0160	38	72,7

The 3-param. normal CDF $F(x) = b0 * [\text{NormalCDF}(b1 - \log_{10}(x)/b2 + zOpt)]$ 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. 31: Estimated parameters of the 3-param. normal CDF with specific interval growth rate at 28 d: 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 ($H_0: b0|b1|b2 = 0$); 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	0,034	0,001	0,033	0,035	54,659	<0.001
b1	2,300	0,050	2,201	2,399	45,772	<0.001
b2	0,205	0,030	0,146	0,264	6,829	<0.001

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

R²: 0,485; adjusted R²: 0,481. Residual standard error: 0,00865. Akaike Criterion (AIC): -2358,252. ShapiroWilk's test on normal distribution of residuals: p = 0,354..

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

Tab. 32: Analysis of Variance and Test for Lack of Fit for the 3-param. normal CDF with specific interval growth rate at 28 d: 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	0,019	2	0,010	129,403	<0.001
Residuals	0,021	275	0,000		
- Lack of Fit	0,000	3	0,000	0,162	0,922
- Pure Error	0,021	272	0,000		
Total	0,040	277			

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. 33: Observed values in specific interval growth rate after 28 d as caused by the test item and predicted values as calculated from the function.

Treatm.[μ g a.s./L]	Observed	Mean Obs.	Predicted
0,001	0,026	0,0336	0,0338
0,001	0,033	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,030	0,0336	0,0338
0,001	0,026	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,038	0,0336	0,0338
0,001	0,037	0,0336	0,0338
0,001	0,049	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,033	0,0336	0,0338
0,001	0,038	0,0336	0,0338
0,001	0,045	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,026	0,0336	0,0338
0,001	0,038	0,0336	0,0338
0,001	0,037	0,0336	0,0338
0,001	0,022	0,0336	0,0338

0,001	0,031	0,0336	0,0338
0,001	0,044	0,0336	0,0338
0,001	0,023	0,0336	0,0338
0,001	0,036	0,0336	0,0338
0,001	0,037	0,0336	0,0338
0,001	0,038	0,0336	0,0338
0,001	0,038	0,0336	0,0338
0,001	0,039	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,039	0,0336	0,0338
0,001	0,032	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,026	0,0336	0,0338
0,001	0,050	0,0336	0,0338
0,001	0,023	0,0336	0,0338
0,001	0,037	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,021	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,029	0,0336	0,0338
0,001	0,033	0,0336	0,0338
0,001	0,026	0,0336	0,0338
0,001	0,040	0,0336	0,0338
0,001	0,023	0,0336	0,0338
0,001	0,031	0,0336	0,0338
0,001	0,030	0,0336	0,0338
0,001	0,042	0,0336	0,0338
0,001	0,030	0,0336	0,0338
0,001	0,030	0,0336	0,0338
0,001	0,034	0,0336	0,0338
0,001	0,044	0,0336	0,0338
0,001	0,039	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,029	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,049	0,0336	0,0338
0,001	0,033	0,0336	0,0338
0,001	0,037	0,0336	0,0338
0,001	0,025	0,0336	0,0338
0,001	0,033	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,032	0,0336	0,0338
0,001	0,034	0,0336	0,0338
0,001	0,038	0,0336	0,0338
0,001	0,039	0,0336	0,0338

0,001	0,028	0,0336	0,0338
0,001	0,030	0,0336	0,0338
0,001	0,034	0,0336	0,0338
0,001	0,040	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,039	0,0336	0,0338
0,001	0,035	0,0336	0,0338
0,001	0,039	0,0336	0,0338
0,001	0,033	0,0336	0,0338
0,001	0,037	0,0336	0,0338
0,001	0,028	0,0336	0,0338
0,001	0,030	0,0336	0,0338
0,001	0,040	0,0336	0,0338
0,001	0,023	0,0336	0,0338
0,001	0,023	0,0336	0,0338
0,001	0,031	0,0336	0,0338
0,001	0,034	0,0336	0,0338
18,000	0,045	0,0335	0,0338
18,000	0,025	0,0335	0,0338
18,000	0,043	0,0335	0,0338
18,000	0,040	0,0335	0,0338
18,000	0,031	0,0335	0,0338
18,000	0,025	0,0335	0,0338
18,000	0,023	0,0335	0,0338
18,000	0,035	0,0335	0,0338
18,000	0,029	0,0335	0,0338
18,000	0,033	0,0335	0,0338
18,000	0,038	0,0335	0,0338
18,000	0,032	0,0335	0,0338
18,000	0,040	0,0335	0,0338
18,000	0,028	0,0335	0,0338
18,000	0,042	0,0335	0,0338
18,000	0,035	0,0335	0,0338
18,000	0,035	0,0335	0,0338
18,000	0,025	0,0335	0,0338
18,000	0,037	0,0335	0,0338
18,000	0,037	0,0335	0,0338
18,000	0,035	0,0335	0,0338
18,000	0,019	0,0335	0,0338
18,000	0,034	0,0335	0,0338
18,000	0,029	0,0335	0,0338
18,000	0,040	0,0335	0,0338
18,000	0,037	0,0335	0,0338
18,000	0,035	0,0335	0,0338
18,000	0,029	0,0335	0,0338

18,000	0,025	0,0335	0,0338
18,000	0,029	0,0335	0,0338
18,000	0,038	0,0335	0,0338
18,000	0,033	0,0335	0,0338
18,000	0,033	0,0335	0,0338
18,000	0,047	0,0335	0,0338
18,000	0,046	0,0335	0,0338
18,000	0,035	0,0335	0,0338
18,000	0,033	0,0335	0,0338
18,000	0,025	0,0335	0,0338
18,000	0,029	0,0335	0,0338
18,000	0,029	0,0335	0,0338
40,000	0,037	0,0336	0,0338
40,000	0,033	0,0336	0,0338
40,000	0,015	0,0336	0,0338
40,000	0,028	0,0336	0,0338
40,000	0,048	0,0336	0,0338
40,000	0,029	0,0336	0,0338
40,000	0,035	0,0336	0,0338
40,000	0,030	0,0336	0,0338
40,000	0,041	0,0336	0,0338
40,000	0,031	0,0336	0,0338
40,000	0,035	0,0336	0,0338
40,000	0,033	0,0336	0,0338
40,000	0,041	0,0336	0,0338
40,000	0,033	0,0336	0,0338
40,000	0,038	0,0336	0,0338
40,000	0,029	0,0336	0,0338
40,000	0,029	0,0336	0,0338
40,000	0,038	0,0336	0,0338
40,000	0,032	0,0336	0,0338
40,000	0,028	0,0336	0,0338
40,000	0,038	0,0336	0,0338
40,000	0,039	0,0336	0,0338
40,000	0,026	0,0336	0,0338
40,000	0,030	0,0336	0,0338
40,000	0,033	0,0336	0,0338
40,000	0,038	0,0336	0,0338
40,000	0,026	0,0336	0,0338
40,000	0,025	0,0336	0,0338
40,000	0,029	0,0336	0,0338
40,000	0,047	0,0336	0,0338
40,000	0,028	0,0336	0,0338
40,000	0,035	0,0336	0,0338
40,000	0,043	0,0336	0,0338

40,000	0,033	0,0336	0,0338
40,000	0,033	0,0336	0,0338
40,000	0,028	0,0336	0,0338
40,000	0,039	0,0336	0,0338
40,000	0,040	0,0336	0,0338
40,000	0,031	0,0336	0,0338
40,000	0,038	0,0336	0,0338
90,000	0,029	0,0346	0,0337
90,000	0,038	0,0346	0,0337
90,000	0,039	0,0346	0,0337
90,000	0,043	0,0346	0,0337
90,000	0,037	0,0346	0,0337
90,000	0,028	0,0346	0,0337
90,000	0,034	0,0346	0,0337
90,000	0,028	0,0346	0,0337
90,000	0,037	0,0346	0,0337
90,000	0,029	0,0346	0,0337
90,000	0,031	0,0346	0,0337
90,000	0,041	0,0346	0,0337
90,000	0,031	0,0346	0,0337
90,000	0,023	0,0346	0,0337
90,000	0,040	0,0346	0,0337
90,000	0,031	0,0346	0,0337
90,000	0,033	0,0346	0,0337
90,000	0,045	0,0346	0,0337
90,000	0,032	0,0346	0,0337
90,000	0,017	0,0346	0,0337
90,000	0,046	0,0346	0,0337
90,000	0,035	0,0346	0,0337
90,000	0,034	0,0346	0,0337
90,000	0,037	0,0346	0,0337
90,000	0,035	0,0346	0,0337
90,000	0,043	0,0346	0,0337
90,000	0,043	0,0346	0,0337
90,000	0,018	0,0346	0,0337
90,000	0,039	0,0346	0,0337
90,000	0,028	0,0346	0,0337
90,000	0,047	0,0346	0,0337
90,000	0,034	0,0346	0,0337
90,000	0,030	0,0346	0,0337
90,000	0,033	0,0346	0,0337
90,000	0,028	0,0346	0,0337
90,000	0,035	0,0346	0,0337
90,000	0,037	0,0346	0,0337
90,000	0,033	0,0346	0,0337

90,000	0,042	0,0346	0,0337
90,000	0,036	0,0346	0,0337
205,000	0,030	0,0300	0,0300
205,000	0,037	0,0300	0,0300
205,000	0,031	0,0300	0,0300
205,000	0,033	0,0300	0,0300
205,000	0,028	0,0300	0,0300
205,000	0,029	0,0300	0,0300
205,000	0,011	0,0300	0,0300
205,000	0,022	0,0300	0,0300
205,000	0,031	0,0300	0,0300
205,000	0,035	0,0300	0,0300
205,000	0,034	0,0300	0,0300
205,000	0,026	0,0300	0,0300
205,000	0,028	0,0300	0,0300
205,000	0,035	0,0300	0,0300
205,000	0,037	0,0300	0,0300
205,000	0,049	0,0300	0,0300
205,000	0,037	0,0300	0,0300
205,000	0,025	0,0300	0,0300
205,000	0,030	0,0300	0,0300
205,000	0,010	0,0300	0,0300
205,000	0,012	0,0300	0,0300
205,000	0,044	0,0300	0,0300
205,000	0,035	0,0300	0,0300
205,000	0,033	0,0300	0,0300
205,000	0,041	0,0300	0,0300
205,000	0,019	0,0300	0,0300
205,000	0,035	0,0300	0,0300
205,000	0,026	0,0300	0,0300
205,000	0,035	0,0300	0,0300
205,000	0,031	0,0300	0,0300
205,000	0,037	0,0300	0,0300
205,000	0,028	0,0300	0,0300
205,000	0,033	0,0300	0,0300
205,000	0,033	0,0300	0,0300
205,000	0,028	0,0300	0,0300
205,000	0,035	0,0300	0,0300
205,000	0,018	0,0300	0,0300
205,000	0,023	0,0300	0,0300
205,000	0,033	0,0300	0,0300
205,000	0,023	0,0300	0,0300
488,000	0,028	0,0091	0,0091
488,000	0,025	0,0091	0,0091
488,000	0,013	0,0091	0,0091

488,000	-0,013	0,0091	0,0091
488,000	0,009	0,0091	0,0091
488,000	0,009	0,0091	0,0091
488,000	0,011	0,0091	0,0091
488,000	0,000	0,0091	0,0091
488,000	-0,018	0,0091	0,0091
488,000	0,017	0,0091	0,0091
488,000	0,022	0,0091	0,0091
488,000	0,031	0,0091	0,0091
488,000	0,025	0,0091	0,0091
488,000	0,000	0,0091	0,0091
488,000	0,038	0,0091	0,0091
488,000	0,016	0,0091	0,0091
488,000	0,005	0,0091	0,0091
488,000	0,012	0,0091	0,0091
488,000	0,000	0,0091	0,0091
488,000	0,006	0,0091	0,0091
488,000	0,026	0,0091	0,0091
488,000	0,019	0,0091	0,0091
488,000	0,020	0,0091	0,0091
488,000	0,007	0,0091	0,0091
488,000	0,021	0,0091	0,0091
488,000	0,021	0,0091	0,0091
488,000	0,000	0,0091	0,0091
488,000	0,003	0,0091	0,0091
488,000	-0,019	0,0091	0,0091
488,000	-0,031	0,0091	0,0091
488,000	0,014	0,0091	0,0091
488,000	-0,003	0,0091	0,0091
488,000	0,019	0,0091	0,0091
488,000	-0,018	0,0091	0,0091
488,000	0,028	0,0091	0,0091
488,000	0,019	0,0091	0,0091
488,000	0,003	0,0091	0,0091
488,000	-0,016	0,0091	0,0091

Point estimates from the 3-param. normal CDF

Tab. 34: Point estimates from the 3-param. normal CDF with specific interval growth rate at 28 d: Selected effective concentrations (ECx) of the test item; cl: confidence limit

Toxicity Metric	EC10	EC20	EC50
Value [µg a.s./L]	199,564	245,674	365,651
lower 95%-cl	158,912	196,789	279,187
upper 95%-cl	250,614	308,903	482,004

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)..

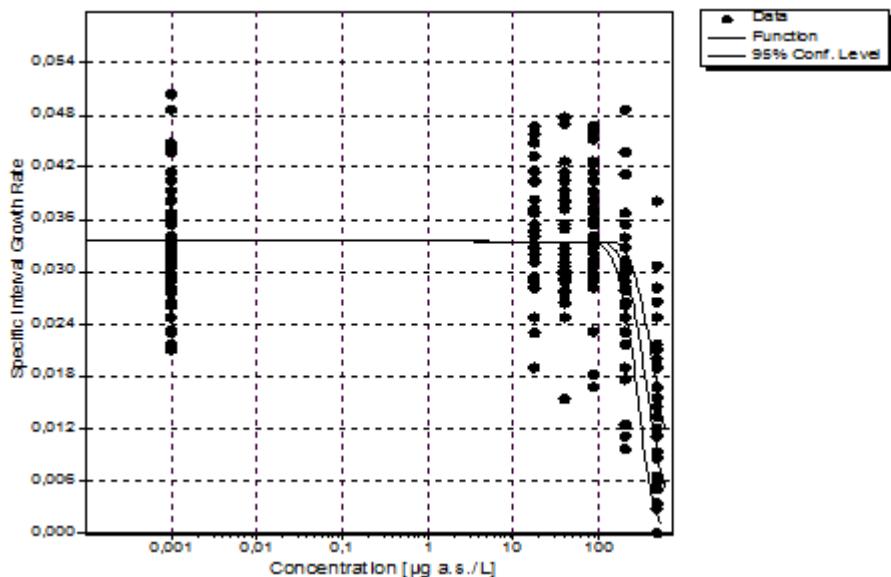


Fig. 6: Concentration-effect curve showing the influence of the test item on specific interval growth rate of the introduced *Oncorhynchus mykiss* as observed after 28 d

Statistical Characteristics of the Samples

Tab. 35: Statistical characteristics with specific interval growth rate at 28 d: 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; $s\%s(X)$: %standard error; 95%l, 95%u: lower, upper 95%-confidence limits.

Treatm. [µg a.s./L]	Mean	Med	Min	Max	n	s	%s	$s(X)$	$s\%s(X)$	95%l	95%u
Pooled Control	0,034	0,034	0,021	0,050	80	0,0064	19,1	0,0007	2,1	0,032	0,035
18,000	0,033	0,033	0,019	0,047	40	0,0066	19,8	0,0010	3,1	0,031	0,036
40,000	0,034	0,033	0,015	0,048	40	0,0064	19,0	0,0010	3,0	0,032	0,036
90,000	0,035	0,035	0,017	0,047	40	0,0068	19,8	0,0011	3,1	0,032	0,037
205,000	0,030	0,031	0,010	0,049	40	0,0083	27,6	0,0013	4,4	0,027	0,033
488,000	0,009	0,012	-0,031	0,038	38	0,0160	174,8	0,0026	28,4	0,004	0,014

Shapiro-Wilk's Test on Normal Distribution

Tab. 36: Shapiro-Wilk's Test on Normal Distribution with specific interval growth rate at 28 d: 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 due to chance). In case p(ShapiroWilk's W) is greater than the chosen significance level, the normality hypothesis(H_0) is accepted.

Treatm. [µg a.s./L]	Mean	s	n
Pooled Control	0,034	0,0064	80
18,000	0,033	0,0066	40
40,000	0,034	0,0064	40
90,000	0,035	0,0068	40
205,000	0,030	0,0083	40
488,000	0,009	0,0160	38

Results:

Number of residuals = 102; Shapiro-Wilk's W = 0,987; p(W) = 0,457; p(W) is greater than the selected significance level of 0,010; thus treatment data do not significantly deviate from normal distribution.

Levene's Test on Variance Homogeneity (with Residuals)

Tab. 37: Levene's Test on Variance Homogeneity (with Residuals) with specific interval growth rate at 28 d: 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	0,00181	5	0,00036	13,380	< 0,001
Residuals	0,00737	272	0,00003		
Total	0,0092	277			

The Levene test indicates variance heterogeneity($p \leq 0,010$)!

Variance homogeneitycheck failed

However, normal distribution requirementsare fulfilled.

The Welch-t-test for non-homogeneousvariances with Bonferroni-Holm-adjustmentsis advisable.

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

Tab. 38: Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm with specific interval growth rate at 28 d: Multiple sequentially rejective comparisons of treatments with "Pooled Control". Significance was Alpha = 0,050, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; MDD: minimum detectable difference to Pooled Control (in percent of Pooled Control); t: sample t; p(t): probability of sample t for Ho: $\mu_1 = \mu_2$; Alpha(i): adjusted significance levels; the differences are significant in case p(t) \leq 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. [µg a.s./L]	Mean	s	df	%MDD	t	p(t)	Alpha(i)	Sign.
Pooled Control	0,034	0,0064						
18,000	0,033	0,0066	76	-8,2	-0,06	0,476	0,017	-
40,000	0,034	0,0064	78	-7,4	0,00	0,502	0,025	-
90,000	0,035	0,0068	73	-6,4	0,77	0,778	0,050	-
205,000	0,030	0,0083	63	-10,2	-2,40	0,010	0,013	+
488,000	0,009	0,0160	42	-19,4	-9,07	< 0,001	0,010	+

+: significant; -: non-significant

A NOEC of 90,000 µg a.s./L is suggested by the program.

Mortality(Data)

Mortality as Dependent on Concentration and Time

Tab. 39: Mortality of *Oncorhynchus mykiss* over time as dependent on concentration of the test item (from RawDataMort)

Treatm. [µg a.s./L]	Control	Solv. Contr.	18,000	40,000	90,000	205,000	488,000
0 d:	0	0	0	0	0	0	0
14 d:	0	0	0	0	0	0	1
28 d:	0	0	0	0	0	0	2

Fisher's Exact Binomial Test

Tab. 40: Fisher's Exact Binomial Test with mortality at 28 d: Two-sample comparisons between the two controls (Alpha is 0,050; two-sided); Ho (no effect) is accepted, if the probability p(exact) > Alpha; p(exact) is the probability that the deviation in category "Dead" observed in the treatment(s) is due to chance.

Treatm.[µg a.s./L]	Introduced	Survived	Dead	% Mortality	p(exact)	sign.
Control	40	40	0	0,0		
Solv. Contr.	40	40	0	0,0	1,000	-

+: significant; -: non-significant

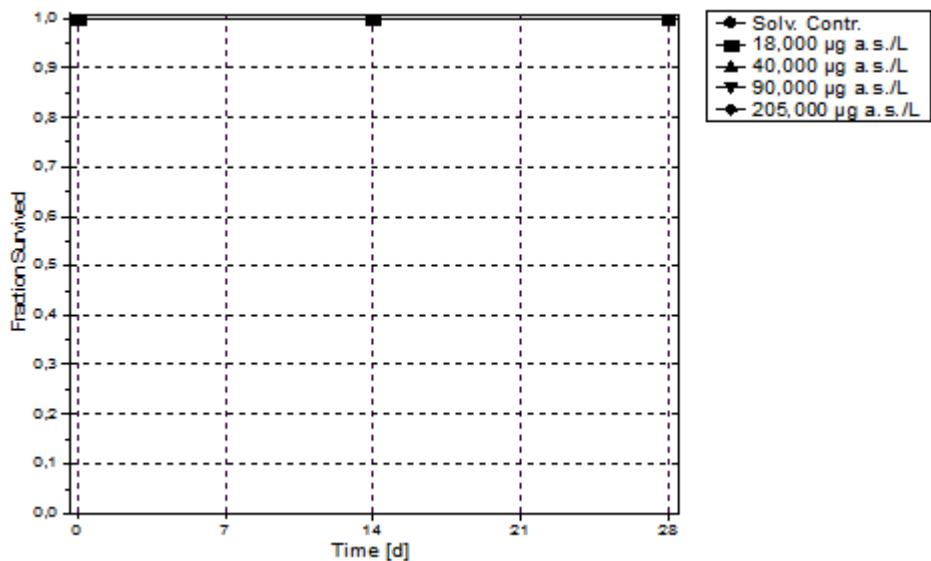


Fig. 7: Mortality of the introduced *Oncorhynchus mykiss* as observed under presence of the test item.

Lethal Concentrations (LC_x) for Mortality at 14 d

Overview Mortality

Tab. 41: Overview Mortality: Overview over the effects on mortality in *Oncorhynchus mykiss* at 14 d

Treatm. [µg a.s./L]	Total	Introduced	Survived	Dead	% Mortality
Solv. Contr.	40	40	40	0	0,0
18,000	40	40	40	0	0,0
40,000	40	40	40	0	0,0
90,000	40	40	40	0	0,0
205,000	40	40	40	0	0,0
488,000	40	40	39	1	2,5

Probit analysis using linear max. likelihood regression

Tab. 42: Probit analysis using linear max. likelihood regression with mortality at 14 d: Determination of the concentration /response function; data is shown which entered the probit analysis; Log(x): logarithm of the concentration; n: number of organisms; Emp. Probit: empirical probit; Reg. Probit: calculated probit for the final function.

Treatm. [µg a.s./L]	Log(x)	% Mortality	n	Emp. Probit	Weight	Reg. Probit
Solv. Contr.		0,0	40			excluded
18,000	1,255	0,0	40	-1,2533	13,904	-1,266
40,000	1,602	0,0	40	-1,2533	14,073	-1,254
90,000	1,954	0,0	40	-1,2533	14,245	-1,241
205,000	2,312	0,0	40	-1,2533	14,420	-1,228
488,000	2,688	2,5	40	-1,1906	14,604	-1,215

excluded: value not in line with the chosen function

Parameters of the probit analysis

Tab. 43: Parameters of the probit analysis with mortality at 14 d: Results of the regression analysis

Parameter	Value
Computation runs:	2

Slope b:	0,10090
Intercept a:	-1,99364
Variance of b:	0,05485
Goodness of Fit	
Chi ² :	0,09602
Degrees of freedom:	3
p(Chi ²):	0,992
Log LC50:	19,75829
SE Log LC50:	41,30055
g-Criterion:	20,69395
F:	5,800
p(F) (df: 1;3):	0,095

Chi² is a goodness of fit measure. If the probability, p(Chi²), is lower or equal than 0,100 data is much scattering round the computed dose/responsefunction. In this case and with quantal data, confidence limits are corrected for heterogeneity(extra-binomial variance).

No statistically significant concentration/response was found ($p(F) > 0.05$; i.e. slope of the relationship is not significantly different from zero).

Due to the lacking concentration/response the shown LCx could not be valid.

Nonetheless, the user decided to show the LCx, confidence limits and graphs if possible.

Results of the probit analysis

Tab. 44: Results of the probit analysis with mortality at 14 d: Selected effective concentrations (LCx) of the test item and their 95%-confidence limits (according to Fieller's theorem).

Toxicity Metric	LC10	LC20	LC50
Value [µg a.s./L]	n.d.	n.d.	n.d.
lower 95%-cl	n.d.	n.d.	n.d.
upper 95%-cl	n.d.	n.d.	n.d.

n.d.: not determined either due to mathematical reasons or value is beyond the tested concentrations by more than factor 1000.

Slope function after Litchfield and Wilcoxon: 8141063168,000 The probability p(F) is greater than 0.05; i.e. the slope was not significantly different from zero. The shown toxic metrics could be meaningless.

(The slope function is derived from the slope, b, of the linearized probit function and computes as $S = 10^{(1/b)}$; please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

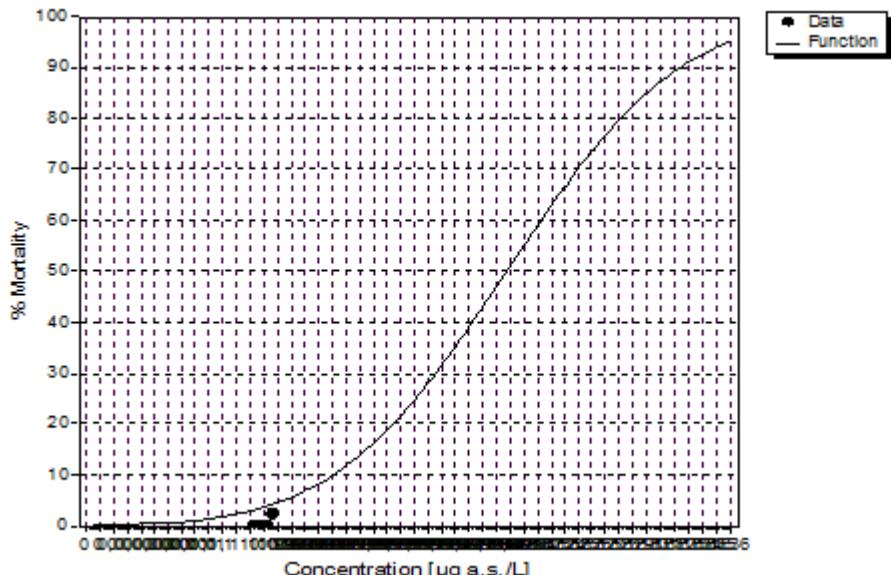


Fig. 8: Concentration-effect curve showing the influence of the test item on mortality of the introduced *Oncorhynchus mykiss* as observed after 14 d

Lethal Concentrations (LCx) for Mortality at 28 d

Overview Mortality

Tab. 45: Overview Mortality: Overview over the effects on mortality in *Oncorhynchus mykiss* at 28 d

Treatm. [µg a.s./L]	Total	Introduced	Survived	Dead	% Mortality
Solv. Contr.	40	40	40	0	0,0
18,000	40	40	40	0	0,0
40,000	40	40	40	0	0,0
90,000	40	40	40	0	0,0
205,000	40	40	40	0	0,0
488,000	40	38	38	2	5,0

Probit analysis using linear max. likelihood regression

Tab. 46: Probit analysis using linear max. likelihood regression with mortality at 28 d: Determination of the concentration /response function; data is shown which entered the probit analysis; Log(x): logarithm of the concentration; n: number of organisms; Emp. Probit: empirical probit; Reg. Probit: calculated probit for the final function.

Treatm. [µg a.s./L]	Log(x)	% Mortality	n	Emp. Probit	Weight	Reg. Probit
Solv. Contr.		0,0	40			excluded
18,000	1,255	0,0	40	-1,2533	13,731	-1,278
40,000	1,602	0,0	40	-1,2533	14,068	-1,254
90,000	1,954	0,0	40	-1,2533	14,412	-1,229
205,000	2,312	0,0	40	-1,2533	14,761	-1,204
488,000	2,688	5,0	40	-1,1280	15,129	-1,177

excluded: value not in line with the chosen function

Parameters of the probit analysis

Tab. 47: Parameters of the probit analysis with mortality at 28 d: Results of the regression analysis

Parameter	Value
Computation runs:	2
Slope b:	0,19644

Intercept a:	-2,14847
Variance of b:	0,05421
Goodness of Fit	
Chi ² :	0,35808
Degrees of freedom:	3
p(Chi ²):	0,949
Log LC50:	10,93726
SE Log LC50:	10,63367
g-Criterion:	5,39631
F:	5,964
p(F) (df: 1;3):	0,092

Chi² is a goodness of fit measure. If the probability, p(Chi²), is lower or equal than 0,100 data is much scattering round the computed dose/responsefunction. In this case and with quantal data, confidence limits are corrected for heterogeneity(extra-binomial variance).

No statistically significant concentration/response was found ($p(F) > 0.05$; i.e. slope of the relationship is not significantly different from zero).

Due to the lacking concentration/response the shown LCx could not be valid.

Nonetheless, the user decided to show the LCx, confidence limits and graphs if possible.

Results of the probit analysis

Tab. 48: Results of the probit analysis with mortality at 28 d: Selected effective concentrations (LCx) of the test item and their 95%-confidence limits (according to Fieller's theorem).

Toxicity Metric	LC10	LC20	LC50
Value [$\mu\text{g a.s./L}$]	n.d.	n.d.	n.d.
lower 95%-cl	n.d.	n.d.	n.d.
upper 95%-cl	n.d.	n.d.	n.d.

n.d.: not determined either due to mathematical reasons or value is beyond the tested concentrations by more than factor 1000.

Slope function after Litchfield and Wilcoxon: 123234,070 The probability p(F) is greater than 0.05; i.e. the slope was not significantly different from zero. The shown toxic metrics could be meaningless.

(The slope function is derived from the slope, b, of the linearizedprobit function and computes as $S = 10^{(1/b)}$; please note that small values refer to a steep concentration/responserelation and large ones to a flat relation.)

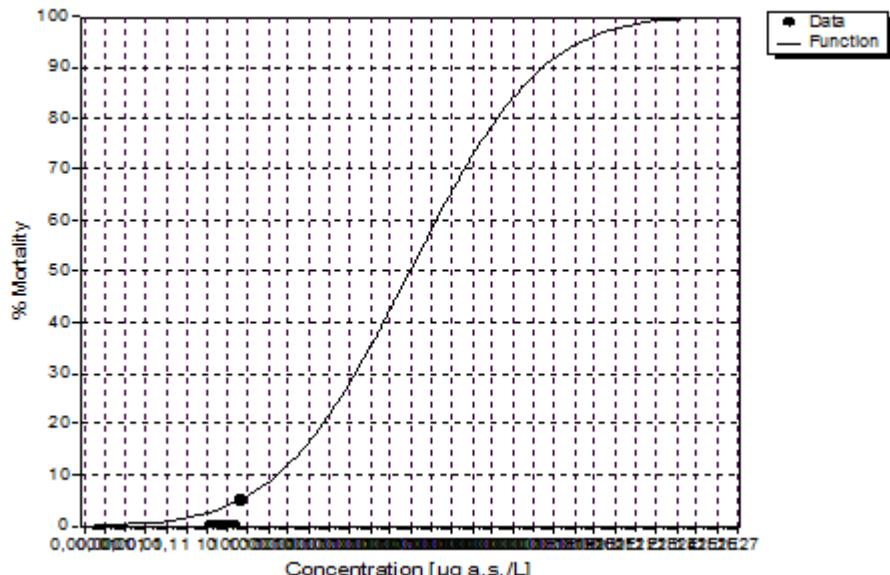


Fig. 9: Concentration-effect curve showing the influence of the test item on mortality of the introduced *Oncorhynchus mykiss* as observed after 28 d

Overview over the LCs of the Test Item on Mortality

Effects on Mortality

Tab. 49: Died fish(M) and percent mortality (%M) as computed from the raw data for test intervals selected; LCxx with mortality: effect levels as selected; lower 95%-cl, upper 95%-cl: lower and upper 95%-confidence limits..

Treatment [µg a.s./L]	0-14 d		0-28 d	
	M	%M	M	%M
Solv. Contr.	40,0	0,0	40,0	0,0
18,000	40,0	0,0	40,0	0,0
40,000	40,0	0,0	40,0	0,0
90,000	40,0	0,0	40,0	0,0
205,000	40,0	0,0	40,0	0,0
488,000	39,0	2,5	38,0	5,0
LC10	n.d.		n.d.	
lower 95%-cl	n.d.		n.d.	
upper 95%-cl	n.d.		n.d.	
LC20	n.d.		n.d.	
lower 95%-cl	n.d.		n.d.	
upper 95%-cl	n.d.		n.d.	
LC50	n.d.		n.d.	
lower 95%-cl	n.d.		n.d.	
upper 95%-cl	n.d.		n.d.	

Threshold concentrations (NOEC) for Mortality at 14 d

To justify the use of the Step-down Cochran-Armitage test at first a trend analysis by contrasts using proportions was performed.

Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 50: Qualitative trend analysis by contrasts (monotonicity of concentration/response) with mortality at 14 d: Psi: total of proportions weighted by contrasts; Var(psi): variance of psi; df: degrees of freedom; Chi²: Chi²-statistic; p(Chi²): 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	Var(psi)	df	Chi ²	p(Chi ²)
Linear	0,1250	0,0152	5	1,026	0,961
Quadratic	0,1250	0,0152	5	1,026	0,961

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 Step-down Cochran-Armitage test was replaced by the Bonferroni Chi² test.

Chi² 2x2 Table Test with Bonferroni Correction

Tab. 51: Chi²-2 x 2 Test with Bonferroni Correction with mortality at 14 d: Comparisons between treatment and control were done with the multiple significance level (Alpha is 0,050; one-sided greater). Two-sample comparisons are performed sequentially using the adjusted Alpha* (=alpha/(k-1) and the standard normal variable z; k: number of comparisons (after Holm 1979)). Ho (no effect) is accepted, if the probability p(z) > Alpha*; p(z) is the probability that the increase in category "Dead" observed in the treatment(s) is due to chance. Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm.[μ g a.s./L]	Introduced	Survived	Dead	% Mortality	z	p(z)	Alpha*sign.
Solv. Contr.	40	40	0	0,0			
18,000	40	40	0	0,0	n.d.	n.d.	n.d. **)
40,000	40	40	0	0,0	n.d.	n.d.	n.d. **)
90,000	40	40	0	0,0	n.d.	n.d.	n.d. **)
205,000	40	40	0	0,0	n.d.	n.d.	n.d. **)
488,000	40	39	1	2,5	1,000	0,159	0,050 -s

+: significant; -: non-significant; *: test could not be performed

s: a small-sample modification of the Chi²-test was applied. **) The prerequisite for a Chi²-test (80% of expected cell frequencies > 4) is not fulfilled. It is recommended to try the corresponding exact test, if possible. The NOEC cannot be determined by the program (expert judgement required).

To justify the use of the Step-down Cochran-Armitage test at first a trend analysis by contrasts using proportions was performed.

Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response)

Tab. 52: Qualitative trend analysis by contrasts (monotonicity of concentration/response) with mortality at 28 d: Psi: total of proportions weighted by contrasts; Var(psi): variance of psi; df: degrees of freedom; Chi²: Chi²-statistic; p(Chi²): 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	Var(psi)	df	Chi ²	p(Chi ²)
Linear	0,2500	0,0297	5	2,105	0,834
Quadratic	0,2500	0,0297	5	2,105	0,834

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 Step-down Cochran-Armitage test was replaced by the Bonferroni Chi² test.

Chi² 2x2 Table Test with Bonferroni Correction

Tab. 53: Chi²-2 x 2 Test with Bonferroni Correction with mortality at 28 d: Comparisons between treatment and control were done with the multiple significance level (Alpha is 0,050; one-sided greater). Two-sample comparisons are performed sequentially using the adjusted Alpha* (=alpha/(k-1) and the standard normal variable z; k: number of comparisons (after Holm 1979)). Ho (no effect) is accepted, if the probability p(z) > Alpha*; p(z) is the probability that the increase in category "Dead" observed in the treatment(s) is due to chance. Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm.[μ g a.s./L]Introduced	Survived	Dead	% Mortality	z	p(z)	Alpha*sign.
Solv. Contr.	40	0	0,0			
18,000	40	0	0,0	n.d.	n.d.	n.d. **)
40,000	40	0	0,0	n.d.	n.d.	n.d. **)
90,000	40	0	0,0	n.d.	n.d.	n.d. **)
205,000	40	0	0,0	n.d.	n.d.	n.d. **)
488,000	38	2	5,0	1,423	0,077	0,050 -s

+: significant; -: non-significant; *: test could not be performed

s: a small-sample modification of the Chi²-test was applied. **) The prerequisite for a Chi²-test (80% of expected cell frequencies > 4) is not fulfilled. It is recommended to try the corresponding exact test, if possible. The NOEC cannot be determined by the program (expert judgement required).

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

Overview over the LOEC and NOEC Determination

Tab. 54: Overview over the LOEC and NOEC Determination with mortality: Survival rates and significance marks as computed for mortality for all inspection intervals (top); bottom part: obtained LOEC and NOEC with indication of statistical test used; *bc: Chi² 2x2 table test with Bonferroni correction, significance level was 0,050, one-sided greater.

Treatm.[μ g a.s./L]	0-14 d	0-28 d
18,000	0,0*	0,0*
40,000	0,0*	0,0*
90,000	0,0*	0,0*
205,000	0,0*	0,0*
488,000	2,5 -	5,0 -

LOEC	n.d. *bc	n.d. *bc
NOEC	n.d. *bc	n.d. *bc

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

Summary of Results for all Endpoints

Tab. 55: 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 [μ g a.s./L]	28 d	
Weight		
	EC10	212,685
95%-CL	lower	164,240
	upper	275,418

Tab. 55 (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	288,154
95%-CL	lower	219,428
	upper	378,864
	EC50	515,154
95%-CL	lower	352,856
	upper	738,635
Weight	LOEC	205,000
	NOEC	90,000
Specific growth rate		
	EC10	199,564
95%-CL	lower	158,912
	upper	250,614
	EC20	245,674
95%-CL	lower	196,789
	upper	308,903
	EC50	365,651
95%-CL	lower	279,187
	upper	482,004
Specific growth rate	LOEC	205,000
	NOEC	90,000
Specific interval growth rate		
	EC10	199,564
95%-CL	lower	158,912
	upper	250,614

Tab. 55 (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	245,674		
95%-CL	lower	196,789		
	upper	308,903		
	EC50	365,651		
95%-CL	lower	279,187		
	upper	482,004		
Specific interval growth rate	LOEC	205,000		
	NOEC	90,000		
		0-14 d	0-28 d	
Mortality				
	LC10	n.d.	n.d.	
95%-CL	lower	n.d.	n.d.	
	upper	n.d.	n.d.	
	LC20	n.d.	n.d.	
95%-CL	lower	n.d.	n.d.	
	upper	n.d.	n.d.	
	LC50	n.d.	n.d.	
95%-CL	lower	n.d.	n.d.	
	upper	n.d.	n.d.	
Mortality	LOEC	n.d.	n.d.	
	NOEC	n.d.	n.d.	

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

Settings Table

Tab. 56:

Area	Item	Default Settings	User Settings
Global	Type of Exposure	Concentration	Concentration
	Extrapolation of LCx	By program	By program
	Show non-significant ECx	YES	YES
	Statistical design		NOEC/ECx
Variables			
Weight	State	Selected for analysis	Selected for analysis
	Data transformation	none	none
	Decimals data	2	2

	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		
		One-wayANOVA	Sig. level 0,05
	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-sidedsmaller	one-sidedsmaller
	ECx computation		
	Selected ECx values	EC10, EC20, EC50	EC10, EC20, EC50
	Selected method	Non-linear Regr.	Non-linearRegr.
	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			
Specific 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
	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-sidedsmaller	one-sidedsmaller
	ECx computation		
	Selected ECx values	EC10, EC20, EC50	EC10, EC20, EC50
	Selected method	Non-linear Regr.	Non-linearRegr.

	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			
Specific Interval 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	
Monotonicity	Contrast Analysis	Contrast Analysis	
Sig. Level	0,05	0,05	
Final testing (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			
Mortality			
State	Selected for analysis	Selected for analysis	
Data transformation	none	ArcSine Square Root(p)	
Decimals data	1	1	
Statistical pre-testing			
Extra-binomial variance	(Not replicated)	Not performed	
Additional tests	None	None	
Final testing (NOEC)			
Test procedures	SD Cochran-Armitage	Bonferroni Chi ²	
Who selected final test	Program	Program	
Additional tests	None	None	
Significance level	0,05	0,05	
Test direction	one-sided greater	one-sided greater	

LCx computation		
Selected LCx values	LC10, LC20, LC50	LC10, LC20, LC50
Selected method	Linear Regression	Linear Regression
Regress. type	Max. Likelihood	Max. Likelihood
Dose/response function	Probit (normal sigmoid)	Probit (normal sigmoid)
Sig. level goodness of fit	0,10	0,10
Data	Treatment mean/total	Treatment totals
Confidence limits	after Fieller	after Fieller
Control mortality	Not compensated	Not compensated