

Fish, Acute Toxicity Test (OECD 203): 96h Acute Toxicity Test

General:

Test identification/project no.	96h Acute Toxicity Test
Test item	Barium (BaCl)
Unit of test item concentration	mg/L
Start of experiment on day	
Date and time of the evaluation	19/09/2018; 1:05:48 PM
Raw data filename: Definitive Test.xls	NewOECD203-Fish Acute Toxicity Test1

Test design

Number of treatments (incl. control(s))	6
Duration of the test	96 h
Test system	Paratya australiensis

Validity of the test

To be a valid test, a maximum control mortality of 10.0% is allowed.

In the present test 0.0% of the introduced animals died.

Thus the test is valid.

Relation of *Paratya australiensis* 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 [mg/L]		0-96 h
Survival		
	EC10	7.881
95%-CL	lower	4.464
	upper	11.028
	EC20	11.449
95%-CL	lower	7.426
	upper	15.013
	EC50	23.388
95%-CL	lower	18.449
	upper	28.857
Survival	LOEC	<=10.000
	NOEC	<10.000

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

Survival of *Paratya australiensis* as Dependent on Concentration

Tab. 2: Survival of *Paratya australiensis* as dependent on concentration of the test item; Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from InputRawData)

Treatm. [mg/L]	Control	10.000	18.000	32.400	58.300	105.000
0 h	7	7	7	7	7	7
	7	7	7	7	7	7
	7	7	7	7	7	7
	7	7	7	7	7	7
Total Introduced:	28	28	28	28	28	28
n:	4	4	4	4	4	4

Tab. 2 (continued): Survival of *Paratya australiensis* as dependent on concentration of the test item;
Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of
variation (from InputRawData)

24 h	7	7	7	7	6	5
	7	7	7	6	7	7
	7	7	7	6	7	6
	7	7	7	7	5	5
Total Survived:	28	28	28	26	25	23
n:	4	4	4	4	4	4
48 h	7	7	6	5	4	1
	7	7	6	4	5	1
	7	7	7	3	4	3
	7	7	5	2	3	1
Total Survived:	28	28	24	14	16	6
n:	4	4	4	4	4	4
72 h	7	7	6	4	1	0
	7	7	6	2	3	0
	7	7	4	2	2	0
	7	7	4	2	1	0
Total Survived:	28	28	20	10	7	0
n:	4	4	4	4	4	4
96 h	7	5	6	4	1	0
	7	5	5	2	2	0
	7	7	3	1	2	0
	7	6	4	2	1	0
Total Survived:	28	23	18	9	6	0
n:	4	4	4	4	4	4

Mortality as Dependent on Concentration and Time

Tab. 3: Cumulative mortality of *Paratya australiensis* as dependent on concentration of the test item
and time (from InputRawData)

Treatm. [mg/L]	Control	10.000	18.000	32.400	58.300	105.000
0 h:	0	0	0	0	0	0

24 h:	0	0	0	2	3	5
48 h:	0	0	4	14	12	22
72 h:	0	0	8	18	21	28
96 h:	0	5	10	19	22	28

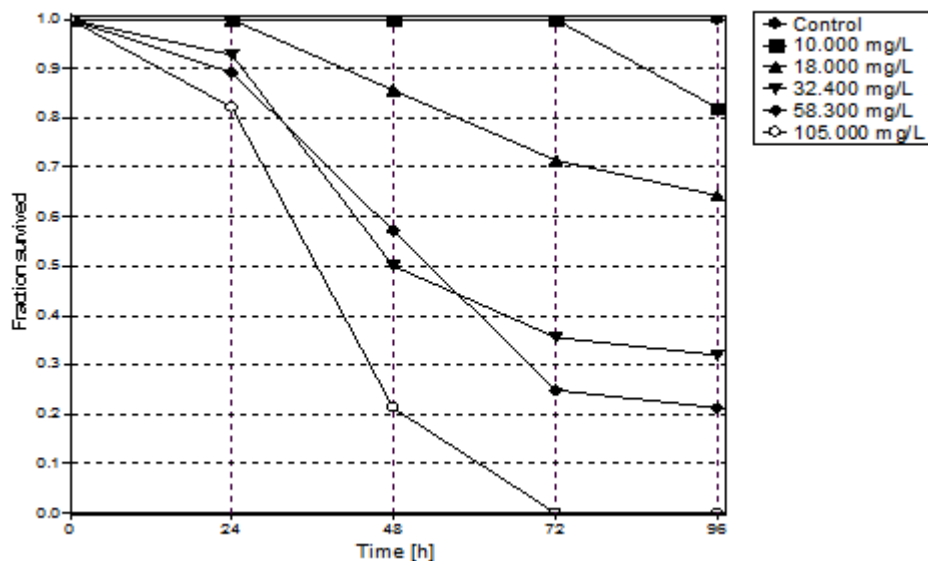


Fig. 1: Survival of the introduced *Paratya australiensis* as observed under presence of the test item.

Overview Survival

Tab. 4: % Mortality caused by the test item at 24 h.

Treatm.[mg/L]	Introduced	Survived	Dead	% Mortality
Control	28	28	0	0.0
10.000	28	28	0	0.0
18.000	28	28	0	0.0
32.400	28	26	2	7.1
58.300	28	25	3	10.7
105.000	28	23	5	17.9

Effective Concentrations (ECx) for Survival at 24 h

Probit analysis using linear max. likelihood regression

Tab. 5: Probit analysis using linear max. likelihood regression: 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. [mg/L]	Log(x)	% Mortality	n	Emp. Probit	WeightReg.
Probit					

ed	Control		0.00	28		exclud
	10.000	1.000	0.00	28	-1.2533	0.990-2.651
	18.000	1.255	0.00	28	-1.2533	2.581-2.198
	32.400	1.511	7.14	28	-1.0743	5.460-1.745
	58.300	1.766	10.71	28	-0.9847	9.479-1.292
	105.000	2.021	17.86	28	-0.8057	13.740-0.839

excluded: value not in line with the chosen function

Parameters of the probit analysis

Tab. 6: Parameters of the probit analysis: Results of the regression analysis

Parameter	Value
Computation runs:	8
Slope b:	1.77424
Intercept a:	-4.42498
Variance of b:	0.39923
Goodness of Fit	
Chi ² :	1.31065
Degrees of freedom:	3
p(Chi ²):	0.72661
Log EC50:	2.49401
SE Log EC50:	0.27727
g-Criterion:	0.48720
F:	18.048
p(F) (df: 1;3):	0.024

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/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).

Results of the probit analysis

Tab. 7: Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller's theorem).

Parameter	EC10	EC20	EC50
Value [mg/L]	59.115	104.629	n.d.
lower 95%-cl	31.948	66.290	n.d.
upper 95%-cl	111.657	498.814	n.d.
lower 99%-cl	26.245	48.274	n.d.
upper 99%-cl	135.921	684.971	n.d.

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

Slope function after Litchfield and Wilcoxon: 3.661

(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/responserelation and large ones to a flat relation.)

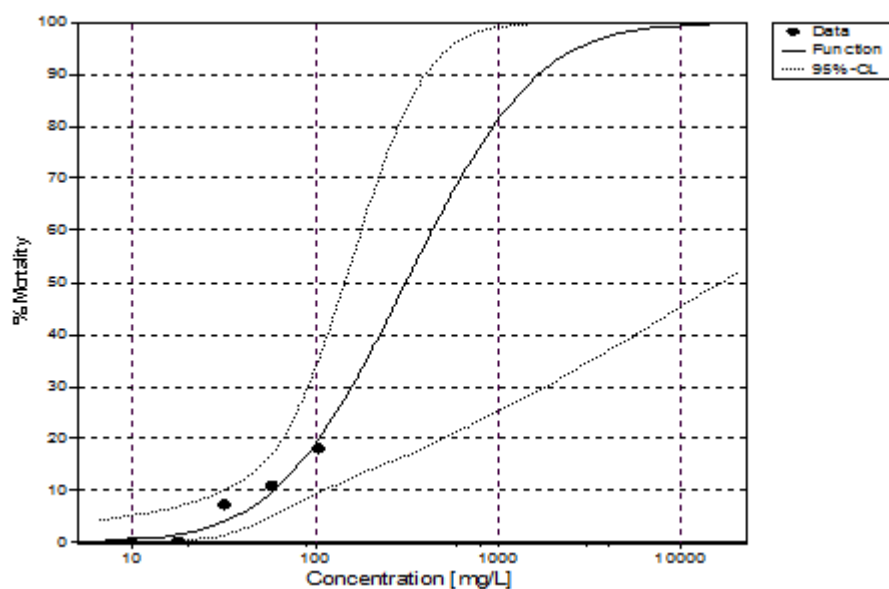


Fig. 2: Concentration-effect curve showing the influence of the test item on survival of the introduced *Paratya australiensis* as observed after 24 h.

Effective Concentrations (ECx) for Survival at 48 h

Probit analysis using linear max. likelihood regression

Tab. 8: Probit analysis using linear max. likelihood regression: 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. [mg/L]	Log(x)	% Mortality	n	Emp. Probit	WeightReg.
Control		0.00	28		excluded
10.000	1.000	0.00	28	-1.2533	5.818-1.700
18.000	1.255	14.29	28	-0.8952	11.504-1.082
32.400	1.511	50.00	28	0.0000	16.474-0.464
58.300	1.766	42.86	28	-0.1790	17.674-0.153
105.000	2.021	78.57	28	0.7162	14.313-0.771

excluded: value not in line with the chosen function

Parameters of the probit analysis

Tab. 9: Parameters of the probit analysis: Results of the regression analysis

Parameter	Value
Computation runs:	6
Slope b:	2.41987
Intercept a:	-4.11973

Variance of b:	0.15111
Goodness of Fit	
Chi²:	7.40877
Degrees of freedom:	3
p(Chi²):	0.05995
Log EC50:	1.70246
SE Log EC50:	0.05352
g-Criterion:	0.64527
F:	15.691
p(F) (df: 1;3):	0.029

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/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).

Results of the probit analysis

Tab. 10: Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller's theorem).

Parameter	EC10	EC20	EC50
Value [mg/L]	14.889	22.629	50.403
lower 95%-cl	0.217	1.606	25.229
upper 95%-cl	28.419	40.696	236.769
lower 99%-cl	0.028	0.416	9.899
upper 99%-cl	217.821	157.066	603.426

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

Slope function after Litchfield and Wilcoxon: 2.590

(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/responderelation and large ones to a flat relation.)

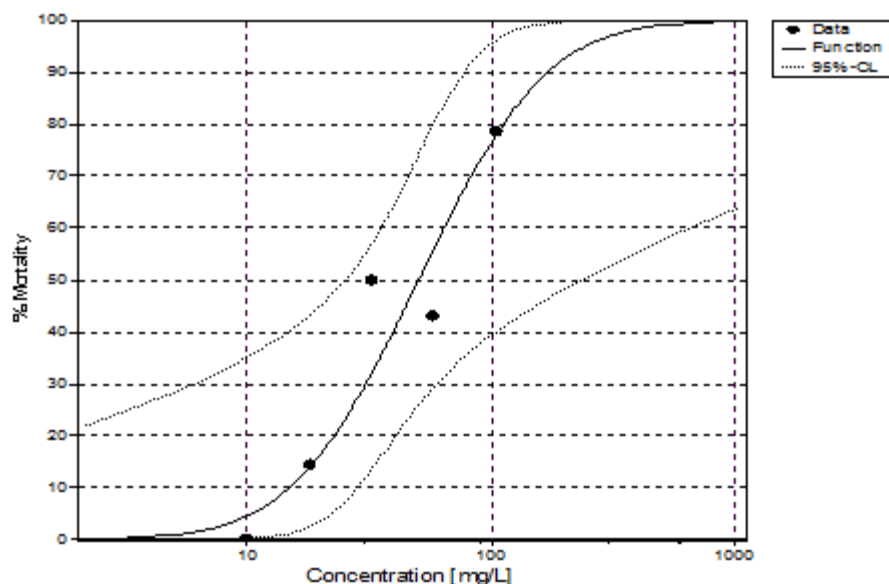


Fig. 3: Concentration-effect curve showing the influence of the test item on survival of the introduced *Paratya australiensis* as observed after 48 h.

Effective Concentrations (ECx) for Survival at 72 h

Probit analysis using linear max. likelihood regression

Tab. 11: Probit analysis using linear max. likelihood regression: 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. [mg/L]	Log(x)	% Mortality	n	Emp. Probit	WeightReg.
Control		0.00	28		excluded
10.000	1.000	0.00	28	-1.2533	5.890-1.691
18.000	1.255	28.57	28	-0.5371	14.327-0.770
32.400	1.511	64.29	28	0.3581	17.6770.152
58.300	1.766	75.00	28	0.6267	11.5941.073
105.000	2.021	100.00	28	1.2533	3.7021.995

excluded: value not in line with the chosen function

Parameters of the probit analysis

Tab. 12: Parameters of the probit analysis: Results of the regression analysis

Parameter	Value
Computation runs:	6
Slope b:	3.60959
Intercept a:	-5.30059
Variance of b:	0.24232
Goodness of Fit	

Chi ² :	6.15233
Degrees of freedom:	3
p(Chi ²):	0.10443
Log EC50:	1.46848
SE Log EC50:	0.03800
g-Criterion:	0.07145
F:	26.218
p(F) (df: 1;3):	0.014

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/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).

Results of the probit analysis

Tab. 13: Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller's theorem).

Parameter	EC10	EC20	EC50
Value [mg/L]	12.985	17.191	29.409
lower 95%-cl	9.027	12.969	24.579
upper 95%-cl	16.423	20.923	35.088
lower 99%-cl	8.217	12.030	23.242
upper 99%-cl	18.043	22.556	37.107

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

Slope function after Litchfield and Wilcoxon: 1.893

(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/responserelation and large ones to a flat relation.)

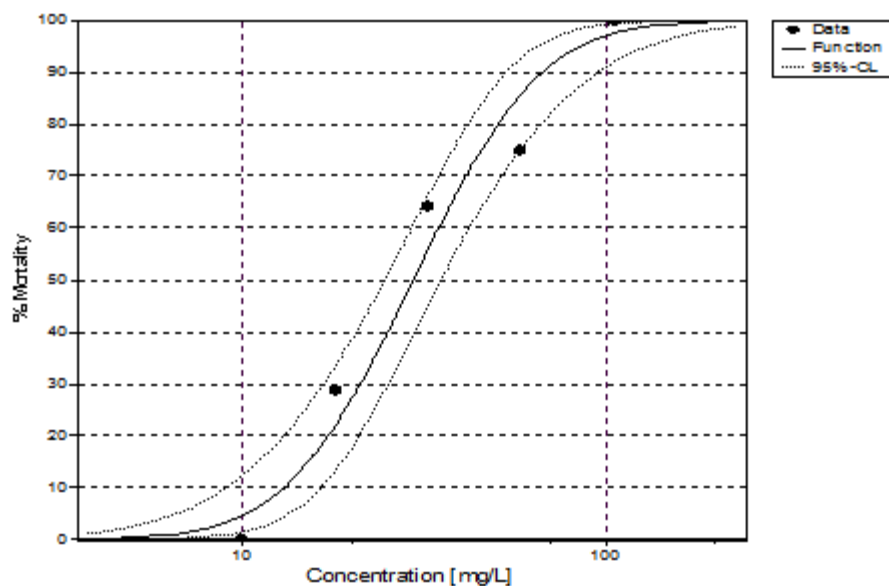


Fig. 4: Concentration-effect curve showing the influence of the test item on survival of the introduced *Paratya australiensis* as observed after 72 h.

Effective Concentrations (ECx) for Survival at 96 h

Probit analysis using linear max. likelihood regression

Tab. 14: Probit analysis using linear max. likelihood regression: 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. [mg/L]	Log(x)	% Mortality	n	Emp. Probit	WeightReg.
Control		0.00	28		excluded
10.000	1.000	17.86	28	-0.8057	12.272-1.001
18.000	1.255	35.71	28	-0.3581	17.218-0.309
32.400	1.511	67.86	28	0.4476	16.892-0.384
58.300	1.766	78.57	28	0.7162	11.561-1.076
105.000	2.021	100.00	28	1.2533	5.270-1.769

excluded: value not in line with the chosen function

Parameters of the probit analysis

Tab. 15: Parameters of the probit analysis: Results of the regression analysis

Parameter	Value
Computation runs:	5
Slope b:	2.71297
Intercept a:	-3.71402
Variance of b:	0.16532
Goodness of Fit	

Chi²:	2.60854
Degrees of freedom:	3
p(Chi²):	0.45599
Log EC50:	1.36899
SE Log EC50:	0.04729
g-Criterion:	0.08629
F:	51.201
p(F) (df: 1;3):	0.006

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/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity(= are made wider; so, check whether these results are reasonable!).

Results of the probit analysis

Tab. 16: Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller's theorem).

Parameter	EC10	EC20	EC50
Value [mg/L]	7.881	11.449	23.388
lower 95%-cl	4.464	7.426	18.449
upper 95%-cl	11.028	15.013	28.857
lower 99%-cl	3.873	6.648	17.197
upper 99%-cl	12.713	16.769	30.959

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

Slope function after Litchfield and Wilcoxon: 2.337

(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/responserelation and large ones to a flat relation.)

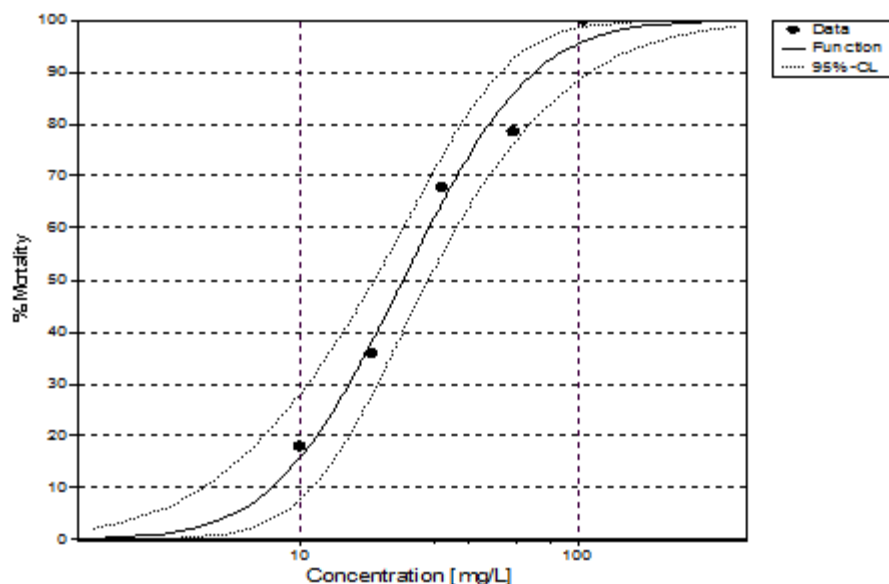


Fig. 5: Concentration-effectcurve showing the influence of the test item on survival of the introduced *Paratya australiensis* as observed after 96 h.

Overview over the ECs of the Test Item on Survival

Effects on Survival

Tab. 17: Survival (S) and percent mortality (%M) as computed from the raw data for test intervals selected; ECxx: effect levels as selected; lower 95%-cl, upper 95%-cl: lower and upper 95%-confidence limits.; *pm: Probit analysis using linear max. likelihood regression.

Treatment	0-24 h		0-48 h		0-72 h		0-96 h	
[mg/L]	S	%M	S	%M	S	%M	S	%M
Control	28	0.0	28	0.0	28	0.0	28	0.0
10.000	28	0.0	28	0.0	28	0.0	23	17.9
18.000	28	0.0	24	14.3	20	28.6	18	35.7
32.400	26	7.1	14	50.0	10	64.3	9	67.9
58.300	25	10.7	16	42.9	7	75.0	6	78.6
105.000	23	17.9	6	78.6	0	100.0	0	100.0
EC10	59.115	*pm 14.889	*pm 12.985		*pm 7.881		*pm	
lower 95%-cl	31.948	0.217	9.027		4.464			
upper 95%-cl	111.657	28.419	16.423		11.028			
EC20	104.629	*pm 22.629	*pm 17.191		*pm 11.449		*pm	
lower 95%-cl	66.290	1.606	12.969		7.426			
upper 95%-cl	498.814	40.696	20.923		15.013			
EC50		n.d. 50.403	*pm 29.409		*pm 23.388		*pm	
lower 95%-cl		n.d. 25.229	24.579		18.449			
upper 95%-cl		n.d. 236.769	35.088		28.857			

Threshold Concentrations (NOEC) for Survival at 24 h

Fisher`s Exact Binomial Test with Bonferroni Correction

Tab. 18: Fisher`s Exact Binomial Test with Bonferroni Correction: Pair-wise comparisons between treatment and control on the multiple significance level (alpha is 0.05; one-sided greater). Pair-wise comparisons are performed sequentially using the adjusted Alpha* (= alpha/(k-1); k: number of comparisons (after Holm 1979)); Ho (no effect) is accepted, if the probability p > Alpha*.

Treatm.[mg/L]	Introduced	Survived	Dead	% Mortality	p	alpha*sig
n.						
Control	28	28	0	0.0		
10.000	28	28	0	0.0	1.000	0.050 -
18.000	28	28	0	0.0	1.000	0.025 -
32.400	28	26	2	7.1	0.245	0.017 -
58.300	28	25	3	10.7	0.118	0.013 -
105.000	28	23	5	17.9	0.026	0.010 -

+: significant; -: non-significant

The NOEC appears to be higher than or equal 105.000 mg/L.

Threshold Concentrations (NOEC) for Survival at 48 h

Fisher`s Exact Binomial Test with Bonferroni Correction

Tab. 19: Fisher`s Exact Binomial Test with Bonferroni Correction: Pair-wise comparisons between treatment and control on the multiple significance level (alpha is 0.05; one-sided greater). Pair-wise comparisons are performed sequentially using the adjusted Alpha* (= alpha/(k-1); k: number of comparisons (after Holm 1979)); Ho (no effect) is accepted, if the probability p > Alpha*.

Treatm.[mg/L]	Introduced	Survived	Dead	% Mortality	p	alpha*sig
n.						
Control	28	28	0	0.0		
10.000	28	28	0	0.0	1.000	0.050 -
18.000	28	24	4	14.3	0.056	0.025 -
32.400	28	14	14	50.0	<0.001	0.013 +
58.300	28	16	12	42.9	<0.001	0.017 +
105.000	28	6	22	78.6	<0.001	0.010 +

+: significant; -: non-significant

A NOEC of 18.000 mg/L is suggested by the program.

Threshold Concentrations (NOEC) for Survival at 72 h

Fisher`s Exact Binomial Test with Bonferroni Correction

Tab. 20: Fisher`s Exact Binomial Test with Bonferroni Correction: Pair-wise comparisons between treatment and control on the multiple significance level (alpha is 0.05; one-sided greater). Pair-wise comparisons are performed sequentially using the adjusted Alpha* (= alpha/(k-1); k: number of comparisons (after Holm 1979)); Ho (no effect) is accepted, if the probability p > Alpha*.

Treatm.[mg/L]	Introduced	Survived	Dead	% Mortality	p	alpha*sig
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n.

Control	28	28	0	0.0			
10.000	28	28	0	0.0	1.000	0.050	-
18.000	28	20	8	28.6	0.002	0.025	+
32.400	28	10	18	64.3	<0.001	0.017	+
58.300	28	7	21	75.0	<0.001	0.013	+
105.000	28	0	28	100.0	<0.001	0.010	+

+: significant; -: non-significant

A NOEC of 10.000 mg/L is suggested by the program.

Threshold Concentrations (NOEC) for Survival at 96 h

Fisher`s Exact Binomial Test with Bonferroni Correction

Tab. 21: Fisher`s Exact Binomial Test with Bonferroni Correction: Pair-wise comparisons between treatment and control on the multiple significance level (alpha is 0.05; one-sided greater). Pair-wise comparisons are performed sequentially using the adjusted Alpha* (= alpha/(k-1); k: number of comparisons (after Holm 1979)); Ho (no effect) is accepted, if the probability p > Alpha*.

Treatm.[mg/L]	Introduced	Survived	Dead	% Mortality	p	alpha*sig
n.						
Control	28	28	0	0.0		
10.000	28	23	5	17.9	0.026	0.050 +
18.000	28	18	10	35.7	<0.001	0.025 +
32.400	28	9	19	67.9	<0.001	0.017 +
58.300	28	6	22	78.6	<0.001	0.013 +
105.000	28	0	28	100.0	<0.001	0.010 +

+: significant; -: non-significant

The NOEC is lower than 10.000 mg/L.

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

Overview over the LOEC and NOEC Determination

Tab. 22: Overview over the LOEC and NOEC Determination: Survival rates and significance marks as computed for survival for all inspection intervals (top); ; bottom part: obtained LOEC and NOEC with indication of statistical test used; *bf: Fisher`s exact binomial test with Bonferroni correction.

Treatm. [mg/L]	0-24 h	0-48 h	0-72 h	0-96 h
10.000	0.0 -	0.0 -	0.0 -	17.9+
18.000	0.0 -	14.3 -	28.6+	35.7+
32.400	7.1 -	50.0+	64.3+	67.9+
58.300	10.7 -	42.9+	75.0+	78.6+
105.000	17.9 -	78.6+	100.0+	100.0+
LOEC	>105.000 *bf	32.400 *bf	18.000 *bf	<=10.000 *bf
NOEC	>=105.000 *bf	18.000 *bf	10.000 *bf	<10.000 *bf

+: Significant difference to control (p <=0.05)

