Daphnia, Acute Immobilisation Test (OECD 202-2004): A Project

<Your company name>
<your adress>
<your adress>
<your adress>

General:

Test identification/project no.	A Project
Test item	A Substance
Unit of test item concentration	mg/L
Start of experiment on day	
Date and time of the evaluation	13.02.2022; 14:25:07
Raw data filename:	OECD202 Daphnia AcuteTest 2004.xls

Test design

Number of treatments (incl. control(s))	8
Duration of the test	48 h
Measurement interval	24 h
Measurement variable	Immobility
Test system	Daphnia magna
Statistical design	Hypothesis testing (NOEC) and regression (LCx)

Validity of the test

To be a valid test, a maximum control mortality of 10% is allowed.
In the present test 0,0% of the introduced animals died.
Thus the test is valid.

Relation of Daphnia magna Endpoints on Concentration

Summary of Results for all Endpoints

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

Critical Conc.s [mg/L]		**0-24 h**	**0-48 h**
Immobility			
	LC10	1,193	0,917
95%-CL	lower	0,928	0,710
	upper	1,392	1,067
	LC20	1,413	1,082
95%-CL	lower	1,169	0,893
	upper	1,623	1,231
	LC50	1,955	1,482
95%-CL	lower	1,705	1,307
	upper	2,323	1,715
Immobility	LOEC	1,000	1,000
	NOEC	0,700	0,700

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

Immobility (Data)

Immobility of Daphnia magna as Dependent on Concentration and Time

Tab. 2: Immobility of Daphnia magna as dependent on concentration of the test item and time (from InputRawData)

Treatm. [mg/L]	**Control**	**0,250**	**0,500**	**0,700**	**1,000**	**1,400**	**2,000**	**4,000**
0 h	5	5	5	5	5	5	5	5
	5	5	5	5	5	5	5	5
	5	5	5	5	5	5	5	5
	5	5	5	5	5	5	5	5
Total Introduced	20	20	20	20	20	20	20	20
n:	4	4	4	4	4	4	4	4

Tab. 2 (continued): Immobility of Daphnia magna as dependent on concentration of the test item and time (from InputRawData)

24 h	0	0	0	0	0	1	3	5
	0	0	0	0	0	1	2	5
	0	0	0	0	0	1	2	5
	0	0	0	0	2	0	2	5
Total Immobile:	0	0	0	0	2	3	9	20
n:	4	4	4	4	4	4	4	4
48 h	0	0	0	0	1	3	4	5
	0	0	0	0	1	2	3	5
	0	0	0	0	1	2	3	5
	0	0	0	0	1	2	5	5
Total Immobile:	0	0	0	0	4	9	15	20
n:	4	4	4	4	4	4	4	4

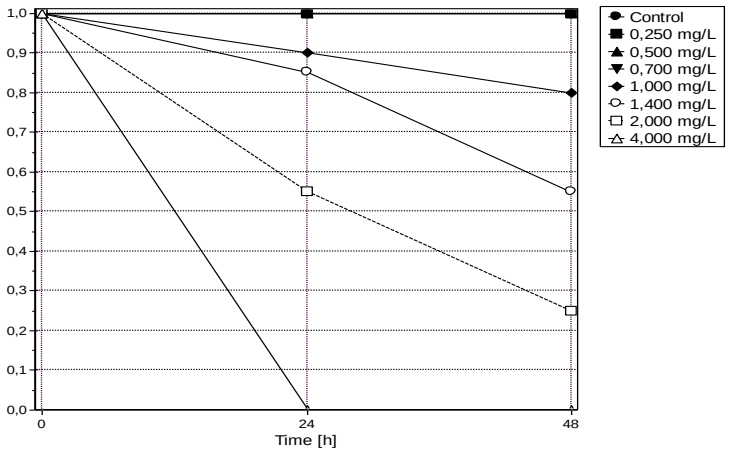

Fig. 1: Immobility of the introduced Daphnia magna as observed under presence of the test item.

Lethal Concentrations (LCx) for Immobility at 24 h

Overview Immobility

Tab. 3: Overview Immobility: Overview over the effects on immobility in Daphnia magna at 24 h

Treatm.[mg/L]	Total Introduced	Mobile	Immobile	% Immobility
Control	20	20	0	0,00
0,250	20	20	0	0,00
0,500	20	20	0	0,00
0,700	20	20	0	0,00
1,000	20	18	2	10,00
1,400	20	17	3	15,00
2,000	20	11	9	45,00
4,000	20	0	20	100,00

Probit analysis using linear max. likelihood regression

Tab. 4: Probit analysis using linear max. likelihood regression with immobility at 24 h: 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)	% Immobility	n	Emp. Probit	Weight	Reg. Probit
Control		0,00	20			excluded
0,250	-0,602	0,00	20	-1,2533	0,000	-5,333
0,500	-0,301	0,00	20	-1,2533	0,058	-3,535
0,700	-0,155	0,00	20	-1,2533	0,686	-2,663
1,000	0,000	10,00	20	-1,0027	3,936	-1,738
1,400	0,146	15,00	20	-0,8773	9,644	-0,866
2,000	0,301	45,00	20	-0,1253	12,717	0,059
4,000	0,602	100,00	20	1,2533	3,309	1,856

excluded: value not in line with the chosen function

Parameters of the probit analysis

Tab. 5: Parameters of the probit analysis with immobility at 24 h: Results of the regression analysis

Parameter	Value
Computation runs:	8
Slope b:	5,96987
Intercept a:	-1,73853
Variance of b:	1,09181
Goodness of Fit	
Chi²:	3,17274
Degrees of freedom:	5
p(Chi²):	0,673
Log LC50:	0,29122
SE Log LC50:	0,03201
g-Criterion:	0,11768
F:	51,442
p(F) (df: 1;5):	<0.001

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 (extra-binomial variance).

A statistically significant concentration/response was found (p(F) <= 0.05; i.e. slope of the relationship is significantly different from zero).

Results of the probit analysis

Tab. 6: Results of the probit analysis with immobility at 24 h: Selected effective concentrations (LCx) of the test item and their 95%-confidence limits (according to Fieller`s theorem).

Toxicity Metric	LC10	LC20	LC50
Value [mg/L]	1,193	1,413	1,955
lower 95%-cl	0,928	1,169	1,705
upper 95%-cl	1,392	1,623	2,323

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

Slope function after Litchfield and Wilcoxon: 1,471

(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.)

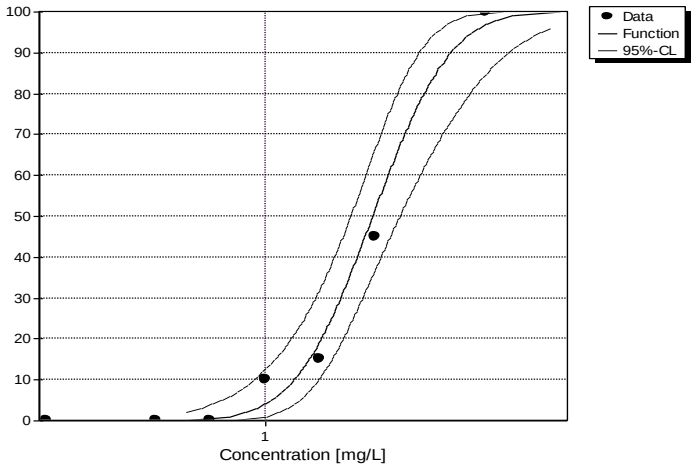

Fig. 2: Concentration-effect curve showing the influence of the test item on immobility of the introduced Daphnia magna as observed after 24 h

Lethal Concentrations (LCx) for Immobility at 48 h

Overview Immobility

Tab. 7: Overview Immobility: Overview over the effects on immobility in Daphnia magna at 48 h

Treatm.[mg/L]	Total Introduced	Mobile	Immobile	% Immobility
Control	20	20	0	0,00
0,250	20	20	0	0,00
0,500	20	20	0	0,00
0,700	20	20	0	0,00
1,000	20	16	4	20,00
1,400	20	11	9	45,00
2,000	20	5	15	75,00
4,000	20	0	20	100,00

Probit analysis using linear max. likelihood regression

Tab. 8: Probit analysis using linear max. likelihood regression with immobility at 48 h: 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)	% Immobility	n	Emp. Probit	Weight	Reg. Probit
Control		0,00	20			excluded
0,250	-0,602	0,00	20	-1,2533	0,000	-4,754
0,500	-0,301	0,00	20	-1,2533	0,378	-2,902
0,700	-0,155	0,00	20	-1,2533	2,606	-2,004
1,000	0,000	20,00	20	-0,7520	8,428	-1,051
1,400	0,146	45,00	20	-0,1253	12,625	-0,153
2,000	0,301	75,00	20	0,6267	10,052	0,800
4,000	0,602	100,00	20	1,2533	0,706	2,651

excluded: value not in line with the chosen function

Parameters of the probit analysis

Tab. 9: Parameters of the probit analysis with immobility at 48 h: Results of the regression analysis

Parameter	Value
Computation runs:	8
Slope b:	6,14986
Intercept a:	-1,05121
Variance of b:	1,14007
Goodness of Fit	
Chi²:	1,21872
Degrees of freedom:	5
p(Chi²):	0,943
Log LC50:	0,17093
SE Log LC50:	0,02818
g-Criterion:	0,11580
F:	136,103
p(F) (df: 1;5):	<0.001

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 (extra-binomial variance).

A statistically significant concentration/response was found (p(F) <= 0.05; i.e. slope of the relationship is significantly different from zero).

Results of the probit analysis

Tab. 10: Results of the probit analysis with immobility at 48 h: Selected effective concentrations (LCx) of the test item and their 95%-confidence limits (according to Fieller`s theorem).

Toxicity Metric	LC10	LC20	LC50
Value [mg/L]	0,917	1,082	1,482
lower 95%-cl	0,710	0,893	1,307
upper 95%-cl	1,067	1,231	1,715

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

Slope function after Litchfield and Wilcoxon: 1,454

(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.)

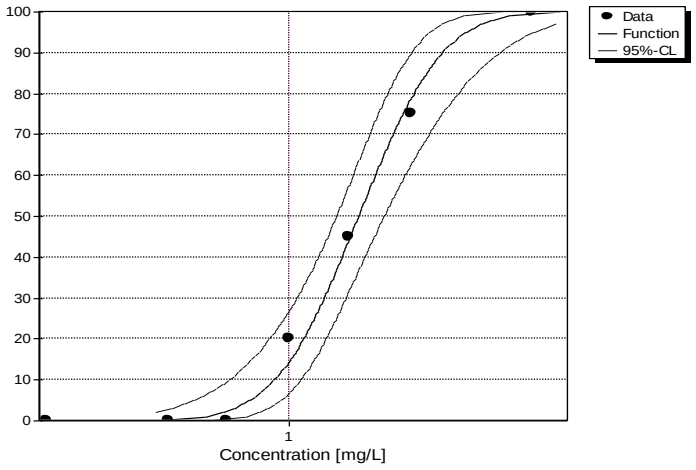

Fig. 3: Concentration-effect curve showing the influence of the test item on immobility of the introduced Daphnia magna as observed after 48 h

Overview over the LCs of the Test Item on Immobility

Increase in Immobility

Tab. 11: Immobility (M) and percent mortality (%M) as computed from the raw data for test intervals selected; LCxx with immobility: 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
[mg/L]	M	%M	M	%M	
Control	20,0	0,00	20,0	0,00	
0,250	20,0	0,00	20,0	0,00	
0,500	20,0	0,00	20,0	0,00	
0,700	20,0	0,00	20,0	0,00	
1,000	18,0	10,00	16,0	20,00	
1,400	17,0	15,00	11,0	45,00	
2,000	11,0	45,00	5,0	75,00	
4,000	0,0	100,00	0,0	100,00	
LC10	1,193	*pm	0,917	*pm	
lower 95%-cl	0,928		0,710		
upper 95%-cl	1,392		1,067		
LC20	1,413	*pm	1,082	*pm	
lower 95%-cl	1,169		0,893		
upper 95%-cl	1,623		1,231		
LC50	1,955	*pm	1,482	*pm	
lower 95%-cl	1,705		1,307		
upper 95%-cl	2,323		1,715		

Threshold concentrations (NOEC) for Immobility at 24 h

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. 12: Qualitative trend analysis by contrasts (monotonicity of concentration/response) with immobility at 24 h: 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	Chi²	p(Chi²)
Linear	9,8000	0,3712	7	258,694	<0.001
Quadratic	6,5000	0,1823	7	231,824	<0.001

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

The analysis of contrasts revealed a linear trend, thus the selected Step-down Cochran-Armitage test was performed.

Ahead of the Cochran-Armitage test Tarone´s test had to be performed to test for extra-binomial variance.

Tarone´s Test Procedure

Tab. 13: Tarone Test with immobility at 24 h: Treatment-wise testing the homgeneity of proportions (Alpha = 0,010). The statistic TZ has an asymptotic chi² distribution with one degree of freedom and measures the deviation from homogeneity. Ho (Phi = 0; i.e. homogeneity) is accepted, if the probability p(TZ) > Alpha; p(TZ) is the probability that the deviation from homogeneity observed in the treatment(s) is due to chance.

Treatm.[mg/L]	Introduced	Mobile	Immobile	TZp(TZ)	sign.
Control	20	20	0	2,5000,114	-
0,250	20	20	0	2,5000,114	-
0,500	20	20	0	2,5000,114	-
0,700	20	20	0	2,5000,114	-
1,000	20	18	2	1,1110,292	-
1,400	20	17	3	1,2460,264	-
2,000	20	11	9	1,8000,180	-
4,000	20	0	20	2,5000,114	-

+: significant; -: non-significant

In treatments no signs of extra-bionmial variance were found.

Step-down Cochran-Armitage Test Procedure

Tab. 14: Step-down Cochran-Armitage Test Procedure with immobility at 24 h: Step-down test to detect an increasing trend in responses (Alpha is 0,050; one-sided greater); Chi²(tot): total (Pearson) Chi²; z(trend): standardized one-sided deviation due to the linear upward trend; Chi²(err): unexplained component of Chi²(tot); p(tot|trend|err): probabilities that the observed results could be due to chance; Ho (no trend) is accepted, if p(trend) > Alpha. Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [mg/L]	Total	Introduced	Immobile	% Immobility	Chi²(tot)	p(tot)	Chi²(err)	p(err)|z|(trend)	p(trend)	Sign.
Control	20	0	0,00							
0,250	20	0	0,00	0,000	1,000	0,000	<0.001	0,000	1,000	-
0,500	20	0	0,00	0,000	1,000	0,000	<0.001	0,000	1,000	-
0,700	20	0	0,00	0,000	1,000	0,000	<0.001	0,000	1,000	-
1,000	20	2	10,00	8,163	0,086	4,082	0,253	2,020	0,022	+
1,400	20	3	15,00	11,061	0,050	3,172	0,529	2,809	0,003	+
2,000	20	9	45,00	36,667	<0.001	12,361	0,030	4,930	<0.001	+
4,000	20	20	100,00	104,426	<0.001	36,103	<0.001	8,266	<0.001	+

+: significant; -: non-significant

A NOEC of 0,700 mg/L is suggested by the program.

Threshold concentrations (NOEC) for Immobility at 48 h

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. 15: Qualitative trend analysis by contrasts (monotonicity of concentration/response) with immobility at 48 h: 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	Chi²	p(Chi²)
Linear	12,3000	0,3537	7	427,675	<0.001
Quadratic	5,4000	0,3207	7	90,912	<0.001

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

The analysis of contrasts revealed a linear trend, thus the selected Step-down Cochran-Armitage test was performed. Ahead of the Cochran-Armitage test Tarone´s test had to be performed to test for extra-binomial variance.

Tarone´s Test Procedure

Tab. 16: Tarone Test with immobility at 48 h: Treatment-wise testing the homgeneity of proportions (Alpha = 0,010). The statistic TZ has an asymptotic chi² distribution with one degree of freedom and measures the deviation from homogeneity. Ho (Phi = 0; i.e. homogeneity) is accepted, if the probability p(TZ) > Alpha; p(TZ) is the probability that the deviation from homogeneity observed in the treatment(s) is due to chance.

Treatm.[mg/L]	Introduced	Mobile	Immobile	TZp(TZ)	sign.
Control	20	20	0	2,5000,114	-
0,250	20	20	0	2,5000,114	-
0,500	20	20	0	2,5000,114	-
0,700	20	20	0	2,5000,114	-
1,000	20	16	4	2,5000,114	-
1,400	20	11	9	1,8000,180	-
2,000	20	5	15	0,1780,673	-
4,000	20	0	20	2,5000,114	-

+: significant; -: non-significant

In treatments no signs of extra-bionmial variance were found.

Step-down Cochran-Armitage Test Procedure

Tab. 17: Step-down Cochran-Armitage Test Procedure with immobility at 48 h: Step-down test to detect an increasing trend in responses (Alpha is 0,050; one-sided greater); Chi²(tot): total (Pearson) Chi²; z(trend): standardized one-sided deviation due to the linear upward trend; Chi²(err): unexplained component of Chi²(tot); p(tot|trend|err): probabilities that the observed results could be due to chance; Ho (no trend) is accepted, if p(trend) > Alpha. Note that the step-down test terminates after the first non-significant treatment is encountered

Treatm. [mg/L]	Total	Introduced	Immobile	% Immobility	Chi²(tot)	p(tot)	Chi²(err)	p(err)|z|(trend)	p(trend)	Sign.
Control	20	0	0,00							
0,250	20	0	0,00	0,000	1,000	0,000	<0.001	0,000	1,000	-
0,500	20	0	0,00	0,000	1,000	0,000	<0.001	0,000	1,000	-
0,700	20	0	0,00	0,000	1,000	0,000	<0.001	0,000	1,000	-
1,000	20	4	20,00	16,667	0,002	8,333	0,040	2,887	0,002	+
1,400	20	9	45,00	35,629	<0.001	11,604	0,021	4,901	<0.001	+
2,000	20	15	75,00	65,625	<0.001	15,525	0,008	7,078	<0.001	+
4,000	20	20	100,00	103,333	<0.001	17,568	0,007	9,261	<0.001	+

+: significant; -: non-significant

A NOEC of 0,700 mg/L is suggested by the program.

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

Overview over the LOEC and NOEC Determination

Tab. 18: Overview over the LOEC and NOEC Determination with immobility: Arithmetic means and significance marks as computed for immobility for all inspection intervals (top); bottom part: obtained LOEC and NOEC with indication of statistical test used; *casd: Step-down Cochran-Armitage test procedure, significance level was 0,050, one-sided greater.

Treatm. [mg/L]	0-24 h	0-48 h
0,250	0,00 -	0,00 -
0,500	0,00 -	0,00 -
0,700	0,00 -	0,00 -
1,000	10,00+	20,00+
1,400	15,00+	45,00+
2,000	45,00+	75,00+
4,000	100,00+	100,00+
LOEC	1,000 *casd	1,000 *casd
NOEC	0,700 *casd	0,700 *casd

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

Summary of Results for all Endpoints

Tab. 19: Summary of Results for all Endpoints: Critical effect and threshold concentration as observed at end of experimental time; LC: Effective concentration for xx% reduction; 95%-CL: 95% Confidence limits; LOEC: Lowest observed effect concentration; NOEC: No observed effect concentration.

Critical Conc.s [mg/L]		0-24 h	0-48 h
Immobility			
	LC10	1,193	0,917
95%-CL	lower	0,928	0,710
	upper	1,392	1,067
	LC20	1,413	1,082
95%-CL	lower	1,169	0,893
	upper	1,623	1,231
	LC50	1,955	1,482
95%-CL	lower	1,705	1,307
	upper	2,323	1,715
Immobility	LOEC	1,000	1,000
	NOEC	0,700	0,700

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

Settings Table

Tab. 20:

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			
Immobility	State	Selected for analysis	Selected for analysis
	Data transformation	none	none
	Decimals data	1	1
	Statistical pre-testing		
	Extra-binomial variance	Tarone test	Deselected
	Monotonicity	Contrast Analysis	Contrast Analysis
	Sig. Level	0,05	0,05
	Additional tests	None	None
	Final testíng (NOEC)		
	Test procedures	SD Cochran-Armitage	SD Cochran-Armitage
	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