Daphnia, Acute Immobilisation Test (OECD 202): Kaliumdichromat

Biotest Company XYZ
Ecotoxicology-Street 123
D-23456 Sample Town

General:

Test identification/project no.	Kaliumdichromat
Test item	
Unit of test item concentration	mg/L
Start of experiment on day	27.1.2011
Date and time of the evaluation	20.04.2011; 09:49:33
Raw data filename:	OECD202 Daphnia AcuteTest.xls

Test design

Number of treatments (incl. control(s))	8
Duration of the test	48 h
Test system	Daphnia magna

Validity of the test

To be a valid test, a maximum control mortality of 10,0% is allowed.
In the present test 10,0% 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; EC: Effective concentration for xx% reduction; 95%-CL: 95% Confidence limits; LOEC: Lowest observed effect concentration; NOEC: No observed effect concentration

Critical Conc.s [mg/L]		**0-24 h**	**0-48 h**
Mobility			
	EC10	1,539	1,331
95%-CL	lower	1,157	1,038
	upper	1,839	1,500
	EC20	1,865	1,487
95%-CL	lower	1,502	1,247
	upper	2,196	1,654
	EC50	2,691	1,839
95%-CL	lower	2,285	1,654
	upper	3,335	2,136
Mobility	LOEC	4,000	2,000
	NOEC	2,000	1,400

Mobility of Daphnia magna as Dependent on Concentration

Tab. 2: Mobility of Daphnia magna 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**	**0,25**	**0,5**	**0,7**	**1,0**	**1,4**	**2,0**	**4,0**
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): Mobility of Daphnia magna 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	0	0	0	0	0	1	2	3
	1	0	0	0	0	0	2	5
	0	0	0	0	0	1	1	5
	0	0	0	0	1	0	2	3
Total Immobile:	1	0	0	0	1	2	7	16
n:	4	4	4	4	4	4	4	4
48 h	0	0	0	0	0	1	3	5
	1	0	0	0	0	1	2	5
	1	0	0	1	0	1	4	5
	0	0	0	0	2	2	4	5
Total Immobile:	2	0	0	1	2	5	13	20
n:	4	4	4	4	4	4	4	4

Mortality as Dependent on Concentration and Time

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

Treatm. [mg/L]	**Control**	**0,25**	**0,5**	**0,7**	**1,0**	**1,4**	**2,0**	**4,0**
0 h:	0	0	0	0	0	0	0	0
24 h:	1	0	0	0	1	2	7	16
48 h:	2	0	0	1	2	5	13	20

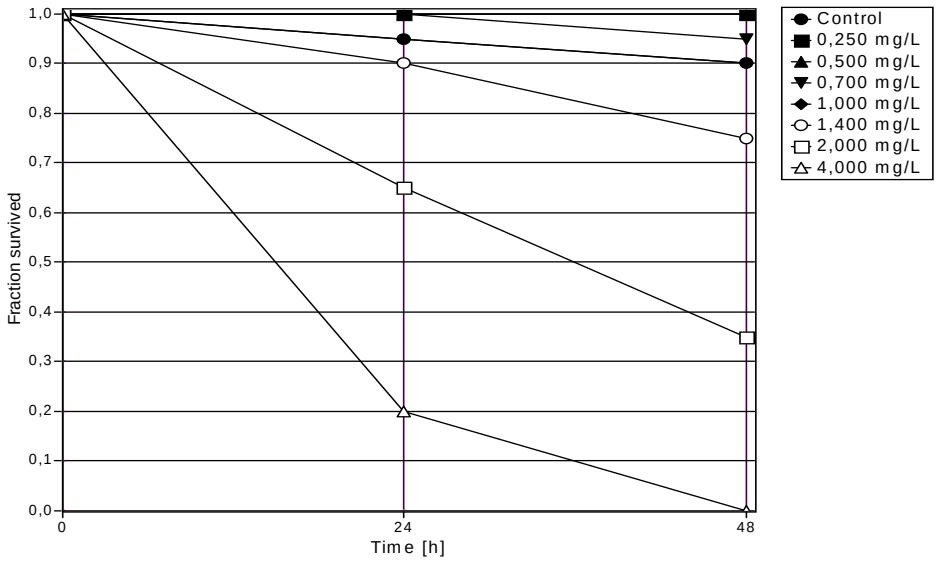

Fig. 1: Mobility of the introduced Daphnia magna as observed under presence of the test item.

Overview Mobility

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

Treatm.[mg/L]	Introduced	Mobile	Immobile	% Immobility
Control	20	19	1	5,00
0,250	20	20	0	0,00
0,500	20	20	0	0,00
0,700	20	20	0	0,00
1,000	20	19	1	5,00
1,400	20	18	2	10,00
2,000	20	13	7	35,00
4,000	20	4	16	80,00

The control mortality of 5,0% at 24 h will be compensated using Abbott`s formula.

The control mortality of 10,0% at 48 h will be compensated using Abbott`s formula.

Effective Concentrations (ECx) for Mobility 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)	% Immobility	n	Emp. Probit	Weight	Reg. Probit
Control		0,00	20			excluded
0,250	-0,602	0,00	20	-1,3852	0,000	-5,452
0,500	-0,301	0,00	20	-1,3852	0,019	-3,862
0,700	-0,155	0,00	20	-1,3852	0,227	-3,090
1,000	0,000	0,00	20	-1,2533	1,600	-2,271
1,400	0,146	5,26	20	-1,1214	5,385	-1,499
2,000	0,301	31,58	20	-0,4617	10,736	-0,681
4,000	0,602	78,95	20	0,7256	9,369	0,909

excluded: value not in line with the chosen function

Inhibition greater than 100% or lower than 0% were replaced by 100% and 0%, respectively.

Parameters of the probit analysis

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

Parameter	Value
Computation runs:	8
Slope b:	5,28326
Intercept a:	-2,27149
Variance of b:	0,90358
Goodness of Fit	
Chi^2:	0,92693
Degrees of freedom:	5
$p(Chi^2)$:	0,96824

Log EC50:	0,42994
SE Log EC50:	0,03883
g-Criterion:	0,12436
F:	166,633
p(F) (df: 1;5):	0,000

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]	1,539	1,865	2,691
lower 95%-cl	1,157	1,502	2,285
upper 95%-cl	1,839	2,196	3,335
lower 99%-cl	1,076	1,414	2,153
upper 99%-cl	1,979	2,332	3,539

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

Slope function after Litchfield and Wilcoxon: 1,546

(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.)

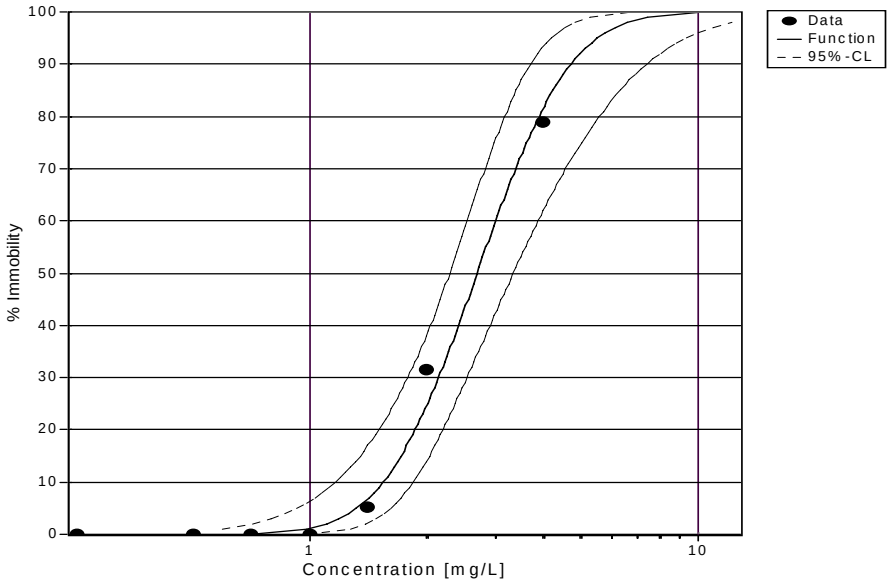

Fig. 2: Concentration-effect curve showing the influence of the test item on mobility of the introduced Daphnia magna as observed after 24 h.

Effective Concentrations (ECx) for Mobility 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)	% Immobility	n	Emp. Probit	Weight	Reg. Probit
Control		0,00	20			excluded
0,250	-0,602	0,00	20	-1,5318	0,000	-7,911
0,500	-0,301	0,00	20	-1,5318	0,000	-5,164
0,700	-0,155	0,00	20	-1,3926	0,021	-3,830
1,000	0,000	0,00	20	-1,2533	1,195	-2,415
1,400	0,146	16,67	20	-0,8355	8,222	-1,081
2,000	0,301	61,11	20	0,2785	12,229	0,333
4,000	0,602	100,00	20	1,2533	0,233	3,081

excluded: value not in line with the chosen function

Inhibition greater than 100% or lower than 0% were replaced by 100% and 0%, respectively.

Parameters of the probit analysis

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

Parameter	Value
Computation runs:	9
Slope b:	9,13003
Intercept a:	-2,41555
Variance of b:	4,58468
Goodness of Fit	
Chi²:	0,33249
Degrees of freedom:	5
p(Chi²):	0,99699
Log EC50:	0,26457
SE Log EC50:	0,02483
g-Criterion:	0,21129
F:	273,422
p(F) (df: 1;5):	0,000

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]	1,331	1,487	1,839
lower 95%-cl	1,038	1,247	1,654
upper 95%-cl	1,500	1,654	2,136
lower 99%-cl	0,979	1,193	1,588
upper 99%-cl	1,590	1,729	2,224

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

Slope function after Litchfield and Wilcoxon: 1,287

(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.)

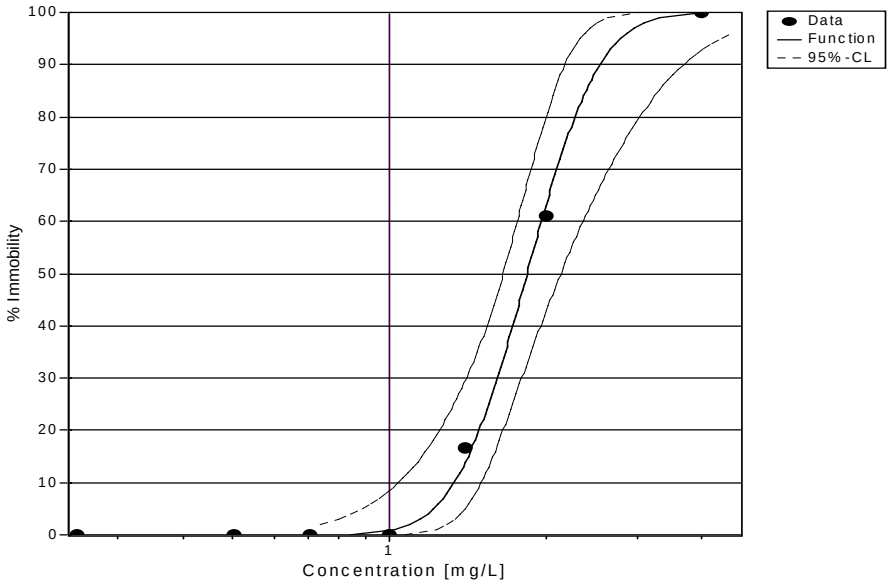

Fig. 3: Concentration-effect curve showing the influence of the test item on mobility of the introduced Daphnia magna as observed after 48 h.

Overview over the ECs of the Test Item on Mobility

Effects on Mobility

Tab. 11: Survival (M) and percent immobility (%I) 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**
[mg/L]	**M**	**%I**	**M**	**%I**
Control	19	5,0	18	10,0
0,250	20	0,0	20	0,0
0,500	20	0,0	20	0,0
0,700	20	0,0	19	5,0
1,000	19	5,0	18	10,0
1,400	18	10,0	15	25,0
2,000	13	35,0	7	65,0
4,000	4	80,0	0	100,0
EC10	1,539	*pm	1,331	*pm
lower 95%-cl	1,157		1,038	
upper 95%-cl	1,839		1,500	
EC20	1,865	*pm	1,487	*pm
lower 95%-cl	1,502		1,247	
upper 95%-cl	2,196		1,654	
EC50	2,691	*pm	1,839	*pm
lower 95%-cl	2,285		1,654	
upper 95%-cl	3,335		2,136	

Threshold Concentrations (NOEC) for Mobility at 24 h

Fisher`s Exact Binomial Test with Bonferroni Correction

Tab. 12: 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	Mobile	Immobile	% Immobility	p	alpha*	sign.
Control	20	19	1	5,00			
0,250	20	20	0	0,00	0,500	0,025	-
0,500	20	20	0	0,00	0,500	0,017	-
0,700	20	20	0	0,00	0,500	0,013	-
1,000	20	19	1	5,00	0,756	0,050	-
1,400	20	18	2	10,00	0,500	0,010	-
2,000	20	13	7	35,00	0,022	0,008	-
4,000	20	4	16	80,00	<0.001	0,007	+

+: significant; -: non-significant

A NOEC of 2,000 mg/L is suggested by the program.

Threshold Concentrations (NOEC) for Mobility at 48 h

Fisher`s Exact Binomial Test with Bonferroni Correction

Tab. 13: 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	Mobile	Immobile	% Immobility	p	alpha*	sign.
Control	20	18	2	10,00			
0,250	20	20	0	0,00	0,244	0,017	-
0,500	20	20	0	0,00	0,244	0,013	-
0,700	20	19	1	5,00	0,885	0,050	-
1,000	20	18	2	10,00	0,698	0,025	-
1,400	20	15	5	25,00	0,204	0,010	-
2,000	20	7	13	65,00	<0.001	0,008	+
4,000	20	0	20	100,00	<0.001	0,007	+

+: significant; -: non-significant

A NOEC of 1,400 mg/L is suggested by the program.

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

Overview over the LOEC and NOEC Determination

Tab. 14: Overview over the LOEC and NOEC Determination: Survival rates and significance marks as computed for mobility 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
0,250	0,00 -	0,00 -
0,500	0,00 -	0,00 -
0,700	0,00 -	5,00 -
1,000	5,00 -	10,00 -
1,400	10,00 -	25,00 -
2,000	35,00 -	65,00+
4,000	80,00+	100,00+
LOEC	4,000 *bf	2,000 *bf
NOEC	2,000 *bf	1,400 *bf

+: Significant difference to control (p <=0,05)