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Report on 21-days full-life cycle *Daphnia magna* test for
the Anolyte produced by the unit EUROSTEL
(chronic toxicity test)

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Protocol of the test [1,2,4].

Daphnia magna was used for 21 day full life-cycle testing measuring two endpoints, mortality and reproduction. *Daphnia*-clone used was of Finnish origin (EF) from the North Savo Regional Environmental Centre in Kuopio, Finland.

Five days old neonates were used to begin the 21-day test. Five *Daphnias* 5 days old were placed in each vessel containing a 100 ml of tested water or control. Each test point was triplicated. Thus, each test was carried out with 15 animals. 15 animals divided in three groups of five animals each were used at each test concentration and five animals for the controls. pH of the concentrations was adjusted prior to the start of experiment. The pH of water was kept optimum for *Daphnias* (ranged between 8.2-8.5). Test chamber size was 120 ml, volume of the sample 100 ml. The concentrations were renewed three times a week [3]. *Daphnias* were fed three times a week with alga *Selenastrum capricornutum*.

During the trials water temperature ranged between 20 and 21° C. An artificial illumination was used with a 16-hour light and 8-hour dark cycle.

Daphnias were counted three times a week and the number neonates born during this time was fixed. Then neonates were removed from the test vessel.

On the basis of the 24 hours acute toxicity tests the maximum ineffective concentrations (MIC) were determined for both Anolytes: Anolyte medium MIC= 1:480; Anolyte strong MIC=1:1300. The concentrations used for the chronic test were < MIC.

The following concentrations (dilutions) were tested:

	Tested concentrations (dilutions)		
Anolyte strong	1:1500	1:2000	1:2500
Anolyte medium	1:500	1:1000	

The parameters of the Anolytes were as following:

	PH	REDOX	Active chlorine, mg/l
Anolyte strong	2.47 ± 0.03	1166 ± 3	580 ± 35
Anolyte medium	2.86 ± 0.06	1165 ± 1	347 ± 26

The control test was carried out for all test series.

Results of the experiments

The concentrations used for the chronic test were < MIC for *D. magna*. The maximum mortality observed was 6.7 % (that means that one animal of 15 died during the 21 days test in the test vessel. See column1 in Table 1). No mortality and no immobility were observed in any other tests. So it can be concluded that there were no significant differences in *D. magna* 21-day mortality between the tested water and control. Calculations made for the reproduction test included only survivors.

Anolyte strong

The experimental data are presented in the supplement (Table i). The number of neonates per adult were calculated from the experimental results. The

results of the test carried out with the Anolyte strong are presented in Table 1 and Figure 1.

There was no tendency for the number of *Daphnia magna* young per surviving female to increase or decrease with the increase of the Anolyte concentration. There were no significant differences in the reproduction of *Daphnias* the tested solutions and the control (see Figure 1). There were no differences observed in the size and weight at the endpoint of experiments between *Daphnias* grown in the tested samples and the control.

Table 1. Results of the 21-day test with Anolyte strong (29.11-20.12).

Dilution	Neonates per adult in 21 day			
	1:1500	1:2000	1:2500	Control
Vessel 1	37.6	38.6	39.4	35.4
Vessel 2	42.9	45.1	38	41.6
Vessel 3	38.4	40.8	40	37.1
Average	39.4	41.5	39.1	37.1
STDV	2.9	3.4	1.0	4.0
Number of <i>Daphnias</i> dead	1	0	0	0

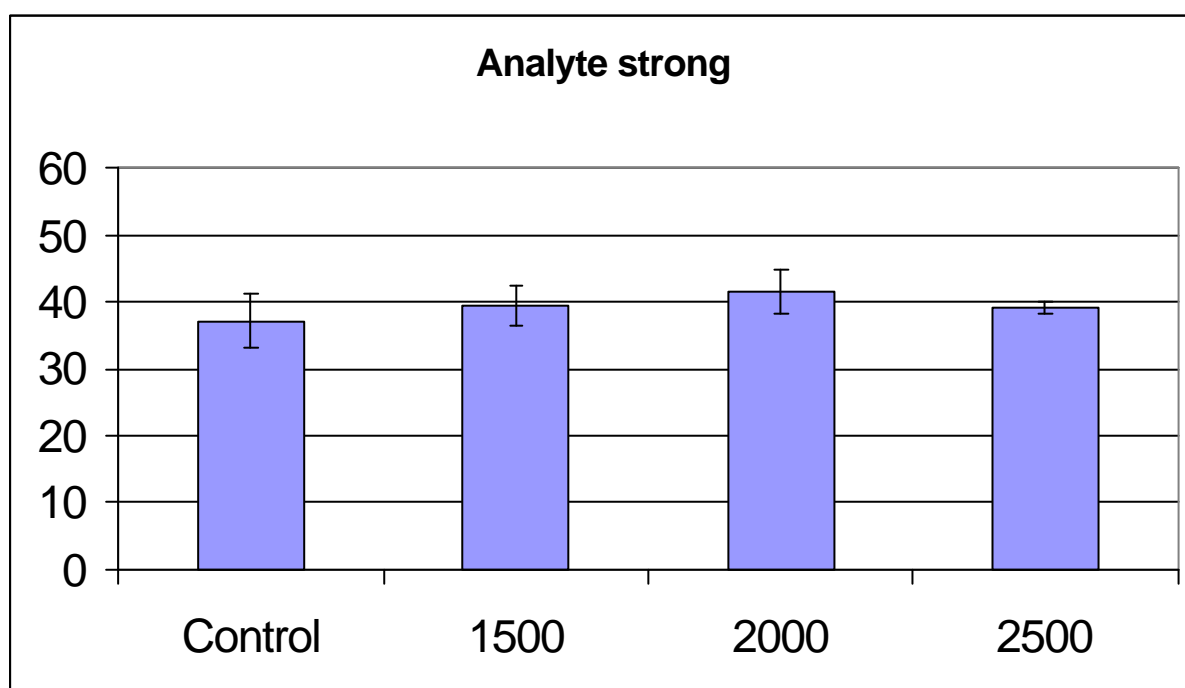


Figure 1. The results of the 21-days full life cycle test with *Daphnia magna* for Anolyte strong.

Anolyte medium.

Two series of experiments were carried out for Anolyte medium. The experimental data are presented in the supplement (Table ii and iii). The number of neonates per adult were calculated from the experimental results. The results of the test carried out with the Anolyte medium are presented in Tables 2, 3 and Figure 2, 3.

There was no tendency for the number of *Daphnia magna* young per surviving female to increase or decrease with the increase of the Anolyte concentration.

There were no significant differences in the reproduction of *Daphnias* the tested solutions and the control (see Figures 2, 3). There were no differences observed in the size and weight at the endpoint of experiments between *Daphnias* grown in the tested samples and the control.

At the dilutions less than 1:500 acute toxic effects were observed.

Table 2. Results of the 21-day test with Anolyte medium (19.11-10.12, series ii).

Dilution	Neonates per adult in 21 day	
	Control	1:500
Vessel 1	54.8	67.2
Vessel 2	70.8	63.2
Vessel 3	67	-
Average	64.2	65.1
STDV	8.4	2.8
Number of <i>Daphnias</i> dead	0	0

Table 3. Results of the 21-day test with Anolyte medium (19.11-10.12, series iii).

Dilution	Neonates per adult in 21 day		
	1:500	1:1000	Control
Vessel 1	59	56	53.8
Vessel 2	51	60.8	54.4
Vessel 3	58.6	53.4	58
Average	56.2	56.7	55.4
STDV	4.5	3.8	2.3
Number of <i>Daphnias</i> dead	0	0	0

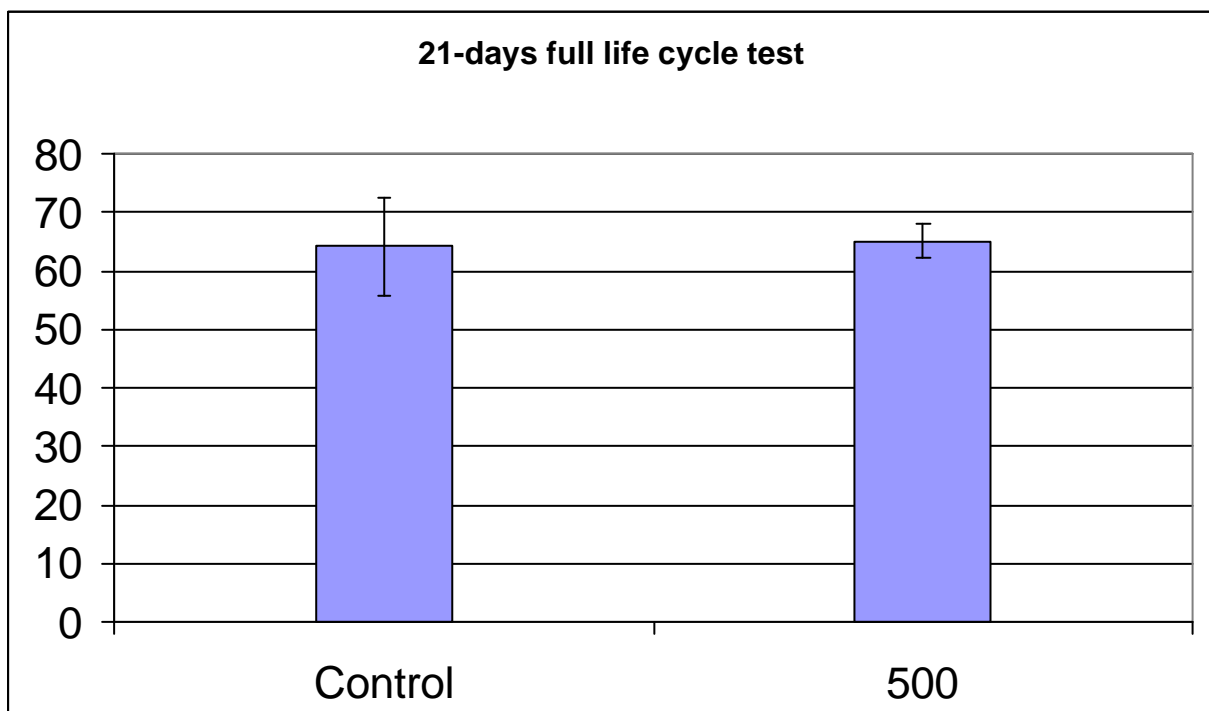


Figure 2. The results of the 21-days full life cycle test with *Daphnia magna* for Anolyte medium (series ii).

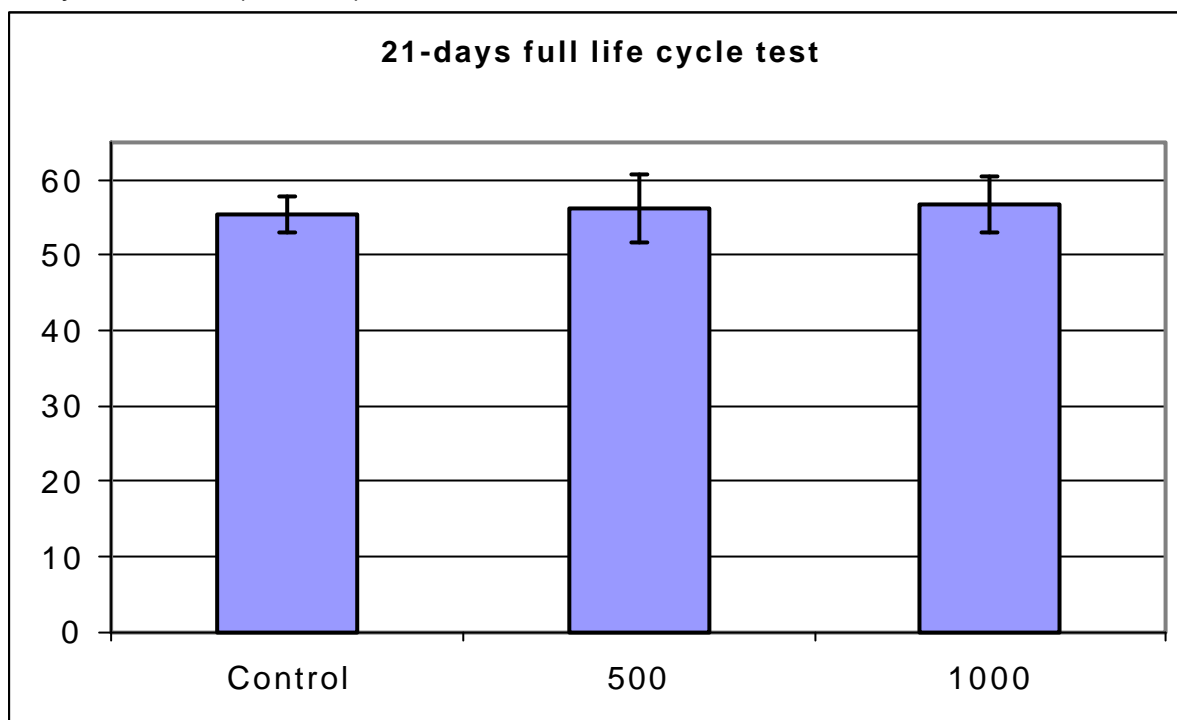


Figure 3. The results of the 21-days full life cycle test with *Daphnia magna* for Anolyte medium (series iii).

Conclusions.

No significant differences were found in the reproduction of *Daphnia magna* in all tested media. The mortality of *Daphnias* in the tests was less than 10 % for sample (only one *Daphnia* died during the experiments). There were no differences observed in the size and weight at the endpoint of experiments between *Daphnia magna* grown in the tested samples and the control.

The results of the current study showed clearly that for both Anolyte medium and Anolyte strong **no chronic toxicity effects were observed**.

References.

1. P.Chapman, T.Moran, T.Kierstead. Refinery water (intake and effluent) quality – update of 1970s with 1990s toxicity testing.
2. ASTM. 1990. Standard Guide for Conducting Renewal life-cycle toxicity test with *Daphnia magna*. 1987. In: Annual Book of ASTM Standards; Water and Environmental Technology, vol. 11.04; E 1193.
3. United States Environmental protection Agency. 10-day chronic toxicity test using *Daphnia magna* or *Daphnia pulex*. 11.16.94, No 2028.
4. Standard methods for the examination of water and wastewater (edited by L.S.Clesceri, A.E.Greenberg, R.R.Trussel). American Public Health Assosication, 17-th edition. 8711 Toxicity test procedure for *Daphnias*.