

CITALOPRAM

Chronic toxicity testing of Citalopram on the crustacean
Daphnia magna



Toxicon Report E22-017
Härslöv October 2022



Chronic toxicity testing of Citalopram on the crustacean *Daphnia magna*

This report accounts for the results obtained from “*Daphnia magna* Reproduction Test” performed by Toxicon AB.

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Study Director: Anders Walstad

Technical Performance: Susanne Fritz (Toxicon AB)

Reference analysis: Ola Svahn (MoLab)

I, the undersigned, hereby declare that this report constitutes a complete, true and accurate representation of the study and its results.

Anders Walstad
Anders Walstad, Study Director

2022-10-31
Date

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Summary

From April to May, 2022, an ecotoxicological test on the test item “Citalopram.HBr” was performed by Toxicon AB, Härslöv, Sweden. The study included one method for ecotoxicological testing. The test item was provided by H. Lundbeck A/S.

The method used was:

- OECD Guidelines for Testing of Chemicals No. 211: *Daphnia magna* Reproduction Test (2012).

The stock solution (26,67 mg/L) for the reproduction test (OECD 211) was made using M7-medium and used within one week, after which a fresh stock solution was prepared. The test was performed on freshly prepared dilutions of the test item using the stock solution. All concentrations mentioned in this report, consider Citalopram without HBr.

In the two highest test concentrations, 3 000 and 938 µg/L Citalopram, the mortality was 100 percent after 5 respectively 12 days.

No inhibition of reproduction output was noticed in the test concentration 293 µg/L Citalopram, but in behaviour there was a difference in this test concentration compared to the lower concentrations. The parent animals were very active and were moving irradicably compared to the parent animals in the control. There was a stimulation of the reproduction in two of the test concentrations, 91.6 and 28.6 µg/L Citalopram. This means that animals subjected to these test concentrations had higher number of live offspring compared to the control.

Results from the reproduction test are presented in Table 1.

Table 1. NOEC, EC₁₀ and EC₅₀ of *Daphnia magna* determined after 21 days of exposure to Citalopram. NOEC is the highest test concentration that was not significantly different from the control ($p>0.05$).

Test item	NOEC (µg/L)	EC ₁₀ (µg/L)	EC ₅₀ (µg/L)
Citalopram	293	361	552

Results from the reference analysis (UPLC-MS/MS) indicate that the test substance did not degrade during a 48-hour incubation time. Results from the reference analysis are presented in Table 2. The measured samples were used for exposure between day 5 and 7 in the test.

Table 2. Nominal and measured test concentrations of Citalopram at day 5 to 7 of exposure.

Test item	Incubation time (hours)	Nominal concentration (µg/L)	Measured concentration (µg/L)
Citalopram	0	8.94	7.0
Citalopram	48	8.94	7.9
Citalopram	0	938	1084.9
Citalopram	48	938	1167.9

Introduction

A sample of the product Citalopram.HBr was received at Toxicon AB on April 6th, 2022 for ecotoxicological test. The test was performed according to OECDs Guidelines for testing of chemicals.

Test item

Chemical name:	Citalopram.HBr
CAS number:	59729-23-7
Batch number:	2001976
Purity:	99.9 %
Appearance:	White to off-white, solid
Molecular formula:	$C_{20}H_{21}FN_2O \cdot HBr$
Relative molecular mass:	405.31 g/mole
Water solubility:	Approximately 30 mg/ml
Stability:	Stable at normal temperature and pressure
Expiry date:	2025-03-24
Toxicon identification code:	E22-017-1
Storage conditions:	Room temperature, in dark storage.

The test item was provided by H. Lundbeck A/S.

Test duration

Experimental starting date: April 20th, 2022.

Experimental completion date: May 11th, 2022.

Test methods

The method used was:

- OECD Guidelines for Testing of Chemicals No. 211: *Daphnia magna* Reproduction Test (2012). Toxicon SOP T 007-7.

***Daphnia magna* Reproduction Test – OECD TG No 211**

The purpose of the test is to determine the effect of the test substrate on a reproductive output of *Daphnia magna*. The method uses newly hatched animals, 6 to 24 hours old, which are exposed to a freshly prepared test item at a range of concentrations. The test duration is 21 days. From the first day offspring are observed, the number of living offspring produced per animal are counted and noted three times per week. At the end of the test the total number of living offspring produced from surviving parent animal is assessed.

Deviations from the standard OECD TG No. 211

There was no deviation from the standard OECD TG No. 211.

Test organism

The *Daphnia magna* Straus culture is a Clone-A originating from the Norwegian Institute of Water Research (NIVA), Norway. The Clone-A has been identified by genotyping. A qualification test on the *D. magna* culture was performed December 16th, 2021, to assure the quality of the test animals. EC₅₀ was found to be 0.9 mg potassium dichromate per Litre, which is between 0.6 and 2.1, confirming that the culture was suitable for testing according to OECD TG No. 202 (*Daphnia* sp. Acute Immobilisation Test), and in extension OECD TG No. 211.

Test medium

M7-medium was used as test medium and prepared according to OECD TG No. 211 (Table 3). The M7-medium had, after equilibration with air, a pH of 7.8±0.5 and a theoretical hardness of 250±25 mg/L (expressed as CaCO₃). The dissolved oxygen concentration should be above 3 mg/L at the beginning and during the test and pH within the range of 6-9.

Table 3. Preparation of M7- medium. The substances were added to reversed osmosis water (< 10µS/cm).

Single substance	Concentration (µg/l)	Single substance	Concentration (µg/l)
CaCl ₂ x 2 H ₂ O	294	CoCl ₂ x 6 H ₂ O	10
MgSO ₄ x 7 H ₂ O	123	KI	3.3
NaHCO ₃	65	Na ₂ SeO ₃	2.2
KCl	5.8	NH ₄ VO ₃	0.6
H ₃ BO ₃	715	Na ₂ EDTA x 2 H ₂ O	625
MnCl ₂ x 4 H ₂ O	90	FeSO ₄ x 7 H ₂ O	249
LiCl	77	Na ₂ SiO ₃	4.3
RbCl	18	NaNO ₃	0.27
SrCl ₂ x 6 H ₂ O	38	KH ₂ PO ₄	0.14
NaBr	4.0	K ₂ HPO ₄	0.18
Na ₂ MoO ₄ x 2 H ₂ O	16	Thiaminhydrochloride	0.075
CuCl ₂ x 2 H ₂ O	4.2	Cyanocobalamine (B12)	0.001
ZnCl ₂	13	Biotine	0.00075

Preparation of test solutions

A fresh stock solution (26.67 mg Citalopram per Litre) was prepared at room temperature (20-22 °C) in normal office lighting. The test item was weighed in on a weighing boat and then applied to a glass bottle containing M7-medium (Table 3). The stock solution was stirred at a speed that created a small vortex on the surface (approximately 100 rpm) until the test item was dissolved. A fresh stock solution was prepared every week and subsequently diluted into the different test concentrations. Part of the diluted test item was used for ecotoxicological testing, and part was stored in freezer for future chemical analysis.

The nominal test concentrations were 8.94; 28.6; 91.6; 293; 938 and 3 000 µg/L.

The test solutions contain Citalopram, M7-medium (Table 3) and algae suspension of *Pseudokirchneriella subcapitata* (0.1–0.2 mg C/animal and day). As control, only M7-medium and algae suspension of *P. subcapitata*, was used.

Ecotoxicological testing

Ten replicates were used for the control as well as for each of the six test concentrations, with one animal in each replicate. Glass E-flasks with 50 ml test solution or control, were used as test vessel, incubated in a room with a constant temperature of 20±1 °C and with a circadian rhythm of 16 hours light and 8 hours darkness. Controls and test solutions were renewed three times per week. The pH (SS-EN ISO 10523), the level of dissolved oxygen (SS-EN ISO 5814) and temperature were measured in all solutions, fresh and old, before the test starts, at every replacement as well as the end of the test after 21 days.

Calculations/Statistics

For the control and test concentrations, the total number of living offspring per surviving parent animal (a measure of the reproduction ability), were calculated for each test vessel (replicate).

EC₁₀ and EC₅₀ were calculated with the logarithmic concentrations plotted against the inhibition of reproduction.

LOEC (Lowest Observed Effect Concentration) was determined as the lowest test concentration that has statistical significance compared to the control regarding reproduction ability ($p < 0.05$). NOEC (No Observed Effect Concentration) was also determined, as the highest test concentration that was not significantly different from the control ($p > 0.05$).

Normal distribution in the data was confirmed using Levene's test and NOEC/LOEC were determined by analysis of variance (ANOVA) followed by Tukey/Kramer ($p < 0.05$).

All calculations were performed in Excel.

Reference analysis

The highest and lowest nominal test concentrations, where animals did survive, were analysed with respect to the measured concentrations and the stability of Citalopram. The analysis was performed using Ultra Performance Liquid Chromatography in combination with tandem mass spectrometry (UPLC-MS/MS) according to the method developed and described by Svahn, O., & Björklund, E. (2016). Samples for analysis were collected at 0 hours and 48 hours exposure at the renewal of test solutions at day 5.

Results

Daphnia magna Reproduction Test – OECD 211

The criteria for the validity of the test according to OECD TG No. 211 were fulfilled:

- The mortality of parent animals in the control did not exceed 20 % at the end of the test.
- The mean number of living offspring produced per parent animal surviving in the control was ≥ 60 at the end of the test.

The mean number of offspring per surviving parent animal in the control, the coefficient of variation for the control regarding the mean of living offspring/ surviving parent animal (reproduction output) together with the same data for all test concentrations of Citalopram are presented in Table 4 and Figure 1.

Offspring from deceased parent animals only occurs in one test concentration (28.6 mg/L) and only from one parent. The death of this parent animal was not considered to be caused by the exposure of Citalopram, and therefore not included in the results. The number of offspring for the parent animal, until the death occurred at day 16 of total 21 days exposure, was 60.

Table 4. The mean number of offspring per surviving parent animal (mean $\pm$ CV %) including % dead parental animal after 21 days exposed for different test concentration of the Citalopram.

Test concentration ($\mu\text{g/L}$)	($\bar{x}$) living offspring/ parent animal surviving Mean $\pm$ CV %	Days until first offspring	Dead parent animal (%)
Control	99 $\pm$ 23	9	10
8.94	119 $\pm$ 15	9	20
28.6	138 $\pm$ 23	9	10
91.6	132 $\pm$ 30	9	20
293	109 $\pm$ 22	9	10
938	0 $\pm$ 0	-	100
3 000	0 $\pm$ 0	-	100

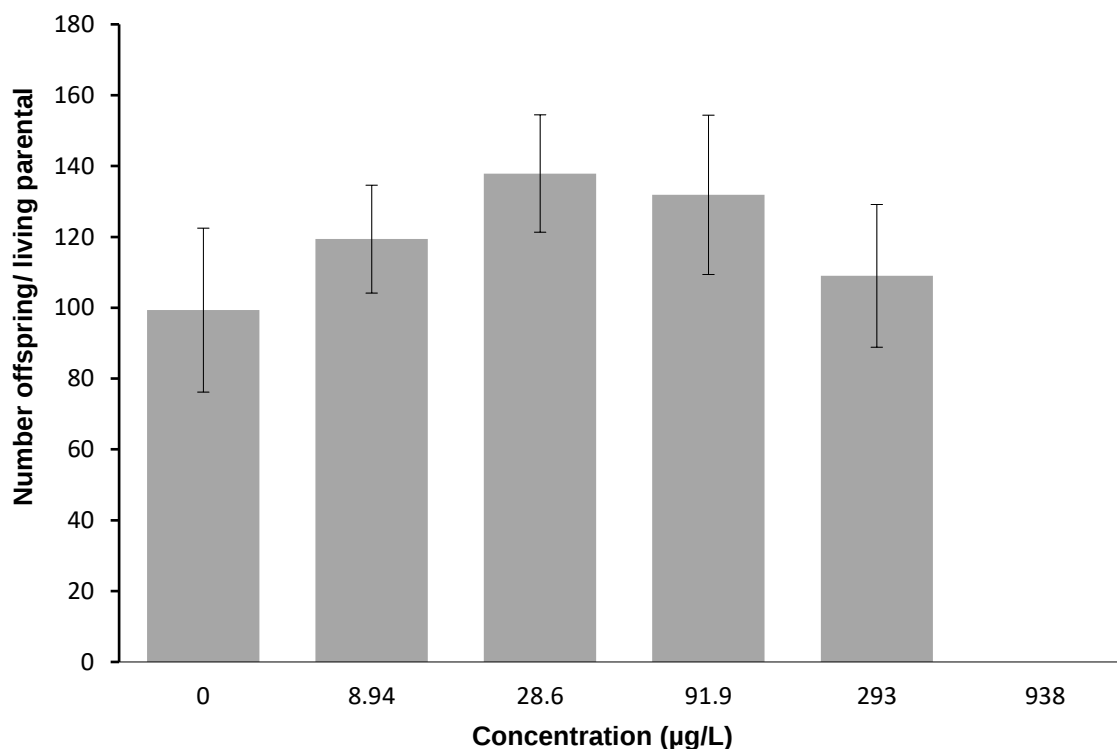


Figure 1. Mean ($\pm$ CV %) of living offspring/ parent animal surviving in different test concentrations of the test item Citalopram. The test concentration 3 000 µg/L is not presented in the figure since there were no surviving parent animals from day 5.

The acute toxicity was very high for the two highest concentration, 938 and 3 000 µg/L. All parent animals died within 12 and 5 days respectively.

NOEC was determined to 293 µg/L, as no difference was statistically found compared to the control regarding reproduction output. But in behaviour there was a difference in this test concentration compared to the lower concentrations, the parent animals were very active and were “spinning” most of the time instead of swimming.

LOEC could not be registered, the only statistical significance shows a stimulation of the reproduction, meaning that these two test concentrations, 28.6 and 91.6 µg/L, have higher number of live offspring compared to the control. The results indicate that exposure to low concentrations of Citalopram is stimulating reproduction. This could possibly be explained by hormesis, which is defined as a phenomenon in which a harmful substance gives stimulating and beneficial effects to living organisms when the quantity of the harmful substance is small.

LC₁₀, LC₅₀ and NOEC are presented in Table 5.

Table 5. LC₁₀ and LC₅₀ for the test item Citalopram were calculated with the logarithmic concentrations plotted against the inhibition of reproduction. NOEC is the highest test concentration that was not significantly different from the control ($p>0.05$) regarding reproductive ability.

Test item	NOEC (µg/L)	LC ₁₀ (µg/L)	LC ₅₀ (µg/L)
Citalopram	293	361	552

Primary data are presented in Appendix 1.

Reference analysis

Results from the reference analysis are presented in Table 6 and in Appendix 2. The measured test concentrations indicate that the test substance Citalopram did not degrade during test.

Table 6. Measured concentrations compared with the nominal test concentration after 0 hours and 48 hours incubation from day 5 to day 7.

Sample	Nominal concentration (µg/L)	Measured concentration (µg/L)
Citalopram, 0 h (day 5)	8.94	7.0
Citalopram, 48 h (day 7)	8.94	7.9
Citalopram, 0 h, (day 5)	938	1084.9
Citalopram, 48 h, (day 7)	938	1167.9

References

1. OECD Guidelines for Testing of Chemicals No. 211: *Daphnia magna* Reproduction Test. Adopted 2012.
2. SS-EN ISO 10523:2012, Determination of pH.
3. SS-EN ISO 5814:2012, Determination of dissolved oxygen.
4. Svahn, O., & Björklund, E. (2016). Increased electrospray ionization intensities and expanded chromatographic possibilities for emerging contaminants using mobile phases of different pH. *Journal of Chromatography B*, 1033, 128-137.

Appendix 1

Protocol for “*Daphnia magna* Reproduction Test”

Titel:			Document no:		
Protocol for reproduction test with <i>Daphnia magna</i> according to ISO 10706 or OECD TG 211			PROT 083-2		
Approved of:	Sign:	Date:	Valid from:	Page (of):	
Eva Helgesson		2016-02-01	2016-02-08	1(1)	

Date: 220420 to 220511 (21 days)

Project no: E22-017

Test laboratory: Toxicon AB

Test item: Citalopram (E22-017-1)

Test leader: Anders Walstad

Concentration: Control

Performance: Susanne Fritz

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
Oxygen (mg/l)*	9.32		9.28			9.25		9.3		9.17			9.09		9.17		8.72			9.34			new	
			11.41			10.51		10.93		11.11			11.13		11.25		9.41			9.9		9.79	old	
pH*	7.72		7.89			8.12		7.79		7.64			7.66		7.74		7.52			7.74			new	
			8.93			8.67		8.76		8.77			9.02		8.91		8.03			8.39		8.37	old	
Temp (°C)*	20.8		21.0			20.9		20.4		20.3			20.6		20.6		20.0			20.3			new	
			21.0			20.9		20.4		20.3			20.6		20.6		20.0			20.3		20.9	old	
Food	x		x			x		x		x			x		x		x			x				
No. of live offspring in vessel																							Total	
1*										5			21				24			27		30	107	
2										7			21				26			30		30	114	
3													12		20		23			23			78	
4										10			19				19			23		27	98	
5													9		27		40			25		1	102	
6													12		26		27			27			92	
7										+	+	+	+	+	+	+	+	+	+	+	+	+	0	
8													13		18		18			21			70	
9													16		23					21		25	85	
10										13			28		29					34		44	148	
Signature	SF		SF			SF		SF		SF			SF		SF		SF			SF		SF	Total	894
*indicates which vessel was used for the measurements of the abiotic components (oxygen, pH and temperature).																						Mean:	99	

Signature: *A WA*

Titel:			Document no:		
Protocol for reproduction test with <i>Daphnia magna</i> according to ISO 10706 or OECD TG 211			PROT 083-2		
Approved of:	Sign:	Date:	Valid from:	Page (of):	
Eva Helgesson		2016-02-01	2016-02-08	1(1)	

Date: 220420 to 220511 (21 days)

Project no: E22-017

Test laboratory: Toxicon AB

Test item: Citalopram (E22-017-1)

Test leader: Anders Walstad

Concentration: 8.94 µg/L

Performance: Susanne Fritz

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
Oxygen (mg/l)*	9.32		9.29			10.01		9.36		9.22			9.23		9.23		9.08			9.67			new
			11.03			11.09		10.78		12.01			11.36		11.2		10.17			9.44		9.42	old
pH*	7.77		7.93			8.24		7.82		7.71			7.71		7.79		7.62			7.83			new
			8.78			8.78		8.77		8.75			8.92		8.77		8.46			8.02		8.21	old
Temp (°C)*	20.8		21.0			20.9		20.4		20.3			20.6		20.6		20.0			20.3			new
			21.0			20.9		20.4		20.3			20.6		20.6		20.0			20.3		20.9	old
Food	x		x			x		x		x			x		x		x			x			
No. of live offspring in vessel																							Total
1*													16		35					40		44	135
2										4			15		22					27		31	99
3						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
4													17		26		30			36			109
5										14			25		29					29		38	135
6													19		34		46			39		1	139
7													14		30		36			29			109
8													+	+	+	+	+	+	+	+	+	+	0
9										15			24				31			37		27	134
10													16		25					22		32	95
Signature	SF		SF			SF		SF		SF			SF		SF		SF			SF		SF	Total 955
*indicates which vessel was used for the measurements of the abiotic components (oxygen, pH and temperature).																							Mean: 119

Signature:.....*A WA*.....

Titel: Protocol for reproduction test with <i>Daphnia magna</i> according to ISO 10706 or OECD TG 211			Document no: PROT 083-2		
Approved of: Eva Helgesson	Sign:	Date: 2016-02-01	Valid from: 2016-02-08	Page (of): 1(1)	

Date: 220420 to 220511 (21 days)

Project no: E22-017

Test laboratory: Toxicon AB

Test item: Citalopram (E22-017-1)

Test leader: Anders Walstad

Concentration: 28.6 µg/L

Performance: Susanne Fritz

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
Oxygen (mg/l)*	9.48		9.29			9.92		9.41		9.22			9.28		9.32		9.23			9.75			new	
			10.93			10.91		10.57		10.62			10.92		11.32		10.35			9.43		9.2	old	
pH*	7.8		7.93			8.24		7.83		7.72			7.72		7.82		7.64			7.83			new	
			8.75			8.83		8.58		8.62			8.8		8.98		8.57			8.01		8.1	old	
Temp (°C)*	20.8		21.0			20.9		20.4		20.3			20.6		20.6		20.0			20.3			new	
			21.0			20.9		20.4		20.3			20.6		20.6		20.0			20.3		20.9	old	
Food	x		x			x		x		x			x		x		x			x				
No. of live offspring in vessel																							Total	
1*										11			21		25					30		35		122
2										11			20				30			40		34		135
3										14			21		25		+	+	+	+	+	+		0
4										13			24		29					35		40		141
5										17			22		30					31		44		144
6										15			32		49					97		1		194
7										12			22		25					30		37		126
8										13			20		24					27		38		122
9													20		35		32			33				120
10										11			23		30					29		44		137
Signature	SF		SF			SF		SF		SF			SF		SF		SF			SF		SF	Total	1241
*indicates which vessel was used for the measurements of the abiotic components (oxygen, pH and temperature).																							Mean:	138

Signature: *A WA*

Titel:			Document no:		
Protocol for reproduction test with <i>Daphnia magna</i> according to ISO 10706 or OECD TG 211			PROT 083-2		
Approved of:	Sign:	Date:	Valid from:	Page (of):	
Eva Helgesson		2016-02-01	2016-02-08	1(1)	

Date: 220420 to 220511 (21 days)

Project no: E22-017

Test laboratory: Toxicon AB

Test item: Citalopram (E22-017-1)

Test leader: Anders Walstad

Concentration: 91.9 µg/L

Performance: Susanne Fritz

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
Oxygen (mg/l)*	9.41		9.38			10.1		9.46		9.41			9.32		9.34		9.37			9.81			new	
			11.43			11.53		10.83		10.62			10.47		11.44		9.83			9.82		9.55	old	
pH*	7.8		7.94			8.27		7.85		7.73			7.74		7.84		7.67			7.84			new	
			8.85			9.00		8.77		8.74			8.65		8.94		8.33			8.24		8.36	old	
Temp (°C)*	20.8		21.0			20.9		20.4		20.3			20.6		20.6		20.0			20.3			new	
			21.0			20.9		20.4		20.3			20.6		20.6		20.0			20.3		20.9	old	
Food	x		x			x		x		x			x		x		x			x				
No. of live offspring in vessel																							Total	
1*													44				37			41		38	160	
2										13			25		32					36		39	145	
3										10			24		1		30			38		38	141	
4										10			26		38					40		43	157	
5						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	
6										10			21				32			38		35	136	
7																	11			25		31	67	
8						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	
9										11			19				29			32		25	116	
10										14			22		28					32		37	133	
Signature	SF		SF			SF		SF		SF			SF		SF		SF			SF		SF	Total	1055
*indicates which vessel was used for the measurements of the abiotic components (oxygen, pH and temperature).																							Mean:	132

Signature:.....A WA.....

Titel: Protocol for reproduction test with <i>Daphnia magna</i> according to ISO 10706 or OECD TG 211			Document no: PROT 083-2		
Approved of: Eva Helgesson	Sign: 	Date: 2016-02-01	Valid from: 2016-02-08	Page (of): 1(1)	

Date: 220420 to 220511 (21 days)

Project no: E22-017

Test laboratory: Toxicon AB

Test item: Citalopram (E22-017-1)

Test leader: Anders Walstad

Concentration: 293 µg/L

Performance: Susanne Fritz

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
Oxygen (mg/l)*	9.48		9.47			10.15		9.4		9.42			9.35		9.37		9.29			9.72			new	
			11.18			11.49		10.72		10.74			11.29		10.68		10.56			9.95		9.17	old	
pH*	7.81		7.96			8.27		7.86		7.74			7.76		7.83		7.66			7.87			new	
			8.81			8.97		8.77		8.8			9.1		8.85		8.77			8.45		8.21	old	
Temp (°C)*	20.8		21.0			20.9		20.4		20.3			20.6		20.6		20.0			20.3			new	
			21.0			20.9		20.4		20.3			20.6		20.6		20.0			20.3		20.9	old	
Food	x		x			x		x		x			x		x		x			x				
No. of live offspring in vessel																							Total	
1*						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	
2*										12			22		30					27		31	122	
3										13			22				24			27		31	117	
4										12			25		31					29		27	124	
5													11		23					26		27	87	
6										14			25				28			29		32	128	
7										10			21		26					30		21	108	
8													13		23					21		29	86	
9										14			22				34			33		33	136	
10										8			21				20			24			73	
Signature	SF		SF			SF		SF		SF			SF		SF		SF			SF		SF	Total	981
*indicates which vessel was used for the measurements of the abiotic components (oxygen, pH and temperature).																							Mean:	109

Signature:.....*A WA*.....

Titel: Protocol for reproduction test with <i>Daphnia magna</i> according to ISO 10706 or OECD TG 211			Document no: PROT 083-2		
Approved of: Eva Helgesson		Sign: 		Date: 2016-02-01	
				Valid from: 2016-02-08	
				Page (of): 1(1)	

Date: 220420 to 220502 (12 days)

Project no: E22-017

Test laboratory: Toxicon AB

Test item: Citalopram (E22-017-1)

Test leader: Anders Walstad

Concentration: 938 µg/L

Performance: Susanne Fritz

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
Oxygen (mg/l)*	9.6		9.43			10.08		9.42		9.57													new
			11.19			10.68		10.87		11.21			11.24										old
pH*	7.84		7.96			8.3		7.87		7.74													new
			8.84			8.68		8.8		8.81			8.8										old
Temp (°C)*	20.8		21.0			20.9		20.4		20.3													new
			21.0			20.9		20.4		20.3			20.6										old
Food	x		x			x		x		x													
No. of live offspring in vessel																							Total
1*								+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
2*													+	+	+	+	+	+	+	+	+	+	0
3						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
4								+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
5										+	+	+	+	+	+	+	+	+	+	+	+	+	0
6										+	+	+	+	+	+	+	+	+	+	+	+	+	0
7						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
8										+	+	+	+	+	+	+	+	+	+	+	+	+	0
9								+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
10										+	+	+	+	+	+	+	+	+	+	+	+	+	0
Signature	SF		SF			SF		SF		SF			SF										Total
																							0

*indicates which vessel was used for the measurements of the abiotic components (oxygen, pH and temperature).

Mean:

0

Signature:..... *A WA*

Titel: Protocol for reproduction test with <i>Daphnia magna</i> according to ISO 10706 or OECD TG 211															Document no: PROT 083-2						
Approved of: Eva Helgesson					Sign: 					Date: 2016-02-01					Valid from: 2016-02-08					Page (of): 1(1)	

Date: 220420 to 220425 (5 days)

Project no: E22-017

Test laboratory: Toxicon AB

Test item: Citalopram (E22-017-1)

Test leader: Anders Walstad

Concentration: 3000 µg/L

Performance: Susanne Fritz

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
Oxygen (mg/l)*	9.5		9.49																				new	
			9.77			9.43																	old	
pH*	7.83		7.95																				new	
			8.24			8.2																	old	
Temp (°C)*	20.8		21.0																				new	
			21.0			20.9																	old	
Food	x		x																					
No. of live offspring in vessel																								Total
1*						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0
2						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0
3			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0
4						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0
5						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0
6						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0
7						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0
8						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0
9						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0
10						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0
Signature	SF		SF			SF																	Total	0
*indicates which vessel was used for the measurements of the abiotic components (oxygen, pH and temperature).																							Mean:	0

Signature:..... *A WA*

Appendix 2

Report from reference analysis.

Sample ID Lab	Sample ID	Citalopram ug/L
<i>Daphna magna</i> reproduction test (OECD TG 211)		
22-33-11	ALS E22-017-1 Dag 5-A-0 h 8,94ug/l 22-04-25	7,0
22-33-12	ALS E22-017-1 Dag 7-A-48 h 8,94 ug/l 22-04-27	7,9
22-33-13	ALS E22-017-1 Dag 5-E-0 h 938 ug/l 22-04-25	1084,9
22-33-14	ALS E22-017-1 Dag 7-E-48 h 938 ug/l 22-04-27	1167,9
LOQ (ng/L)		1,2
RS (%)		1,8

Analysis by Ola Svahn (PhD), MoLab, Stridsvagnsvägen 14, Kristianstad (2022-09-28)

Method description: Thawing of frozen samples. Analysis of the samples were performed using chromatography in combination with mass spectrometry (UPLC-MS/MS). The analysis method for citalopram is developed as described by Svahn, O., & Björklund, E. (2016)* and validated according to Standard method 1694 (US EPA, 2007).

*) Svahn, O., & Björklund, E. (2016). *Increased electrospray ionization intensities and expanded chromatographic possibilities for emerging contaminants using mobile phases of different pH. Journal of Chromatography B*, 1033, 128-137.