

Fish, Early-life Stage Toxicity Test with Citalopram on the early life stages of zebrafish (*Danio rerio*)

DHI Accredited report No.: 213



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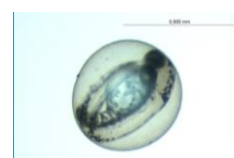
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Approved by

Signed by: Gitte Petersen

Fish, Early-life Stage Toxicity Test with Citalopram on the early life stages of zebrafish (*Danio rerio*)

Prepared for NIRAS
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Zebra fish egg

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1 Introduction

DHI has conducted an ecotoxicological toxicity test of the test item Citalopram on the early life stages of zebrafish (*Danio rerio*). The test performed was a chronic test investigating the lethal and the sublethal effects of the test item on embryos and sac-fry stages of the freshwater fish zebrafish (*Danio rerio*).

The study was performed in accordance with the OECD Guideline for the Testing of Chemicals No. 210 "Fish, Early-life Stage Toxicity Test" /1/.

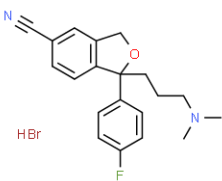
The test was carried out as an accredited study in compliance with ISO 17025 /2/.

2 Test item

The test item, Citalopram, is a psychopharmacological drug which acts as selective-serotonin-re-uptake-inhibitor (SSRI), and which is used in treatment of depressions by inhibition of the re-uptake of synaptic serotonin (5-HT) in the brain.

The test item was provided by H. Lundbeck A/S as Citalopram HBr. The test item was received at DHI on March 3rd, 2022. The certificate of analysis and the safety data sheet are attached in Annex C and information about the test item is summarized in the table below.

Table 2.1 Information regarding the test item

Product Name:	Citalopram, HBr
Purity	99.9%
Synonyms	Citalopram hydrobromide
Structural formula	
Product Number:	Lu 10-171-B
Batch Number:	2001976
CAS Number:	59729-32-7
Formula:	C ₂₀ H ₂₂ BrFN ₂ O
Average mass	405.35 g/mol
Water solubility	30 g/L
Storage	Store at room temperature, protected from light
Expiry date	01-Mar-2025

3 Test method

The chronic toxicity of Citalopram to early life stages of *Danio rerio* (zebra fish) was examined in accordance with the OECD Guideline for the Testing of Chemicals No. 210 /1/.

The test concentrations were prepared as dilutions of a stock solution of the test item with laboratory medium (as described in Table 3.1). A stock solution of the test item in ISO medium was prepared once a week and stored cold and dark. The test item received was Citalopram hydrobromide, but the nominal test concentrations were based only on the molar weight of Citalopram. The stock solution was used to prepare the dilution series: 0; 0.01; 0.03; 0.10; 0.30 and 1.0 mg/L.

The test was performed as a semi-static test with renewal of all test solutions three times per week, after which surviving eggs and larvae were gently transferred to the new vessels in a small volume of old solution to avoid exposure to air. After two weeks post hatch organisms were retained in the test vessels whilst a proportion (at least two thirds) of the test solution / control volume was changed.

The pH, temperature and dissolved oxygen concentrations were measured in each test container at the initiation of the test, before and after renewal of test solutions (three times per week) and at the termination of the test. Temperature was additionally measured continuously by thermologger placed in a separate container with control media and no eggs.

Duplicate samples for chemical analysis were collected from each test concentration and the control at the initiation of the test, before (named old) and after each renewal (named new) and at the termination of the test. Two times 5 mL sample were collected (polyethylene vials (HDPE) and stored frozen at $-20 \pm 2.0^{\circ}\text{C}$. Although samples were collected in duplicate, only one replicate was analysed. The other replicates serve as spare samples and will be discarded after the approval of the report. The samples were shipped on dry ice to the chemical laboratory at Molab, Kristiansand.

The test conditions are summarized in Table 3.1.

Table 3.1 Test conditions for the early life stage toxicity test with *Danio rerio*.

Test guideline	OECD 210 /1/
Test organism	<i>Danio rerio</i> (Zebra fish)
Test organism source	Department of Biology at University of Southern Denmark
Test organism life stage	The test was stated as soon as possible after the eggs had been fertilised (6-8 hours after fertilisation).
Test concentrations of Citalopram	0; 0.01; 0.03; 0.1; 0.3 and 1.0 mg/L
Test duration	30 days post-hatch (approx. 32-34 days).
Test container	The test was initiated in 180-mL Pyrex crystallizing dish with approximately 100 mL of test solution (synthetic medium and test item to obtain the test concentration or synthetic medium for controls). From 10 days post hatch and onwards the test was conducted in 2-L glass containers with 1.5 L test solution.
Replicates	• 4 × laboratory control • 4 × test concentration

Eggs per container and eggs per concentration	30 eggs per replicate. After 24 hours of exposure the number of eggs was reduced to 20 eggs per replicate and thus 80 eggs per concentration
Renewal of test solutions	The test solutions were renewed three times a week
Observations	• Stage of embryonic development at the beginning of the exposure • Number of hatched larvae per day • Cumulative mortality (embryos larvae and juvenile fish) • Abnormal morphological appearance • Abnormal behaviour (hyperventilation; uncoordinated swimming; atypical feeding behaviour etc.) • Length and weight of surviving animals at the end of the test
Laboratory control medium	Freshly produced synthetic medium was used in the test. The medium was prepared from Millipore water according to ISO 7346 /3/, with a pH value of 7.8 ± 0.2 and hardness of 225 ± 50 mg/L expressed as CaCO_3 .
Food regime	Feeding was initiated on day 2 post hatch and continued daily throughout the study (once per day during weekends). Food, hatched brine shrimp nauplii, were provided daily together with flake food. Surplus food and faeces were removed as necessary, to avoid accumulation of waste.
Photoperiod	12:12 hours
Temperature	$26 \pm 1.5^\circ\text{C}$
Initial pH	In the laboratory control medium: 7.8 ± 0.2
Validity criteria	• Hatching success in the laboratory control: $\geq 70\%$ • Survival of hatched larvae in the laboratory control: $\geq 75\%$ • Dissolved oxygen concentration: $>60\%$ • Water temperature differing between test chambers or between successive days: $\pm 1.5^\circ\text{C}$

3.1 Statistical analysis

All the analyses were run using the free software R /4/. The corresponding packages used for the different analysis are described in Section 6, References.

The LOEC/NOEC values were determined by use of the Dunnett's test /5/. The NOEC values ($p>0.05$) were determined as the highest tested concentration, at which no significant negative effect was observed compared with the control. The LOEC ($t<0$ and $p<0.05$) is the concentration just above the NOEC.

To comply with the conditions of the Dunnett's test, the normality of the data was assessed but only by a visual description using a boxplot /6/. The variance was analysed with a Levene's test /7/.

When a significant effect was observed associated with a dose response, a Probit analysis was conducted to estimate the EC/LC10 or EC/LC25.

The EC/LC values were calculated to attain the closest possible curve fit with following equations: LL.3 /8/, W1.3 /9/ and W2.3 /9/.

4 Results

4.1 Results from chemical analysis

Verification of the test item stability in the test system was verified by analysing the lowest and the highest test concentration at day 28 (new solution) and the same solutions at day 31 (old solutions) (Table 4.1 and Annex B). The measured concentrations were higher than expected but the stability was satisfactory maintained.

Table 4.1 Results from chemical analysis

Nominal concentration	Actual measured concentration		Average measured concentration (µg/L)	Average measured concentration in percent of nominal
Citalopram µg/L	Day 28 (new)	Day 31 (old)		
10	10.9	11.9	11.4	114
1000	1305.8	1309.5	1307.7	131

4.2 Ecotoxicological results

The primary data are presented in Appendix A. The results of this test are summarized in Table 5.1. All validity criteria prescribed in the OECD 210 guideline /1/ were fulfilled.

4.2.1 Hatching success

No significant effects on the endpoint hatching success were observed (Table 4.2). NOEC and LOEC values are thus estimated to be 1 mg/L and >1 mg/L, respectively.

As no significant effect or any dose-response relationship was observed, the EC10 and EC50 for the endpoint hatching success were estimated to be higher than >1 mg/L.

Table 4.2 Hatching success of *Danio rerio* after exposure to Citalopram.

Concentration (nominal)	Hatching success mean after 3 days	Standard deviation
0	100%	0%
0.01	98%	3%
0.03	96%	5%
0.1	96%	5%
0.3	89%	13%
1.0	98%	5%

4.2.2 Mortality

Significant effects on the endpoint mortality were observed at the highest tested concentration of 1 mg/L (Table 4.3). NOEC and LOEC values are thus estimated to be 0.3 mg/L and 1 mg/L, respectively.

The LC10 and LC50 for the endpoint mortality were estimated to be 0.20 mg/L and >1 mg/L, respectively.

Table 4.3 Mortality of *Danio rerio* after exposure to Citalopram.

Citalopram	Mortality, embryos and larvae, mean	Standard deviation
0	16.3%	13.8%
0.01	8.8%	5.0%
0.03	13.8%	8.5%
0.1	22.5%	8.7%
0.3	26.3%	11.1%
1.0	42.5%	8.7%

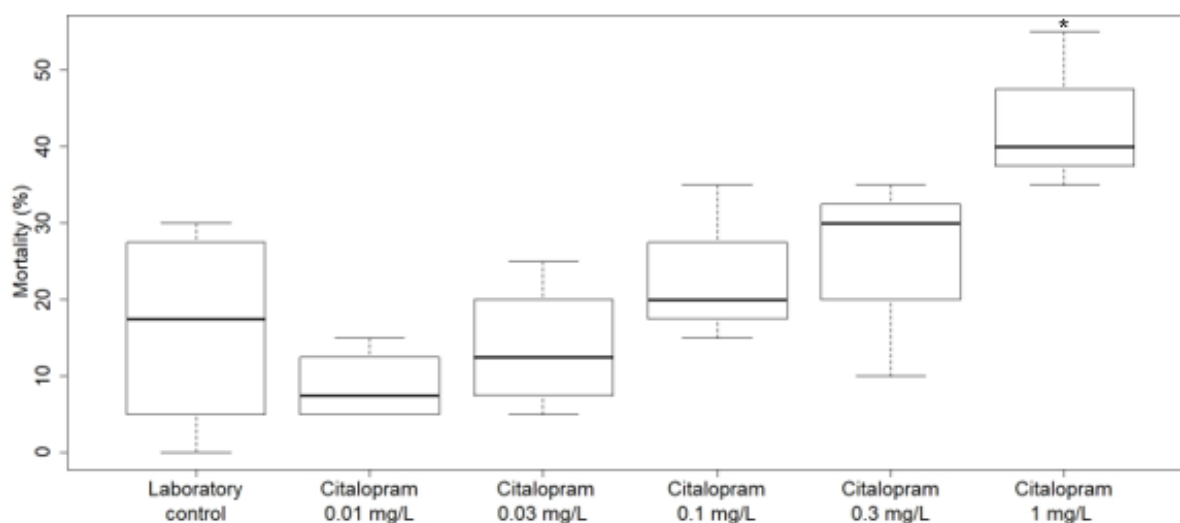


Figure 4.1 Boxplot of the mortality of embryos and larvae after exposure to Citalopram. The line in the middle of the box is the median. The box itself represents the middle 50% of the data. The box edges are the 25th and 75th percentiles. An asterisk in the plot indicates a significant difference between the test group and the control group.

4.2.3 Body length

Significant effects on the body length were observed at the two highest test concentrations (Table 4.4). NOEC and LOEC values are thus estimated to be 0.1 mg/L and 0.3 mg/L, respectively.

The EC10 and EC50 for the body length were estimated to be 0.77 mg/L and >1 mg/L, respectively.

Table 4.4 Average body length of *Danio rerio* larvae after exposure to Citalopram.

Concentration	Body length (mm)	Standard deviation
0	11.7	1.7
0.01	11.4	1.5
0.03	11.3	1.8
0.1	11.1	1.5
0.3	10.7	1.9
1.0	10.4	2.5

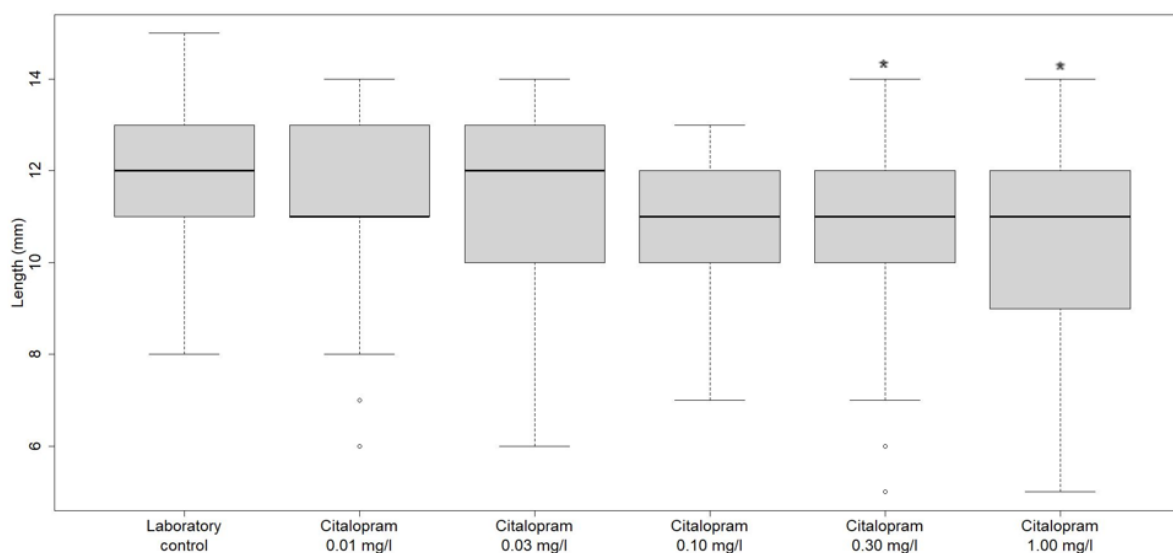


Figure 4.2 Boxplot of the body length of *Danio rerio* embryos and larvae after exposure to Citalopram. The box itself represents the middle 50% of the data. The box edges are the 25th and 75th percentiles. An asterisk in the plot indicates a significant difference between the test group and the control group.

4.2.4 Body weight

No significant effects on the body weight were observed (Table 4.5). NOEC and LOEC values are thus estimated to be 1 mg/L and >1 mg/L, respectively.

As no significant effect or any dose-response relationship was observed, the EC10 and EC50 for the body length were estimated to be >1 mg/L.

Table 4.5 Average body weight of *Danio rerio* larvae after exposure to Citalopram

Concentration	Body weight (mg)	Standard deviation
0	19.9	7.7
0.01	17.4	6.2

0.03	17.0	6.5
0.1	17.8	6.6
0.3	16.6	7.3
1.0	17.4	8.5

5 Conclusion

All validity criteria were fulfilled, and the stability of the test item was satisfactory maintained. The endpoints obtained in the study is summarised in Table 5.1 and given in mg Citalopram/L (nominal concentrations).

Table 5.1 Results (in mg Citalopram/L) of the *Danio rerio* toxicity test after exposure to Citalopram. 95% confidence intervals are indicated in brackets. No confidence limits could be calculated or was not within the tested concentrations

Endpoint	NOEC	LOEC	EC/LC10	EC/LC50
Hatching success	1	>1	>1	>1
Mortality	0.3	1	0.16	>1
Body length	0.1	0.3	0.77	>1
Body weight	1	>1	>1	>1

6 References

- /1/ OECD Guideline for Testing of Chemicals No. 210: Fish, Early-life Stage Toxicity Test. 2013.07.26.
- /2/ ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
- /3/ ISO 7346-2 (1996): "Water Quality - Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)]. Part 2: Semi static method".
- /4/ R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- /5/ Frank Bretz, Torsten Hothorn and Peter Westfall (2010) (Dunnett's test), Multiple Comparisons Using R, CRC Press, Boca Raton.
- /6/ Fox, J. (2005) The R Commander: A Basic Statistics Graphical User Interface to R. Journal of Statistical Software, 14(9): 1–42
- /7/ Fox, J. and Weisberg, S. (2011) *An R Companion to Applied Regression*, Second Edition, Sage.

- /8/ Seber, G. A. F. and Wild, C. J (1989) Nonlinear Regression, New York: Wiley & Sons (p. 330).
- /9/ Ritz, C (2010). Towards a unified approach to dose-response modelling in ecotoxicology. *Environmental Toxicology & Chemistry*, 29, 220–229.

APPENDICES

APPENDIX A

Raw data and statistical evaluation

A Raw data and statistical evaluation

A.1.1 Primary data on chronic toxicity test

Table A.1 Raw data of the number and larvae stages of organisms generated in the chronic toxicity test with Citalopram. Test period 2022/03/04 to 2022/04/06.

Date		4 March, 2022								Accumulated day(s)								0			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	20	20	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.01	80	20	20	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.03	80	20	20	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1	80	20	20	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.3	80	20	20	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.0	80	20	20	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Date		5 March, 2022								Accumulated day(s)								1			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	20	20	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.01	80	20	20	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.03	80	20	20	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1	80	20	20	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.3	80	20	20	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.0	80	20	20	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Date		6 March, 2022								Accumulated day(s)								2			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	19	20	19	19	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0
0.01	80	18	20	18	20	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0
0.03	80	17	17	13	18	0	0	0	0	3	3	7	2	0	0	0	0	0	0	0	0
0.1	80	19	20	20	19	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
0.3	80	16	19	19	19	0	0	0	0	4	1	1	1	0	0	0	0	0	0	0	0
1.0	80	19	20	18	19	0	0	0	0	1	0	2	1	0	0	0	0	0	0	0	0

Date		7 March, 2022								Accumulated day(s)								3			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0
0.01	80	-	1	-	1	-	0	-	0	20	19	20	19	0	0	0	0	0	0	0	0
0.03	80	-	1	-	2	-	0	-	0	20	19	20	18	0	0	0	0	0	0	0	0
0.1	80	2	1	-	-	0	0	-	-	18	19	20	20	0	0	0	0	0	0	0	0
0.3	80	1	6	2	-	0	0	0	-	19	14	18	20	0	0	0	0	0	0	0	0
1.0	80	-	-	2	-	-	-	0	-	20	20	18	20	0	0	0	0	0	0	0	0

Date		8 March, 2022								Accumulated day(s)								4			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	1	-	-	20	19	20	20	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	1	-	-	20	19	20	20	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	1	-	20	20	19	20	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0

Date		9 March, 2022								Accumulated day(s)								5			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	19	19	20	20	1*	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	20	19	20	20	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	20	20	19	20	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0

* Dead at the renewal of the test solution

Date		10 March, 2022								Accumulated day(s)								6			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	19	19	20	20	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	20	19	20	20	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	20	20	19	20	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0

Date		11 March, 2022								Accumulated day(s)								7			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	19	19	20	20	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	20	19	20	18	0	0	0	2	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	17	20	20	20	3	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	20	20	18	19	0	0	1	1	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0

Date		12 March, 2022								Accumulated day(s)								8			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	19	19	20	20	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	20	19	20	18	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	17	20	20	20	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	20	20	18	19	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0

Date		13 March, 2022								Accumulated day(s)								9			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	20	20	20	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	19	19	20	20	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	20	19	20	18	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	16	20	20	20	1	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	20	20	18	19	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	19	20	20	19	1	0	0	1	0	0	0	0

Date		14 March, 2022								Accumulated day(s)								10			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	19	20	19	20	1	0	1	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	19	19	20	20	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	20	19	20	18	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	16	19	20	20	0	1	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	19	20	18	19	1	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	19	20	20	19	0	0	0	0	0	0	0	0

Date		15 March, 2022								Accumulated day(s)								11			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	17	20	17	20	2	0	2	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	20	20	1	1	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	20	16	20	18	0	3	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	20	19	1	1	0	1	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	18	20	17	18	1	0	1	1	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	18	17	18	19	1	3	2	0	0	0	0	0

Date		16 March, 2022								Accumulated day(s)								12			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	17	20	1	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	19	20	0	0	1	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	20	16	19	18	0	0	1	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	18	18	0	0	2	1	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	18	20	17	17	0	0	0	1	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	18	17	18	19	0	0	0	0	0	0	0	0

Date		17 March, 2022								Accumulated day(s)								13			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	17	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	19	20	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	20	15	19	18	0	1	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	18	0	0	1	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	16	20	15	17	2	0	2	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	17	16	17	18	1	1	1	1	0	0	0	0

Date		18 March, 2022								Accumulated day(s)								14			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	17	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	19	19	0	0	0	1	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	20	15	19	17	0	0	0	1	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	17	0	0	0	1	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	20	15	16	2	0	0	1	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	15	14	14	16	2	2	3	2	0	0	0	0

Date		19 March, 2022								Accumulated day(s)								15			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	17	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	19	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	1	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	17	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	20	15	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	15	14	13	15	0	0	1	1	0	0	0	0

Date		20 March, 2022								Accumulated day(s)								16			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	17	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	19	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	17	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	15	16	0	1	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	15	14	12	14	0	0	1	1	0	0	0	0

Date		21 March, 2022								Accumulated day(s)								17			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	17	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	19	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	17	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	15	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	15	14	11	14	0	0	1	0	0	0	0	0

Date		22 March, 2022								Accumulated day(s)								18			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	17	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	19	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	17	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	15	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	14	10	13	1	0	1	1	0	0	0	0

Date		23 March, 2022								Accumulated day(s)								19			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	17	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	19	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	16	0	0	0	1	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	15	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	14	10	12	0	0	0	1	0	0	0	0

Date		24 March, 2022								Accumulated day(s)								20			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	17	20	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	19	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	16	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	15	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	14	10	12	0	0	0	0	0	0	0	0

Date		25 March, 2022								Accumulated day(s)								21			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	14	19	0	0	3	1	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	19	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	16	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	15	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	14	9	12	0	0	1	0	0	0	0	0

Date		26 March, 2022								Accumulated day(s)								22			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	14	19	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	19	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	16	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	15	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	14	9	12	0	0	0	0	0	0	0	0

Date		27 March, 2022								Accumulated day(s)								23			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	14	19	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	19	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	16	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	15	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	14	9	12	0	0	0	0	0	0	0	0

Date		28 March, 2022								Accumulated day(s)								24			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	14	19	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	18	19	0	0	1	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	16	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	15	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	14	9	12	0	0	0	0	0	0	0	0

Date		29 March, 2022								Accumulated day(s)								25			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	14	19	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	18	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	16	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	15	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	14	9	12	0	0	0	0	0	0	0	0

Date		30 March, 2022								Accumulated day(s)								26			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	14	19	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	18	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	16	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	15	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	14	9	12	0	0	0	0	0	0	0	0

Date		31 March, 2022								Accumulated day(s)								27			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	16	20	14	19	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	18	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	19	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	15	18	17	16	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	15	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	14	9	12	0	0	0	0	0	0	0	0

Date		1 April, 2022								Accumulated day(s)								28			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	15	20	14	19	1	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	18	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	18	15	19	17	1	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	14	18	17	16	1	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	14	16	0	0	1	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	14	9	12	0	0	0	0	0	0	0	0

Date		2 April, 2022								Accumulated day(s)								29			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	15	20	14	19	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	18	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	18	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	14	18	17	16	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	14	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	13	9	12	0	1	0	0	0	0	0	0

Date		3 April, 2022								Accumulated day(s)								30			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	15	20	14	19	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	18	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	18	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	14	18	17	16	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	14	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	13	9	12	0	0	0	0	0	0	0	0

Date		4 April, 2022								Accumulated day(s)								31			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	15	20	14	19	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	18	18	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	18	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	14	18	17	16	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	19	14	16	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	14	13	9	12	0	0	0	0	0	0	0	0

Date		5 April, 2022								Accumulated day(s)								32			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	15	20	14	18	0	0	0	1	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	17	17	19	0	1	1	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	18	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	13	16	17	16	1	2	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	18	14	13	0	1	0	3	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	12	13	9	12	2	0	0	0	0	0	0	0

Date		6 April, 2022								Accumulated day(s)								33			
Conc. (mg/L)	Initial eggs per test group	Daily number of :																			
		Eggs				Eggs that will never hatch today				Live larvae				Dead larvae				Malformed larvae			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	-	-	-	-	-	-	-	-	15	20	14	18	0	0	0	0	0	0	0	0
0.01	80	-	-	-	-	-	-	-	-	18	17	17	19	0	0	0	0	0	0	0	0
0.03	80	-	-	-	-	-	-	-	-	18	15	19	17	0	0	0	0	0	0	0	0
0.1	80	-	-	-	-	-	-	-	-	13	16	17	16	0	0	0	0	0	0	0	0
0.3	80	-	-	-	-	-	-	-	-	14	18	14	13	0	0	0	0	0	0	0	0
1.0	80	-	-	-	-	-	-	-	-	12	13	9	12	0	0	0	0	0	0	0	0

A.1.2 Physical parameters

Table A.2 Measurements of temperature, dissolved oxygen and pH generated in the chronic toxicity test with Citalopram. Test period 2022/03/04 to 2022/04/06.

Date	4 March, 2022				Remark				Start of the test			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	24.6	-	-	-	100	-	-	-	7.7	-	-	-
0.01	24.8	-	-	-	100	-	-	-	7.8	-	-	-
0.03	24.8	-	-	-	100	-	-	-	7.8	-	-	-
0.1	24.8	-	-	-	100	-	-	-	7.8	-	-	-
0.3	24.8	-	-	-	100	-	-	-	7.8	-	-	-
1.0	24.7	-	-	-	100	-	-	-	7.9	-	-	-

Date	7 March, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	24.6	24.7	24.9	24.8	95	99	99	100	8.0	8.0	8.0	8.0
0.01	24.4	24.5	24.8	25.0	100	100	100	100	8.0	8.0	8.0	8.0
0.03	25.0	25.1	25.2	25.3	100	100	100	100	7.9	7.9	7.9	7.9
0.1	25.1	25.2	25.4	25.5	100	100	100	100	7.9	7.9	7.9	7.9
0.3	25.4	25.5	25.5	25.5	100	100	100	100	7.9	7.9	7.9	7.9
1.0	25.5	25.6	25.6	25.6	100	100	100	100	7.9	7.9	7.9	7.9

Date	7 March, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.0	25.0	25.1	25.2	100	100	100	100	7.7	7.7	7.7	7.8
0.01	25.3	25.4	25.4	25.4	100	100	100	100	7.8	7.8	7.7	7.7
0.03	25.4	25.4	25.5	25.5	100	100	100	100	7.7	7.7	7.7	7.7
0.1	25.5	25.5	25.6	25.6	100	100	100	100	7.7	7.7	7.8	7.8
0.3	25.5	25.5	25.6	25.6	100	100	100	100	7.8	7.8	7.8	7.8
1.0	25.6	25.6	25.6	25.5	100	100	100	100	7.8	7.8	7.8	7.8

Date	9 March, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.4	25.5	25.7	25.8	100	98	97	98	7.6	7.6	7.6	7.6
0.01	25.8	25.8	25.8	25.9	100	100	100	100	7.7	7.6	7.7	7.6
0.03	26.0	26.0	26.0	26.0	100	100	100	100	7.6	7.7	7.7	7.7
0.1	26.0	26.1	26.1	26.2	100	100	98	98	7.7	7.7	7.7	7.7
0.3	26.2	26.3	26.3	26.3	98	99	98	98	7.7	7.7	7.7	7.7
1.0	26.3	26.3	26.3	26.4	98	99	99	98	7.7	7.7	7.7	7.7

Date	9 March, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.2	25.2	25.3	25.5	100	100	100	100	7.7	7.7	7.7	7.7
0.01	25.5	25.5	25.6	25.6	100	100	100	100	7.7	7.7	7.8	7.7
0.03	25.7	25.7	25.7	25.7	100	100	100	100	7.7	7.8	7.8	7.8
0.1	25.5	25.5	25.7	25.6	100	100	100	100	7.8	7.8	7.8	7.8
0.3	25.7	25.7	25.7	25.6	100	100	100	99	7.8	7.8	7.8	7.8
1.0	25.6	25.7	25.7	25.7	100	100	100	100	7.8	7.8	7.8	7.8

Date	11 March, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.6	25.6	25.6	25.6	96	97	96	96	7.6	7.6	7.6	7.7
0.01	25.6	25.6	25.7	25.7	96	96	96	96	7.6	7.6	7.6	7.6
0.03	25.6	25.8	25.9	25.9	96	96	96	97	7.6	7.6	7.7	7.6
0.1	25.8	25.7	25.8	25.9	99	98	98	96	7.7	7.7	7.8	7.6
0.3	25.9	25.9	25.9	25.9	97	97	97	96	7.7	7.7	7.7	7.7
1.0	26.0	26.0	26.0	26.0	97	98	97	97	7.7	7.7	7.7	7.7

Date	11 March, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.8	25.6	25.7	25.7	99	100	100	100	7.6	7.6	7.6	7.6
0.01	25.6	25.6	25.7	25.7	100	100	100	100	7.6	7.6	7.6	7.7
0.03	25.3	25.3	25.5	25.6	100	100	100	99	7.7	7.7	7.7	7.7
0.1	25.7	25.7	25.7	25.7	99	99	99	99	7.7	7.7	7.7	7.7
0.3	25.6	25.6	25.5	25.4	99	99	98	98	7.7	7.7	7.7	7.8
1.0	25.2	25.3	25.2	25.1	99	99	98	98	7.8	7.7	7.8	7.8

Date	14 March, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	24.9	25.4	25.7	25.8	100	97	95	96	7.5	7.5	7.5	7.5
0.01	25.9	26.0	26.0	26.0	97	97	98	98	7.6	7.6	7.6	7.6
0.03	26.1	26.3	26.3	26.3	99	100	100	100	7.6	7.6	7.6	7.6
0.1	26.3	26.3	26.3	26.4	100	99	98	98	7.6	7.6	7.6	7.6
0.3	26.4	26.4	26.4	26.5	98	97	97	97	7.6	7.6	7.6	7.6
1.0	26.5	26.5	26.5	26.6	98	99	100	99	7.7	7.7	7.7	7.7

Date	14 March, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.0	24.9	25.1	25.2	97	98	98	98	7.6	7.6	7.6	7.6
0.01	25.3	25.3	25.4	25.4	98	99	99	99	7.6	7.6	7.6	7.6
0.03	25.4	25.4	25.5	25.5	100	100	100	100	7.6	7.7	7.7	7.7
0.1	25.6	25.6	25.6	25.5	100	99	99	100	7.7	7.7	7.7	7.7
0.3	25.2	25.2	25.3	25.3	100	100	99	99	7.7	7.7	7.7	7.7
1.0	25.2	25.3	25.3	25.3	100	99	99	99	7.8	7.8	7.8	7.8

Date	16 March, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.3	25.3	25.7	26.0	93	92	88	89	7.9	7.9	7.9	7.9
0.01	26.0	26.0	26.1	26.2	94	95	93	93	7.9	7.9	7.9	7.9
0.03	26.2	26.2	26.2	26.3	95	96	96	97	7.9	7.9	7.9	7.9
0.1	26.1	26.2	26.2	26.1	96	96	97	99	7.9	7.9	7.9	7.9
0.3	26.0	26.0	26.0	26.1	98	97	97	98	7.9	7.9	7.9	7.9
1.0	26.1	26.0	26.1	26.1	100	100	98	98	7.9	7.9	7.9	7.9

Date	16 March, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.7	25.6	25.5	25.4	100	100	100	99	7.7	7.7	7.8	7.8
0.01	25.3	25.2	25.0	24.9	100	100	100	99	7.8	7.8	7.9	7.9
0.03	24.8	24.9	24.8	24.8	99	99	99	99	7.9	7.9	7.9	7.9
0.1	24.9	24.9	24.7	24.7	99	99	100	100	7.9	7.9	8.0	7.9
0.3	24.7	24.6	24.7	24.7	99	100	100	100	7.9	7.9	7.8	7.8
1.0	24.7	24.7	24.6	24.7	100	100	100	100	7.8	7.9	7.8	7.8

Date	18 March, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.1	25.1	25.3	25.4	92	87	85	85	7.5	7.5	7.5	7.5
0.01	25.2	25.3	25.5	25.5	93	91	89	88	7.5	7.5	7.5	7.5
0.03	25.5	25.6	25.6	25.6	87	86	87	87	7.5	7.5	7.5	7.5
0.1	25.6	25.5	25.4	25.3	88	89	95	93	7.6	7.6	7.6	7.6
0.3	25.4	25.4	25.3	25.3	92	91	92	92	7.6	7.6	7.6	7.6
1.0	25.0	25.0	25.0	25.0	91	90	88	89	7.6	7.6	7.6	7.6

Date	18 March, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.4	25.3	25.3	25.3	100	99	98	98	7.7	7.7	7.7	7.7
0.01	25.3	25.3	25.0	25.0	97	97	96	96	7.7	7.7	7.7	7.7
0.03	25.0	24.8	24.8	24.8	96	96	97	97	7.7	7.7	7.7	7.7
0.1	24.8	24.8	24.7	24.6	97	98	99	98	7.7	7.7	7.7	7.7
0.3	24.7	24.7	24.7	24.7	98	99	99	99	7.7	7.7	7.7	7.7
1.0	24.6	24.6	24.6	24.6	99	99	99	99	7.7	7.8	7.8	7.8

Date	21 March, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.6	25.6	25.5	25.5	95	92	90	89	7.6	7.6	7.6	7.6
0.01	25.5	25.5	25.4	25.4	89	88	86	84	7.6	7.6	7.6	7.6
0.03	25.5	25.5	25.5	25.6	84	84	83	81	7.5	7.6	7.5	7.5
0.1	25.6	25.6	25.4	25.1	81	83	94	92	7.5	7.5	7.6	7.6
0.3	25.1	25.0	24.9	24.9	92	91	90	88	7.6	7.6	7.6	7.6
1.0	24.9	24.8	24.9	24.9	90	92	90	91	7.6	7.6	7.6	7.6

Date	21 March, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.3	25.3	25.2	25.2	100	99	99	99	7.7	7.7	7.7	7.7
0.01	25.1	25.0	25.0	25.1	99	99	98	98	7.8	7.8	7.8	7.8
0.03	25.1	25.2	25.2	25.2	99	99	99	99	7.8	7.8	7.8	7.8
0.1	25.2	25.2	25.1	24.9	98	98	99	99	7.8	7.8	7.8	7.8
0.3	24.8	24.9	24.8	24.7	99	99	99	99	7.8	7.8	7.8	7.8
1.0	24.8	24.6	24.6	24.6	99	99	99	99	7.8	7.8	7.8	7.8

Date	23 March, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.4	25.4	25.2	25.1	80	78	88	84	7.6	7.5	7.6	7.6
0.01	25.1	25.2	25.3	25.4	82	79	80	79	7.6	7.5	7.6	7.5
0.03	25.5	25.7	25.7	25.7	81	81	81	82	7.5	7.5	7.5	7.6
0.1	25.7	25.8	25.6	25.3	83	83	89	88	7.6	7.6	7.6	7.6
0.3	25.2	25.1	25.1	25.0	88	88	88	88	7.6	7.6	7.6	7.6
1.0	25.0	24.8	24.9	24.8	89	89	89	89	7.6	7.6	7.6	7.7

Date	23 March, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.0	25.0	25.0	25.0	95	95	96	96	7.9	7.8	7.9	7.9
0.01	25.0	25.1	25.2	25.2	96	95	95	96	7.9	7.8	7.8	7.9
0.03	25.2	25.2	25.1	25.1	96	96	96	96	7.9	7.9	7.9	7.9
0.1	25.1	25.1	25.0	24.7	96	96	100	99	7.9	7.9	7.9	7.9
0.3	24.7	24.7	24.7	24.6	99	98	98	99	7.9	7.9	7.9	8.0
1.0	24.7	24.7	24.6	24.5	99	99	99	99	8.0	8.0	8.0	8.0

Date	25 March, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.7	25.7	25.8	25.8	84	84	85	83	7.5	7.5	7.5	7.5
0.01	25.8	25.8	25.8	25.8	80	80	80	80	7.5	7.5	7.5	7.5
0.03	25.8	25.7	25.7	25.6	81	82	86	84	7.5	7.5	7.5	7.5
0.1	25.6	25.7	25.3	25.1	84	83	91	89	7.5	7.5	7.6	7.6
0.3	25.1	25.0	25.1	25.0	88	86	90	90	7.6	7.6	7.6	7.6
1.0	24.9	24.9	25.0	25.0	87	86	85	84	7.6	7.6	7.6	7.6

Date	25 March, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.6	25.6	25.6	25.5	100	96	96	97	7.8	8.0	8.0	8.0
0.01	25.2	25.2	25.2	25.2	97	96	96	97	8.0	8.0	8.0	8.0
0.03	25.2	25.2	25.2	25.1	98	98	98	97	8.0	8.0	8.0	8.0
0.1	25.2	25.1	25.0	24.9	98	98	99	99	8.0	8.0	8.0	8.0
0.3	24.9	24.8	24.8	24.7	99	99	99	99	8.0	8.0	8.0	8.0
1.0	24.7	24.6	24.6	24.7	99	99	99	99	8.0	8.0	8.0	8.0

Date	28 March, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.5	25.6	25.6	25.5	64	69	70	71	7.4	7.4	7.4	7.4
0.01	25.5	25.5	25.5	25.5	72	74	75	76	7.4	7.4	7.4	7.5
0.03	25.5	25.5	25.6	25.6	76	76	72	73	7.5	7.5	7.4	7.4
0.1	25.5	25.6	25.6	25.6	73	72	71	70	7.4	7.4	7.4	7.4
0.3	25.6	25.6	25.6	25.5	72	73	73	73	7.4	7.4	7.4	7.4
1.0	25.5	25.5	25.5	25.5	72	74	76	77	7.4	7.5	7.5	7.5

Date	28 March, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.4	25.5	25.5	25.4	95	94	94	95	7.9	7.9	7.8	7.9
0.01	25.3	25.2	25.3	25.2	97	96	96	97	7.8	7.9	7.9	7.9
0.03	25.2	25.2	25.2	25.1	97	97	97	97	7.9	7.9	7.9	7.9
0.1	25.2	25.2	24.6	24.6	96	96	99	98	7.9	7.9	7.9	7.9
0.3	24.6	24.5	24.7	24.7	98	98	98	98	7.9	7.9	7.9	7.9
1.0	24.8	24.7	24.7	24.8	98	98	98	99	7.9	8.0	8.0	8.0

Date	30 March, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.7	25.7	25.8	25.7	93	96	92	95	7.8	7.8	7.8	7.8
0.01	25.6	25.4	25.3	25.3	96	96	98	98	7.8	7.8	7.8	7.8
0.03	25.3	25.3	25.4	25.4	97	98	98	98	7.9	7.9	7.9	7.9
0.1	25.4	25.3	25.3	25.3	98	98	97	96	7.9	7.9	7.8	7.8
0.3	25.3	25.3	25.2	25.2	97	95	95	96	7.8	7.8	7.8	7.8
1.0	25.1	25.1	25.2	25.1	97	97	96	96	7.8	7.8	7.8	7.8

Date	30 March, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.7	25.7	25.5	25.4	99	99	99	99	7.9	7.9	7.9	7.9
0.01	25.4	25.3	25.0	25.1	100	100	100	100	7.9	7.9	7.9	7.9
0.03	25.2	25.1	25.1	25.1	100	100	100	99	8.0	7.9	7.9	8.0
0.1	24.9	24.8	24.7	24.6	100	100	100	100	8.0	7.9	8.0	7.9
0.3	24.6	24.5	24.5	24.6	100	100	100	100	8.0	8.0	8.0	8.0
1.0	24.5	24.5	24.6	24.6	100	100	100	100	8.0	8.0	8.0	8.0

Date	1 April, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.7	25.6	25.5	25.5	97	97	97	97	7.8	7.8	7.8	7.9
0.01	25.4	25.5	25.5	25.5	97	97	97	97	7.9	7.9	7.9	7.9
0.03	25.5	25.5	25.6	25.6	98	98	98	98	7.9	7.9	7.9	7.9
0.1	25.5	25.4	25.3	25.3	98	97	99	98	7.9	7.9	7.9	7.9
0.3	25.3	25.2	25.1	25.0	98	98	98	98	7.9	7.9	7.9	7.9
1.0	24.9	25.0	25.0	24.9	98	98	97	98	7.9	7.9	7.9	7.9

Date	1 April, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.5	25.5	25.4	25.3	97	97	98	98	7.9	7.9	7.9	7.9
0.01	25.4	25.3	25.2	25.2	98	98	99	98	7.9	7.9	7.9	7.9
0.03	25.2	25.1	25.0	25.9	98	98	99	99	7.9	7.9	7.9	7.9
0.1	24.8	24.7	24.6	24.6	98	98	99	99	7.9	7.9	7.9	7.9
0.3	24.6	24.6	24.6	24.6	99	99	99	99	7.9	7.9	7.9	7.9
1.0	24.6	24.5	24.5	24.5	99	99	99	99	7.9	8.0	8.0	8.0

Date	4 April, 2022				Remark				Before renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	26.1	26.0	25.9	25.8	99	97	96	96	7.9	7.9	7.9	7.9
0.01	25.6	25.7	25.8	25.9	96	96	94	94	7.9	7.9	7.8	7.8
0.03	26.0	26.0	25.8	26.0	92	92	94	95	7.8	7.8	7.8	7.9
0.1	26.0	26.0	26.0	25.9	96	96	96	95	7.9	7.9	7.9	7.9
0.3	25.6	25.6	25.6	25.6	96	96	96	96	7.9	7.9	7.9	7.9
1.0	25.6	25.7	25.7	25.7	96	95	94	93	7.9	7.9	7.8	7.8

Date	4 April, 2022				Remark				After renewal			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	25.8	25.8	25.7	25.7	99	99	98	98	7.9	7.9	7.9	7.9
0.01	25.5	25.4	25.0	25.1	98	97	99	98	7.9	7.9	7.9	7.9
0.03	25.2	25.1	25.0	25.0	98	99	99	99	7.9	7.9	7.9	7.9
0.1	24.7	24.8	24.8	24.8	100	99	100	99	7.9	7.9	7.9	7.9
0.3	24.8	24.8	24.8	24.9	100	100	100	100	7.9	7.9	7.9	7.9
1.0	24.8	24.8	24.9	24.9	100	100	100	100	7.9	7.9	7.9	8.0

Date	6 April, 2022				Remark				End of test			
Conc. (mg/L)	Temperature (°C)				Oxygen (% sat)				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
Control	26.0	25.8	25.7	25.6	99	98	98	97	7.9	7.9	7.9	7.9
0.01	25.4	25.4	25.3	25.4	94	94	96	96	7.8	7.8	7.8	7.8
0.03	25.5	25.6	25.5	25.5	95	95	96	97	7.8	7.8	7.8	7.9
0.1	25.5	25.7	25.5	25.6	98	98	98	97	7.9	7.9	7.9	7.9
0.3	25.6	25.5	25.5	25.5	98	97	97	97	7.9	7.9	7.9	7.9
1.0	25.5	25.4	25.4	25.3	98	97	97	94	7.9	7.9	7.9	7.8

A.1.3 Statistical analysis

A.1.3.1 Embryonic development, hatching time and survival of embryo and larvae

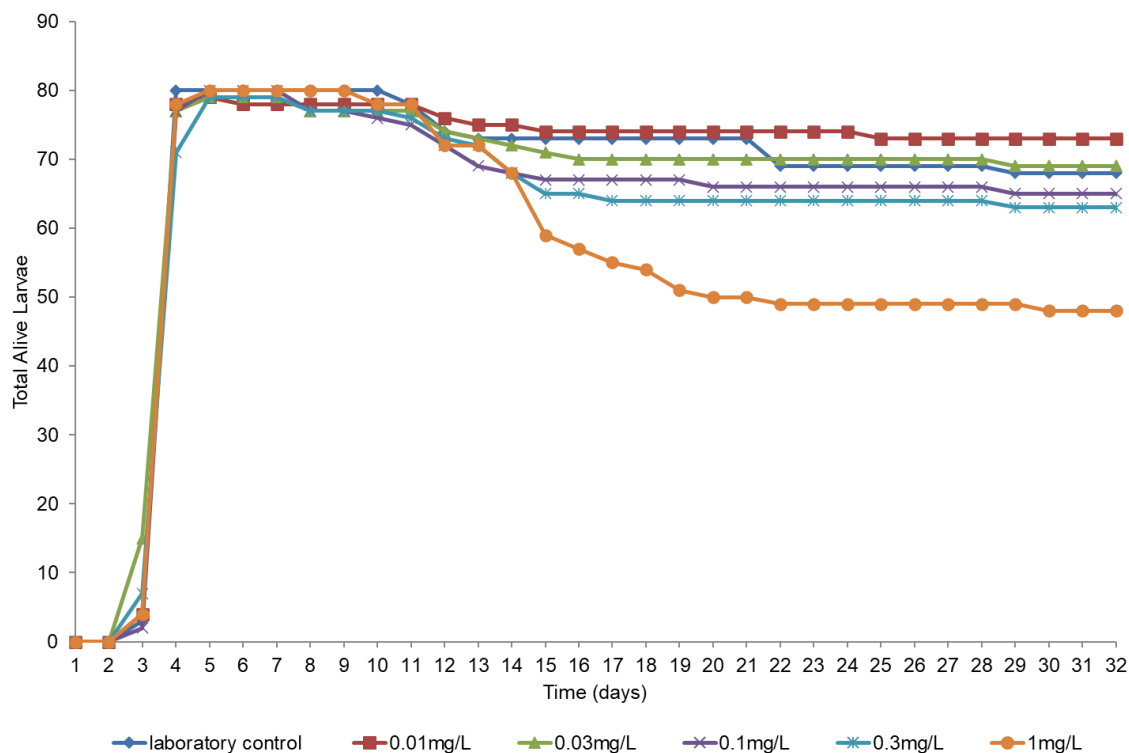


Figure A.1 Embryonic development and number of hatched larvae alive of *Danio rerio* observed against the time with different concentration of the test item.

A.1.3.2 Determination of NOEC and LOEC for the hatching success

Table A.3 Experimental data of the hatching success (%)

Replicate No.	Laboratory control	0.01 mg Citalopram/L	0.03 mg Citalopram/L	0.1 mg Citalopram/L	0.3 mg Citalopram/L	1.0 mg Citalopram/L
1	100	100	100	100	100	100
2	100	95	95	100	100	100
3	100	100	100	100	95	100
4	100	100	100	100	100	100
Count	4	4	4	4	4	4
Mean	100	99	99	100	99	100

- Dunnett's test

Dunnett's test tests the null hypothesis that the averages of each test group are not different from the control group (Table A.4).

Table A.4 Multiple comparisons of means: Dunnett's contrasts.

Mean Comparisons	Estimate	Std. Error	t value	Pr(> t)	Significance ¹⁾
Citalopram-0.01-mg/L vs Citalopram-0-mg/L	-0.01	0.01	-1.00	0.77	-
Citalopram-0.03-mg/L vs Citalopram-0-mg/L	-0.01	0.01	-1.00	0.77	-
Citalopram-0.1-mg/L vs Citalopram-0-mg/L	0.00	0.01	0.00	1.00	-
Citalopram-0.3-mg/L vs Citalopram-0-mg/L	-0.05	0.01	-1.00	0.77	-
Citalopram-1-mg/L vs Citalopram-0-mg/L	0.00	0.01	0.00	1.00	-

1) Signif. Codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ''1 (Adjusted p values reported – single-step method)

None of the exposure groups was significantly different from the control group, and therefore the NOEC for hatching success equals the highest tested concentration. Table A.4

Table A.5 Hatching success endpoint: estimations of NOEC and LOEC, Dunnett's test.

Endpoint	NOEC	LOEC	Unit
Hatching success	1	>1	mg Citalopram/L

A.1.3.3 Results of the Probit analysis

As no difference was observed among the test groups, the data to estimate the ECX on the hatching success were not computed.

Table A.6 Hatching success endpoint, estimations of EC10, EC50, Probit analysis.

Endpoint	EC10	EC50	Unit
Hatching success	>1	>1	mg Citalopram/L

A.1.3.4 Determination of NOEC and LOEC for survival

Table A.7 Experimental data for survival (%)

Replicate No.	Laboratory control	0.01 mg Citalopram/L	0.03 mg Citalopram/L	0.1 mg Citalopram/L	0.3 mg Citalopram/L	1.0 mg Citalopram/L
1	75	90	90	65	70	60
2	100	95	75	80	90	65
3	70	85	95	85	70	45
4	90	95	85	80	65	60
Count	4	4	4	4	4	4
Mean	84	91	86	78	74	58

- Dunnett's test

Dunnett's test tests the null hypothesis that the averages of each test group are not different from the control group (Table A.8).

Table A.8 Multiple comparisons of means: Dunnett's contrasts.

Mean Comparisons	Estimate	Std. Error	t value	Pr(> t)	Significance ¹⁾
Citalopram-0.01-mg/L vs Citalopram-0-mg/L	-0.08	0.07	-1.1	0.71	-
Citalopram-0.03-mg/L vs Citalopram-0-mg/L	-0.03	0.07	-0.37	1.00	-
Citalopram-0.1-mg/L vs Citalopram-0-mg/L	0.06	0.07	0.92	0.82	-
Citalopram-0.3-mg/L vs Citalopram-0-mg/L	0.10	0.07	1.5	0.47	-
Citalopram-1-mg/L vs Citalopram-0-mg/L	0.26	0.07	3.8	0.01	**

1) Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 (Adjusted p values reported - single-step method)

The hypothesis that the mean of any test groups with Citalopram is significantly lower than the laboratory control was verified ($P < 0.05$) at 1 mg Citalopram/L, which means that LOEC (mortality) is 1 mg/L. Table A.4

Table A.9 Mortality: estimations of NOEC and LOEC, Dunnett's test.

Endpoint	NOEC	LOEC	Unit
Mortality	0.3	1	mg Citalopram/L

A.1.3.5 Results of the Probit analysis

The ECx values were estimated by use of the probit analysis.

Table A.10 ECx estimates, Probit analysis with R (W1.3 {drc} /8/ and /9/) of the survival after 32 days.

ECx	Citalopram concentration mg/L	Std. Error	95% confidence interval	
			Lower	Upper
EC05	0.06	0.06	-0.05	0.18
EC10	0.16	0.62	-0.04	0.37
EC25	0.61	0.19	0.24	0.98
EC50	>1	0.77	0.40	>1

Table A.11 Mortality endpoint, estimations of LC10 and LC50

Endpoint	LC10	LC50	Unit
Mortality	0.16 (*-0.37)	>1 (0.40 - *)	mg Citalopram/L

A.1.3.6 Determination of NOEC and LOEC for the body length with Citalopram

Table A.12 Experimental data of the body length (mm) in the laboratory control group and the Citalopram exposure groups.

Replicate No.	Laboratory control	Citalopram 0.01 mg/L	Citalopram 0.03 mg/L	Citalopram 0.1 mg/L	Citalopram 0.3 mg/L	Citalopram 1.0 mg/L
1	12	13	12	9	12	5
2	12	11	11	11	9	7
3	11	10	12	13	13	13
4	13	11	11	13	11	11
5	13	11	9	12	10	14
6	12	12	13	13	11	12
7	9	12	12	10	12	11
8	13	7	13	10	11	10
9	14	11	12	12	12	10
10	11	12	8	12	7	8
11	13	9	11	12	12	9
12	10	13	6	7	11	9
13	12	13	12	12	10	11
14	13	11	12	10	10	12
15	9	14	13	13	5	10
16	11	12	13	10	8	12
17	10	11	13	13	7	12
18	13	13	11	11	12	12

Replicate No.	Laboratory control	Citalopram 0.01 mg/L	Citalopram 0.03 mg/L	Citalopram 0.1 mg/L	Citalopram 0.3 mg/L	Citalopram 1.0 mg/L
19	13	14	12	10	9	11
20	13	11	8	13	10	10
21	11	10	12	9	9	12
22	11	11	13	11	12	8
23	11	11	13	9	11	11
24	10	13	11	11	12	7
25	13	11	13	10	8	5
26	10	11	13	11	8	9
27	12	13	13	12	10	12
28	15	10	9	10	12	14
29	11	12	11	13	10	13
30	11	11	8	8	14	13
31	13	13	10	12	13	12
32	11	12	10	12	13	11
33	12	13	13	12	13	9
34	14	11	12	13	11	5
35	12	8	10	12	13	12
36	14	13	12	9	12	10
37	12	11	9	10	11	14
38	14	12	12	13	10	13
39	13	12	12	13	12	13
40	12	11	11	13	10	10
41	12	10	10	9	10	10
42	11	10	10	11	6	9
43	13	14	11	10	13	12
44	12	13	9	12	9	8
45	8	12	12	9	12	12
46	9	11	7	13	12	5
47	11	11	11	12	13	-
48	8	11	12	13	12	-
49	13	10	12	11	11	-
50	10	14	12	11	11	-
51	11	11	11	11	10	-
52	9	11	14	9	10	-
53	9	13	8	13	13	-
54	11	11	11	13	10	-
55	13	12	13	12	7	-
56	12	12	13	12	9	-
57	14	10	12	9	12	-

Replicate No.	Laboratory control	Citalopram 0.01 mg/L	Citalopram 0.03 mg/L	Citalopram 0.1 mg/L	Citalopram 0.3 mg/L	Citalopram 1.0 mg/L
58	12	13	8	11	12	-
59	10	13	12	10	11	-
60	14	13	12	9	-	-
61	11	6	12	11	-	-
62	13	13	11	11	-	-
63	13	11	13	-	-	-
64	8	11	11	-	-	-
65	12	11	13	-	-	-
66	9	9	9	-	-	-
67	14	12	10	-	-	-
68	-	10	13	-	-	-
69	-	12	14	-	-	-
70	-	11	-	-	-	-
71	-	11	-	-	-	-
Count	67	71	69	62	59	46
Mean	11.7	11.4	11.3	11.1	10.7	10.4

- Dunnett's test

Dunnett's test tests the null hypothesis that the averages of each test groups are not different from the control group (Table A.13).

Table A.13 Multiple comparisons of means: Dunnett's contrasts.

Mean comparisons	Estimate	Std. Error	t value	Pr(> t)	Significance ¹⁾
Citalopram-0.01-mg/L vs Citalopram-0-mg/L	-0.08	0.25	-0.30	0.76	
Citalopram-0.03-mg/L vs Citalopram-0-mg/L	-0.40	0.32	-1.2	0.57	
Citalopram-0.1-mg/L vs Citalopram-0-mg/L	-0.58	0.38	-1.5	0.38	
Citalopram-0.3-mg/L vs Citalopram-0-mg/L	-1.0	0.34	-3.0	0.01	*
Citalopram-1-mg/L vs Citalopram-0-mg/L	-1.3	0.36	-3.5	0.00	**

1) Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 (Adjusted p values reported - single-step method)

The hypothesis that the mean of any test groups with Citalopram is significantly lower than the laboratory control was verified ($P < 0.05$) at 0.3 mg/L and above. Table A.4

Table A.14 Body length endpoint: estimations of NOEC and LOEC, Dunnett's test.

Endpoint	NOEC	LOEC	Unit
Body length	0.1	0.3	mg Citalopram/L

A.1.3.7 Results of the Probit analysis

Table A. 15 ECx estimates, Probit analysis with R (LL.3 {drc} /8/ and /9/) of the body lengths after 32 days.

ECx	Citalopram concentration mg/L	Std. Error	95% confidence interval	
			Lower	Upper
EC10	0.77	0.30	0.17	>1
EC25	>1	-	-	-

Table A. 16 Body length endpoint, estimations of EC10 and EC50.

Endpoint	EC10	EC50	Unit
Body length	0.77 (0.17 - *)	>1	mg Citalopram/L

A.1.3.8 Determination of NOEC and LOEC for the body weight with Citalopram

Table A.17 Experimental data of the body weight (mg) in the laboratory control group and the Citalopram exposure groups.

Replicate No.	Laboratory control	Citalopram 0.01 mg/L	Citalopram 0.03 mg/L	Citalopram 0.1 mg/L	Citalopram 0.3 mg/L	Citalopram 1.0 mg/L
1	19.17	27.58	21.56	9.83	25.14	3.18
2	24.90	18.13	13.92	19.52	9.20	5.81
3	19.83	13.33	22.79	34.60	28.86	25.35
4	24.94	20.81	15.40	25.87	17.08	15.24
5	25.18	18.49	8.22	21.76	15.30	39.54
6	23.34	18.83	18.02	19.40	14.64	18.80
7	10.31	17.46	21.69	14.60	28.55	20.41
8	30.90	3.31	20.58	13.50	17.17	17.23
9	36.74	13.42	16.88	21.41	25.96	18.55
10	16.45	17.85	7.34	27.24	5.76	8.04
11	30.75	6.92	14.14	25.64	21.93	14.91
12	11.70	25.64	0.50	6.58	24.20	13.33
13	16.56	18.99	16.94	28.94	10.27	19.14
14	28.44	13.89	15.74	13.30	14.60	25.20
15	10.55	25.64	18.50	23.68	1.86	11.42
16	15.94	15.63	23.05	14.28	6.74	20.34
17	9.95	15.23	23.11	24.24	6.31	21.99
18	21.91	19.18	11.23	17.94	17.84	24.44
19	23.27	29.82	18.83	11.52	7.20	20.30
20	26.29	16.97	4.72	29.04	16.19	17.95
21	17.44	12.55	21.42	8.63	7.96	18.78

Replicate No.	Laboratory control	Citalopram 0.01 mg/L	Citalopram 0.03 mg/L	Citalopram 0.1 mg/L	Citalopram 0.3 mg/L	Citalopram 1.0 mg/L
22	15.76	17.41	17.96	17.36	19.27	7.96
23	15.67	16.48	27.60	8.92	18.40	16.32
24	8.97	23.74	17.82	13.15	20.99	7.52
25	20.88	15.21	27.74	11.28	5.20	2.21
26	10.09	15.67	28.19	14.65	6.69	13.79
27	20.06	20.70	30.15	19.24	14.82	23.24
28	34.70	10.64	10.95	12.62	24.38	30.92
29	18.23	20.21	15.57	28.03	11.81	26.44
30	15.37	15.78	6.99	8.36	31.10	31.14
31	24.03	26.56	12.99	17.55	23.85	21.18
32	15.16	20.24	13.24	21.20	22.58	1.17
33	16.04	20.60	23.72	20.60	29.04	8.19
34	28.29	12.90	19.67	28.87	19.04	16.27
35	14.41	5.68	12.19	26.09	25.08	26.23
36	29.90	23.74	16.89	12.48	20.66	11.52
37	22.13	19.86	9.01	9.73	18.18	26.28
38	29.38	18.22	21.24	23.31	12.93	23.66
39	30.62	17.23	19.23	23.94	20.44	28.20
40	22.48	17.69	19.40	21.07	11.56	21.77
41	19.59	12.48	15.18	9.83	11.46	15.50
42	16.76	8.00	11.96	14.18	4.61	15.27
43	28.29	29.73	15.85	13.47	21.24	20.62
44	30.45	17.71	9.68	11.05	7.10	5.77
45	6.98	18.89	22.07	19.05	20.53	16.40
46	9.40	11.87	3.62	7.58	22.82	3.00
47	15.37	17.80	10.87	24.63	27.14	-
48	9.24	13.17	24.72	24.16	18.41	-
49	31.36	14.12	21.26	15.62	15.08	-
50	13.78	31.60	20.29	16.52	15.46	-
51	14.22	17.07	15.85	14.78	14.36	-
52	8.46	14.21	21.94	10.03	11.11	-
53	9.50	18.75	9.93	25.92	24.82	-
54	16.50	12.65	16.65	22.89	11.43	-
55	22.18	19.29	21.32	19.02	7.20	-
56	19.73	21.71	25.70	19.52	11.55	-
57	30.94	10.29	15.04	19.20	22.99	-
58	19.37	28.96	3.24	10.05	19.32	-
59	11.40	26.48	19.35	17.01	11.42	-
60	33.35	31.38	22.50	11.15	-	-

Replicate No.	Laboratory control	Citalopram 0.01 mg/L	Citalopram 0.03 mg/L	Citalopram 0.1 mg/L	Citalopram 0.3 mg/L	Citalopram 1.0 mg/L
61	15.44	2.08	16.39	8.99	-	-
62	24.82	19.70	24.58	16.91	-	-
63	23.63	13.35	12.88	-	-	-
64	7.53	13.87	26.24	-	-	-
65	17.91	17.45	15.09	-	-	-
66	10.45	9.81	25.00	-	-	-
67	28.73	19.90	9.54	-	-	-
68	-	13.31	11.99	-	-	-
69	-	15.04	20.52	-	-	-
70	-	17.18	-	-	-	-
71	-	10.74	-	-	-	-
Count	67	71	69	62	59	46
Mean	19.88	17.42	17.02	17.77	16.56	17.40

Dunnett's test tests the null hypothesis that the averages of each test groups are not different from the control group (Table A.13Table A.17).

Table A.18 Multiple comparisons of means: Dunnett's contrasts.

Mean comparisons	Estimate	Std. Error	t value	Pr(> t)	Significance ¹⁾
Citalopram-0.01-mg/L vs Citalopram-0-mg/L	-0.13	0.09	-1.5	0.46	-
Citalopram-0.03-mg/L vs Citalopram-0-mg/L	-0.19	0.09	-2.1	0.13	-
Citalopram-0.1-mg/L vs Citalopram-0-mg/L	-0.10	0.09	-1.1	0.71	-
Citalopram-0.3-mg/L vs Citalopram-0-mg/L	-0.23	0.10	-2.4	0.07	.
Citalopram-1-mg/L vs Citalopram-0-mg/L	-0.24	0.10	-2.3	0.08	.

1) Signif. codes: 0 '****' 0.001 '***' 0.01 '**' 0.05 '.' 0.1 ' ' 1 (Adjusted p values reported - single-step method)

None of the exposure groups was significantly different from the control group. Table A.4

Table A.19 Body weight endpoint: estimations of NOEC and LOEC, Dunnett's test.

Endpoint	NOEC	LOEC	Unit
Body weight	1	>1	mg Citalopram/L

A.1.3.9 Results of the Probit analysis

As no difference was observed among the test groups, the data to estimate the ECX on the body weight were not computed.

Table A.20 Body weight endpoint, estimations of EC10 and EC50.

Endpoint	EC10	EC50	Unit
Body weight	>1	>1	mg Citalopram/L

A.1.4 Validity criteria

Table A. 21 Validity criteria of the test according to the OECD 210 /1/ in the development test with *Danio rerio*.

Criteria	Target Value	Fulfilled
Survival of hatched larvae in the control group	> 75% (observed: 84%)	Yes
Hatching success in the control group	> 70% (observed: 100%)	Yes
Dissolved oxygen concentration	> 60% (minimum observed: 64%)	Yes
Water temperature and maximum difference at any time	26 °C ± 1.5 °C (Observed average: 25.4 ± 0.4 °C)	Yes

APPENDIX B– Chemical analysis

Results from chemical analyses

B Results from chemical analyses

Sample ID Lab	Sample ID	Citalopram ug/L
Zebrafish test (Method OECD TG 210)		
22-33-7	DHI Zebrafish OECD 210 11827338 ÖT-22-1276 0,01 mg/l A T=28 New 01-04-2022/MAL	10,9
22-33-8	DHI Zebrafish OECD 210 11827338 ÖT-22-1276 1 mg/l A T=28 New 01-04-2022/MAL	1305,8
22-33-9	DHI Zebrafish OECD 210 11827338 ÖT-22-1276 0,01 mg/l A T=31 Old 04-04-2022/MAL	11,9
22-33-10	DHI Zebrafish OECD 210 11827338 ÖT-22-1276 1 mg/l A T=31 Old 04-04-2022/MAL	1309,5
	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.

APPENDIX C – CoA and MSDS

Certificate of Analysis (CoA) and Material Safety Data Sheet
(MSDS)

C CoA and MSDS



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Certificate of Analysis

Product Name and Code No. (including Salt Form): Citalopram Hydrobromide Lu 10-171-B			Citalopram, HBr Lu 10-171-B Batch: 2001976 4g Store at RT, protected from light. Expiry date: 01-Mar-2025 Sample defrosted: 01-Mar-2022/Tini H. Lundbeck A/S, Copenhagen-DK
Batch No.: 2001976	Analysis No.: 559901	Date of analysis: 30-Apr-2020	
Date of manufacture: 14-may-2003	Special storage conditions: -		

Test:	Acceptance criteria:	Results:
Description	White to off-white crystalline material	*White power
Identification		
NMR-spectrum	Conforms to structure	Conforms to structure
NIR	Conforms to standard	Conforms to standard
FT-IR spectrum	Conforms to standard	Conforms to standard
Residue on ignition, %	Max. 0.1	0.0
Heavy metals, ppm	< 20	< 20
Purity, HPLC, w/w %, 239 nm		*99.9
Impurities, HPLC, w/w %, 239 nm		
Lu 18-027	Max. 0.1	*<LOQ
Lu 18-022	Max. 0.2	*<0.02
Lu 11-305	Max. 0.1	*<LOQ
Lu 14-017	Max. 0.1	*<LOQ
Lu 29-075	Max. 0.1	*<0.02
Lu 29-215	Max. 0.1	*<LOQ
Lu 11-109	Max. 0.1	*<0.01
Unknown each	Max. 0.10	*0.03
Impurities in total	Max. 0.5	*0.08
Residual solvents, %		
Acetone	Max. 0.15	0.056
Hexan	Max. 0.06	0.015
Methanol	Max. 0.05	< LOQ
2-Propanol	Max. 0.05	< LOQ
Heptan	Max. 0.1	< LOQ
Total	Max. 0.2	0.1
Water content, KF, % w/w →150°	Max. 0.5	*<0.1
Melting point, DSC, °C		
Onset:		186.2
Peak max.		188.3

Drug Substance
 Short Title: Lu 10-171-B
 Date: 19-May-2020

Certificate of Analysis - Reference Materials

Status: Final

CMC_007654

v. 6.0

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Test:	Acceptance criteria:	Results:
Elemental analysis (CHN), %	Theoretical:	
Carbon:	59.27	59.36
Hydrogen:	5.47	5.50
Nitrogen	6.91	6.88
Potency, %:		*99.8 (100.0-0.08-0.1-<0.1-0.0)
Comments	*Reanalysis is performed	

This Certificate of Analysis has been produced and signed electronically and is valid without a signature.

Prepared by: Corporate Product Quality Control

Controlled by: Corporate Product Quality Control

Drug Substance
Short Title: Lu 10-171-B
Date: 19-May-2020

Certificate of Analysis - Reference Materials

Status: Final

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Safety data sheet
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version 4

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SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifier

Trade name:	Citalopram.HBr
Synonyms	1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrilmonohydrobromid Lu 10-171-B
SDS number:	88
CAS Number:	59729-32-7
EC number:	261-890-6

1.2 Relevant identified uses of the substance or mixture and uses advised against

Sector of Use	SU3 Industrial uses: Uses of substances as such or in preparations at industrial sites
---------------	--

Application of the substance / the mixture	Active substance
--	------------------

1.3 Details of the supplier of the safety data sheet

Manufacturer/Supplier:	H. Lundbeck A/S Ottiliavej 9 DK-2500 Valby Tel.: +45 36 30 13 11
------------------------	---

Further information obtainable from:	This SDS is created in Lundbeck-layout by the department for Health, Safety & Environment (Corp. HS&E). For further information please contact corp.hse@lundbeck.com. Edition number: 12
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1.4 Emergency telephone number:	+45 36301311 (Opening hours Monday to Friday 9 am - 4 pm (DK time)) +45 36301311 (Opening hours Monday to Friday 9 am - 4 pm (DK time))
--	--

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008	
Acute Tox. 4	H302 Harmful if swallowed.
Skin Sens. 1	H317 May cause an allergic skin reaction.
Aquatic Chronic 1	H410 Very toxic to aquatic life with long lasting effects.

2.2 Label elements

Labelling according to Regulation (EC) No 1272/2008	The substance is classified and labelled according to the CLP regulation.
---	---

Hazard pictograms



GHS07 GHS09

Signal word

Warning

Hazard statements

H302 Harmful if swallowed.
 H317 May cause an allergic skin reaction.
 H410 Very toxic to aquatic life with long lasting effects.

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Trade name: Citalopram.HBr

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Precautionary statements

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
P273 Avoid release to the environment.
P280 Wear protective gloves.
P301+P312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.
P330 Rinse mouth.
P501 Dispose of contents/container in accordance with local regulations.

2.3 Other hazards

The substance is a solid. Dust may form explosive mixtures with air.
Citalopram hydrobromide is the active substance of antidepressant drugs with a normal daily dosage of 20 to max. 60 mg, with reduced dosage to elderly and to persons with reduced liver or kidney functions.

Results of PBT and vPvB assessment

PBT: Not applicable.
vPvB: Not applicable.

SECTION 3: Composition/information on ingredients

3.1 Substances

CAS No. Description 59729-32-7 Citalopram.HBr
Identification number(s)
EC number: 261-890-6

SECTION 4: First aid measures

4.1 Description of first aid measures

General information: When seeking medical attention, show this material safety data sheet or the packing with the label.
After inhalation: Immediately remove patient from exposure and move to fresh air. Rinse mouth and nose with water. Obtain medical attention if irritation occurs.
After skin contact: Remove contaminated clothing. Wash affected area with plenty of water. Obtain medical attention if irritation persists.
After eye contact: Remove contact lenses if any. Immediately rinse affected eye(s) with water for up to 15 minutes. Obtain medical attention if irritation/discomfort persists. Continue to rinse with water during transport.
After swallowing: Flush mouth with water and give milk or water to conscious person. Try to induce vomiting. Obtain medical attention. In case of unconsciousness provide artificial respiration.

4.2 Most important symptoms and effects, both acute and delayed

No further relevant information available.

4.3 Indication of any immediate medical attention and special treatment needed

No further relevant information available.

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SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing agents: Water, dry chemical, CO₂ or foam.

5.2 Special hazards arising from the substance or mixture

Formation of toxic and corrosive gases is possible during heating or in case of fire (CO_x, NO_x, HF, HBr).
 Avoid inhalation of fumes. Persons who have inhaled fumes must obtain medical attention (risk of delayed lung oedema).
 The substance is combustible and the dust may form explosive mixtures with air.

5.3 Advice for firefighters

Protective equipment: A self-contained breathing apparatus and full protective clothing should be worn when fighting the fire.

Additional information: Evacuate area.
 Remove substance/product from fire threatened area, if possible.
 Stop leakage. Water from extinction of fires may be hazardous waste. Must not enter sewage or water systems.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Use respirator, protective gloves and goggles/face shield during clean-up.
 Avoid contact with skin and eyes and inhalation of dust.

6.2 Environmental precautions:

Do not allow product to reach sewage system or any water course.

6.3 Methods and material for containment and cleaning up:

Small release: Wipe up spillages with moist cloth or paper.
 Major release:
 Pick up mechanically and dispose of according to regulation on hazardous waste.
 Dust generation may be reduced by carefully moistening or by covering with plastic film.
 Wash the spillage area with plenty of water, and prevent entry into drains.

6.4 Reference to other sections

See Section 7 for information on safe handling.
 See Section 8 for information on personal protection equipment.
 See Section 13 for disposal information.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Persons, who handle this material, should be familiar with the hazards of the material and the necessary safety precautions, including this safety data sheet.
 The substance should preferably be used in closed systems and handled under well-ventilated conditions.
 Wash hands after work, before eating and after using the bathroom.
 Immediately remove contaminated clothes.
 Avoid inhalation of dust. Avoid contact with skin and eyes. Avoid prolonged and/or repeated exposure.

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Information about fire - and explosion protection:

Prevent formation of dust.

If hazardous, explosive atmospheres may form, the work area must be classified and organised according to the regulations on hazardous, explosive atmosphere. All work must be carried out in compliance with these regulations.
Dust can combine with air to form an explosive mixture (dust explosion).

7.2 Conditions for safe storage, including any incompatibilities

Storage:

General requirements:

Store in a well-closed container.
Must be stored securely and separate from food, feedstuff, medicine and the like.
Store separated from substances that may cause hazardous reactions (see section 10).

7.3 Specific end use(s)

No further relevant information available.

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with limit values that require monitoring at the workplace:

No occupational exposure limits were found.

8.2 Exposure controls

Appropriate engineering controls

For further information - see item 7 (Information for safe handling).

Individual protection measures, such as personal protective equipment

Respiratory protection:

In case of insufficient ventilation, use a self-contained breathing apparatus or mask (with filter P2). The filters have a limited usage time and should be changed regularly.

Hand protection

Use gloves e.g. of the type: nitrile or butyl rubber.

Eye/face protection

Use safety glasses/goggles or face shield at the risk of eye contact.

Body protection:

Use protective clothing at the risk of skin contact.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

General Information

Physical state

Solid

Colour:

White to off-white

Odour:

Weak

Odour threshold:

Not determined

Melting point/freezing point:

185-188 °C

Boiling point or initial boiling point and boiling range

Not available

Flammability

Not determined

Lower and upper explosion limit

Not determined

Lower:

Not determined

Upper:

Not determined

Flash point:

Not available

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Auto-ignition temperature:	Not determined.
Decomposition temperature:	Not determined
pH	Not applicable
Viscosity:	
Kinematic viscosity	Not applicable
Dynamic:	Not applicable
Solubility	30 mg/ml (water), 50 mg/ml (ethanol), 270 mg/ml (chloroform)
Water:	Not determined
Partition coefficient n-octanol/water (log value)	Not determined
Vapour pressure:	Not applicable
Density and/or relative density	
Density:	Not determined.
Relative density	Not determined
Vapour density	Not applicable

9.2 Other information

Appearance:	
Form:	Crystalline
Auto-ignition temperature:	Not determined
Explosive properties:	High concentrations of dust increases the risk of dust explosion.
Change in condition	
Softening point/range	
Oxidising properties	Not determined
Evaporation rate	Not applicable

Information with regard to physical hazard classes

Explosives	None
Flammable gases	None
Aerosols	None
Oxidising gases	None
Gases under pressure	None
Flammable liquids	None
Flammable solids	None
Self-reactive substances and mixtures	None
Pyrophoric liquids	None
Pyrophoric solids	None
Self-heating substances and mixtures	None
Substances and mixtures, which emit flammable gases in contact with water	None
Oxidising liquids	None

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Oxidising solids	None
Organic peroxides	None
Corrosive to metals	None
Desensitised explosives	None

SECTION 10: Stability and reactivity

10.1 Reactivity	No further relevant information available.
10.2 Chemical stability	Stable at normal temperature and pressure.
10.3 Possibility of hazardous reactions	The substance is expected to be combustible. Dust may form explosive mixtures with air.
10.4 Conditions to avoid	No further relevant information available.
10.5 Incompatible materials:	No further relevant information available.
10.6 Hazardous decomposition products:	Formation of toxic and corrosive gases is possible during heating or in case of fire (COx, NOx, HF, HBr).

SECTION 11: Toxicological information

11.1 Information on hazard classes as defined in Regulation (EC) No 1272/2008

Acute toxicity	The substance can be absorbed by inhalation, skin contact and ingestion. The substance can be absorbed by inhalation and ingestion. Pharmacological effects occur at an exposure far lower than acute toxicity effects. Harmful if swallowed.
----------------	--

Acute toxicity - data

Oral	LDlow	9.8 mg/kg (Rat)
	LD50	900-1,710 mg/kg (Rat)

Symptoms of exposure:	Nausea, hand tremor, disturbed sleep, dizziness, lethargy, loss of consciousness.
Skin corrosion/irritation	Based on available data, the classification criteria are not met.
Serious eye damage/irritation	Based on available data, the classification criteria are not met.
Respiratory or skin sensitisation	May cause an allergic skin reaction.

11.2 Additional toxicological information:

Information:	Persons under treatment with or exposed to isocaboxazide should not be exposed
IARC (International Agency for Research on Cancer)	Substance is not listed.

Sensibilisation - data

Sensitisation	Skin sensitization	1.2-8.9 - (Mouse) (OECD 429)
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Endocrine disrupting properties

Substance is not listed.

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SECTION 12: Ecological information

12.1 Toxicity Very toxic to aquatic life with long lasting effects.

EC50	5.19 mg/l (Alga) (Algae toxicity, OECD 201 (72h))
NOEC	0.921 mg/l (Alga) (Algae toxicity, OECD 201 (72h))
	0.105 mg/l (Daphnia) (Daphnia toxicity, OECD 211 (21 days))
	0.0268 mg/l (Fish) (Fish toxicity, OECD 210 (28 days))

12.2 Persistence and degradability The substance is not readily biodegradable.
Not determined. Not to be released to the environment.

biodegradability (-) (Biodegradability (OECD 301))

12.3 Bioaccumulative potential Log Kow < 3, indicates that the substance does not bioaccumulate.
Log Kow 1 (-) (Partition Coefficient, shake flask method)

12.4 Mobility in soil No further relevant information available.

12.5 Results of PBT and vPvB assessment

PBT: Not applicable.
vPvB: Not applicable.

12.6 Endocrine disrupting properties For information on endocrine disrupting properties see section 11.

12.7 Other adverse effects

Bioaccumulation potential:
General notes:

Water hazard class 3 (German Regulation) (Self-assessment):
extremely hazardous for water
Do not allow product to reach ground water, water course or
sewage system, even in small quantities.
Danger to drinking water if even extremely small quantities leak
into the ground.

SECTION 13: Disposal considerations

13.1 Waste treatment methods The product should be disposed of according to local regulations.

SECTION 14: Transport information

14.1 UN number or ID number ADR, IMDG, IATA	UN3077
14.2 UN proper shipping name ADR IMDG, IATA	3077 ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (Citalopram.HBr) ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (Citalopram.HBr)
14.3 Transport hazard class(es) ADR, IMDG, IATA Class Label	- 9 Miscellaneous dangerous substances and articles. 9

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14.4 Packing group ADR, IMDG, IATA	III
14.5 Environmental hazards:	Environmentally hazardous substance, solid
14.6 Special precautions for user Hazard identification number (Kemler code): EMS Number: Stowage Category Stowage Code	Warning: Miscellaneous dangerous substances and articles. 90 F-A,S-A A SW23 When transported in BK3 bulk container, see 7.6.2.12 and 7.7.3.9.
14.7 Maritime transport in bulk according to IMO instruments	Not applicable.
Transport/Additional information:	
ADR Limited quantities (LQ) Excepted quantities (EQ) Transport category Tunnel restriction code	5 kg Code: E1 Maximum net quantity per inner packaging: 30 g Maximum net quantity per outer packaging: 1000 g 3 E
IMDG Limited quantities (LQ) Excepted quantities (EQ)	5 kg Code: E1 Maximum net quantity per inner packaging: 30 g Maximum net quantity per outer packaging: 1000 g
UN "Model Regulation":	UN 3077 ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (CITALOPRAM.HBR), 9, III

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

	This safety data sheet has been prepared according to Regulation (EU) no. 1907/2006.
Australian Inventory of Industrial Chemicals	Substance is not listed.
Directive 2012/18/EU	
Named dangerous substances - ANNEX I	Substance is not listed.
Seveso category	E1 Hazardous to the Aquatic Environment
Qualifying quantity (tonnes) for the application of lower-tier requirements	100 t
Qualifying quantity (tonnes) for the application of upper-tier requirements	200 t
National regulations:	
Information about limitation of use:	Not available

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15.2 Chemical safety assessment: A Chemical Safety Assessment has not been carried out.

SECTION 16: Other information

This information is based on our present knowledge of the substance/product.

Department issuing SDS: Corp. HS&E

Abbreviations and acronyms: ADR: Accord relatif au transport international des marchandises dangereuses par route (European Agreement Concerning the International Carriage of Dangerous Goods by Road)
 IMDG: International Maritime Code for Dangerous Goods
 IATA: International Air Transport Association
 GHS: Globally Harmonised System of Classification and Labelling of Chemicals
 EINECS: European Inventory of Existing Commercial Chemical Substances
 CAS: Chemical Abstracts Service (division of the American Chemical Society)
 LC50: Lethal concentration, 50 percent
 LD50: Lethal dose, 50 percent
 PBT: Persistent, Bioaccumulative and Toxic
 vPvB: very Persistent and very Bioaccumulative
 Acute Tox. 4: Acute toxicity – Category 4
 Skin Sens. 1: Skin sensitisation – Category 1
 Aquatic Chronic 1: Hazardous to the aquatic environment - long-term aquatic hazard – Category 1

Sources Investigators Brochure report for Citalopram, January 2000.

Data compared to the previous version altered. See * marking.

GB

APPENDIX D

Accreditation Certificate

D Accreditation Certificate



ACCREDITATION

 for testing

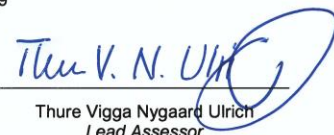
Reg. No 26

Company	DHI A/S Agern Allé 5, DK-2970 Hørsholm CVR: 36466871 DHI Maritime Technology Evaluation Facility Færgevejen 18 DK-3390 Hundested
Scope of accreditation	• Testing, as specified in annex 1
Validity	24-06-2019 to 31-07-2023
Replaces document of	02-07-2015
Basis for accreditation	DS/EN ISO/IEC 17025:2017 The laboratory complies with the requirements in DS/EN ISO/IEC 17025:2017 - General requirements for the competence of testing and calibration laboratories, together with the relevant provisions as an accredited body for the above mentioned scope of accreditation.

24-06-2019



 Jesper Hey
 Director



 Thure Vigga Nygaard Ulrich
 Lead Assessor

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Annex 1 to accreditation document of 24-06-2019**Testing**

Accreditation for testing in compliance with requirements in DS/EN ISO/IEC 17025:2017.

Detailed list of methods is available via

http://english.danak.dk/English/database_eng/testeng/

Fields of Testing

Biological and biochemical testing

Microbiological testing

Ionising radiation and radiochemistry

Sampling