

Effect of Sodium Isobutyl Xanthate (SIBX) on the early life stages of the marine fish, sheepshead minnow (*Cyprinodon variegatus*) and the marine copepod *Acartia tonsa*.

24.02.2020

Final report

This report has been prepared under the DHI Business Management System
certified by Bureau Veritas to comply with ISO 9001 (Quality Management)



Approved by

24-02-2020

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APPENDICES

APPENDIX A

Raw data: Early-life stage test with *Acartia tonsa*

APPENDIX B

Raw data: Fish, early-life stage toxicity test

1 Study objective

The objective of the two studies was to investigate the effect of Sodium Isobutyl Xanthate (SIBX) on the early-life stages of the marine copepod *Acartia tonsa* and the marine fish, sheepshead minnow (*Cyprinodon variegatus*).

The studies were performed in accordance with the procedures in the ISO Standard ISO 16778 "Water quality - Calanoid copepod early-life stage test with *Acartia tonsa*" /1/ and the OECD Guideline for the Testing of Chemicals No. 210 "Fish, Early-life Stage Toxicity Test" /2/.

2 Test item

The test item was Sodium Isobutyl Xanthate (SIBX) provided by Nordic Rutile.

The following information on the test item was received from Principal Consultant Marte Rusten (DNV-GL) on behalf of Nordic Rutile:

Trade name:	Xanthates
Chemical name:	Sodium O-isobutyl dithiocarbonate
CAS No. active ingredient:	25306-75-6
Molecular formula:	C ₅ H ₉ NaOS ₂
Molecular weight:	172 g/mol
Purity:	Unknown
Form:	Yellowish solid, powder
Water solubility at 20-25 °C:	0.66-510 g/L
Log P _{ow} at 20°C:	-1.33
Density:	1.24 (relative at 20°C)
Melting point:	202.59°C
Boiling point:	478.58°C
Stability in water (half-life by hydrolysis at 25°C):	10.833 days
Origin:	LightDeepEarth, South Africa

The test item had room temperature when received at DHI on 14. November 2019 and was stored in refrigerator until use. Unused test item will be returned to the client.

3 Client

Nordic Rutile
c/o Nordic Mining ASA
Vika Atrium
Munkedamsveien 45
N-0250 Oslo
Norway

Att.: Mona Schanche

4 Test facility

DHI A/S, Agern Allé 5, DK-2970 Hørsholm, Denmark. Project Manager Anja Kamper (M.Sc.).

5 Test principle

5.1 *Acartia tonsa*, early-life stage test

Filtered sea water (0.22 µm) collected in the North Sea with a salinity of approx. 32 PSU was used in the test as control and dilution media.

The test was initiated in 250-mL Pyrex beakers with 40 mL of test solution. After 3 days 40 ml freshly prepared test solution was added to the beakers. The marine algae *Rhodomonas salina* was both days added to a concentration of 50,000 cells/mL as food.

The test consisted of 12 replicates of the control group without any test item and 6 replicates of each of 5 concentrations of the test item. The test item was tested at the following concentrations: 0.032; 0.1; 0.32; 1.0 and 3.2 mg/L.

A stock solution of 60 mg/L was prepared by weighing out the test item and dispersing it in filtered sea water. The pH of the stock solution was within the range of 8.0 ± 0.2 and was therefore not adjusted. The stock solution was diluted in filtered sea water to obtain the desired test concentrations. The entire procedure was repeated after 3 days when fresh test solutions is added.

At the initiation of the test the number of eggs added to each beaker, 60-90 eggs, and the time were recorded. The test were carried out at $19.4 \pm 0.1^\circ\text{C}$ with a light:dark cycle of 16:8 hours. Temperature was measured continuously by thermologger placed in a separate beaker. The pH, salinity and dissolved oxygen was measured in each test concentration at the initiation of the test, before and after addition of new test solutions and at the termination of the test.

The test was terminated after 6 days when an average of 40-80% of the total control animals present had reached a copepodite stage, a larval development ration (LDR) of 0.4-0.8. The number of unhatched eggs, nauplii and copepodites was counted in each test beaker after fixation with Lugols solution, in the same time interval as the eggs were added, and subsequent filtration.

5.2 Fish, early-life stage test

Filtered sea water (0.22 µm) collected in the North Sea with a salinity of approx. 32 PSU were used in the test as control and dilution media.

The test was initiated in 180-mL Pyrex crystallizing dishes with approximately 100 mL of test solution. From Day 8 and onwards the test was conducted in 2-L glass containers with 1.5 L test solution.

The test item was tested at the following concentrations: 0.10; 0.32; 1.0; 3.2 and 10 mg/L and a control without test item.

Stock solutions of 50 or 100 mg/L was prepared by weighing out the test item and dispersing it in filtered sea water. The pH of the stock solution was within the range of 8.0 ± 0.2 and was therefore not adjusted. The stock solution was applied to obtain the desired test concentrations by serial dilutions in filtered sea water. The entire procedure was repeated at test solution renewal three times per week.

At Day 5 more than 50% of the eggs in the control group were hatched and therefore Day 5 was defined as the hatching day and exposure was continued for 27 days post-hatch. Resulting in a total exposure period of 32 days.

The sheepshead minnow eggs were deposited at a commercial supplier at a salinity of approx. 28 PSU four days before the test was started. 80 fertilized eggs, divided equally with 20 eggs in each of four replicate containers, were used per concentration and for the control group. The test was performed as a semi-static test with renewal of all test solutions three times per week, after which surviving eggs, larvae and fish were gently transferred to the new vessels in a small volume of old solution, avoiding exposure to air.

From Day 8 organisms were retained in the test vessels whilst a proportion (at least two thirds) of the test solutions / control volumes were changed. pH, salinity, temperature and dissolved oxygen concentrations were measured in each test container at the initiation of the test, before and after renewal and at the termination of the test. Temperature was also measured continuously by thermologger placed in a separate container with control media and no eggs.

The test was carried out at $24.6 \pm 0.4^{\circ}\text{C}$ with a light:dark cycle of 14:10 hours.

Feeding was initiated at Day 6 and continued on a daily basis throughout the study (3 times during weekdays and once a day during weekends). Food, hatched brine shrimp nauplii (*Artemia*), was provided daily together with flake food. Surplus food and faeces were removed as necessary, to avoid accumulation of waste. Dead eggs, larvae and fish were removed immediately from the test containers.

Observations were conducted on a daily basis and included: stage of embryonic development, hatching and survival, abnormal appearance and behaviour and weight and length of the fish at the end of the test.

At the termination of the exposure, the fish were euthanised with Ethomidate and their weight (wet weight, blotted dry) and length measured. In-extremis i.e. where deformities and associated abnormal behaviour were considered so severe that there was considerable suffering to the organism, and it had reached a point beyond which it would not recover, it was removed from the test. Such animals were euthanised applying Ethomidate and treated as mortalities for subsequent data analysis.

6 Results

All primary data is found in Appendixes A and B.

6.1 Calanoid copepod early-life stage test with *Acartia tonsa*

The primary data from the early-life stage toxicity test with *Acartia tonsa* are presented in Appendix A, and the results are summarized in Table 6.1. All validity criteria prescribed in the ISO 16778 standard were fulfilled.

Furthermore, the biological results are presented as box-and-whisker plots for the endpoint hatching, survival and larval development ratio in Figures 6.1-6.3. 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.

Table 6.1 Results of the *Acartia tonsa* early-life stage test after exposure to the SIBX.

Endpoint	NOEC (mg/L)	LOEC (mg/L)	EC/LC10 (mg/L)	EC/LC50 (mg/L)
Hatching success	3.2	>3.2	>3.2	>3.2
Early-life stage mortality	0.32	1.0	0.34 (0.27 - 0.41)	0.58 (0.49 - 0.68)
Larval development ratio (LDR)	0.32	1.0	0.36 (0.23 - 0.50)	0.63 (0.50 - 0.62)

The early-life stage toxicity test with *Acartia tonsa* showed no significant effects of the test item on the endpoint hatching success (Table 6.2). The NOEC were, therefore, equivalent to the highest tested concentration of 3.2 mg/L, whereas the LOEC were estimated above 3.2 mg/L.

As no significant differences were observed, the data were not computed to establish a dose-response curve for the hatching success. The EC10 and EC50 for the endpoint hatching success were therefore estimated to be higher than 3.2 mg/L.

Table 6.2 Hatching success of *Acartia tonsa* after exposure to SIBX.

Concentration (mg/L)	Hatching success mean (%)	Standard deviation
Control	98.3	1.6
0.032	98.4	1.6
0.1	98.1	1.1
0.32	99.4	0.7
1.0	98.4	1.0
3.2	97.1	1.0

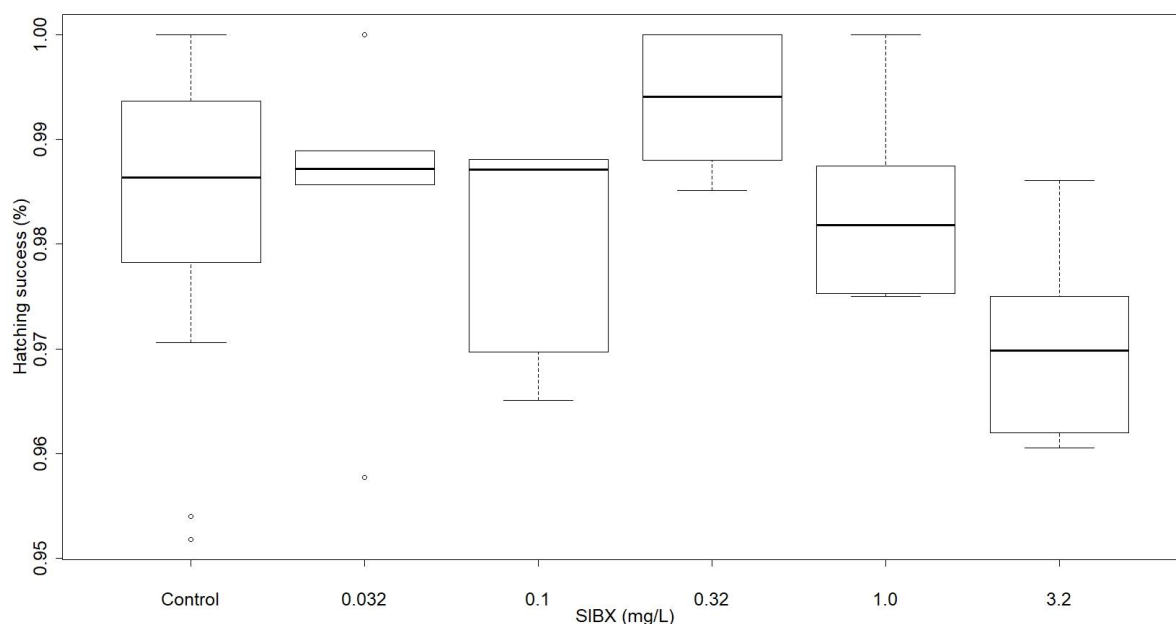


Figure 6.1 Hatching success in LDR test with SIBX. A hatching success of 1.0 corresponds to 100% hatching.

The early-life stage toxicity test with *Acartia tonsa* showed significant effects of the test item on the endpoints early-life stage mortality and larval development ratio at the two highest tested concentrations (Table 6.3 and Table 6.4). The NOEC were, therefore, 0.32 mg/L, whereas the LOEC were estimated to 1.0 mg/L.

The EC10 and EC50 was calculated for the early-life stage mortality and larval development ratio (Table 6.1).

Table 6.3 Mortality of early-life stages of *Acartia tonsa* after exposure to SIBX.

Concentration (mg/L)	Early Life Stage mortality mean	Standard deviation
0	16.3	9.4
0.032	24.8	17.1
0.1	19.2	6.3
0.32	22.8	6.9
1.0	83.8 ¹⁾	8.7
3.2	99.6 ¹⁾	0.7

1) The mean for this concentration was significantly higher than the control mean ($t < 0$ and $P < 0.05$, Table A. 12).

Table 6.4 Larval development ratio of *Acartia tonsa* after exposure to SIBX.

Concentration (mg/L)	Larval development ratio mean	Standard deviation
0	53.0	6.3
0.032	57.7	3.8
0.1	55.4	6.3
0.32	49.3	8.9
1.0	2.1 ¹⁾	5.1
3.2	0.0 ¹⁾	0.0

1) The mean for this concentration was significantly lower than the control mean ($t < 0$ and $P < 0.05$, Table A. 19)

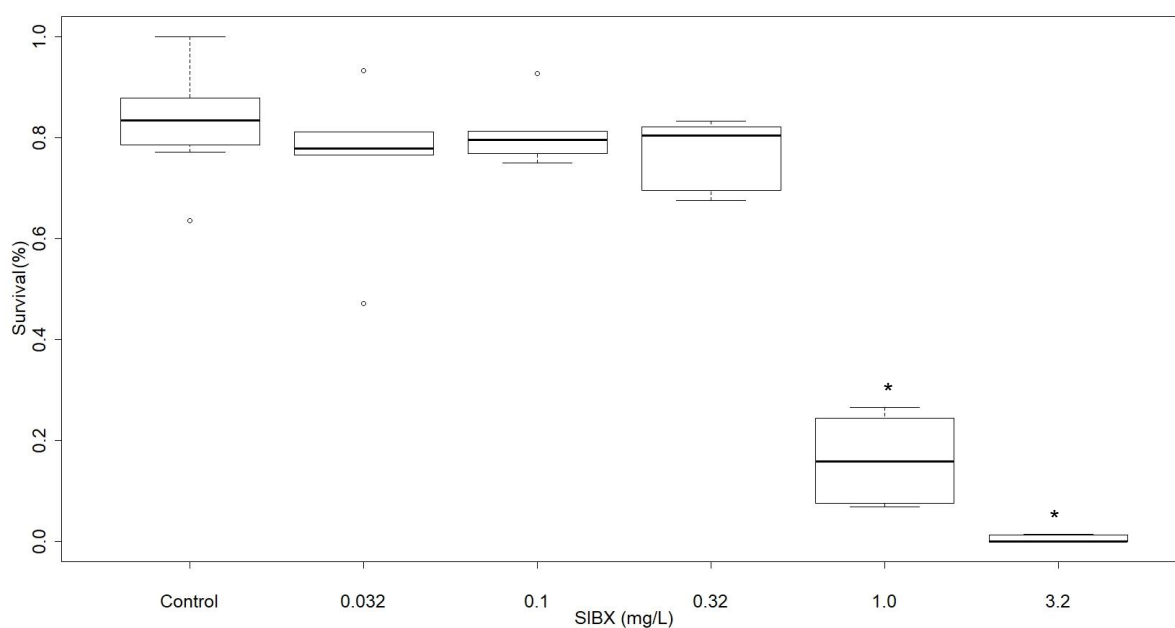


Figure 6.2 Survival in LDR test with SIBX. A survival of 1.0 corresponds to 100% survival. An asterix in the boxplot indicates a significant difference between the test group and the control group ($p < 0.05$).

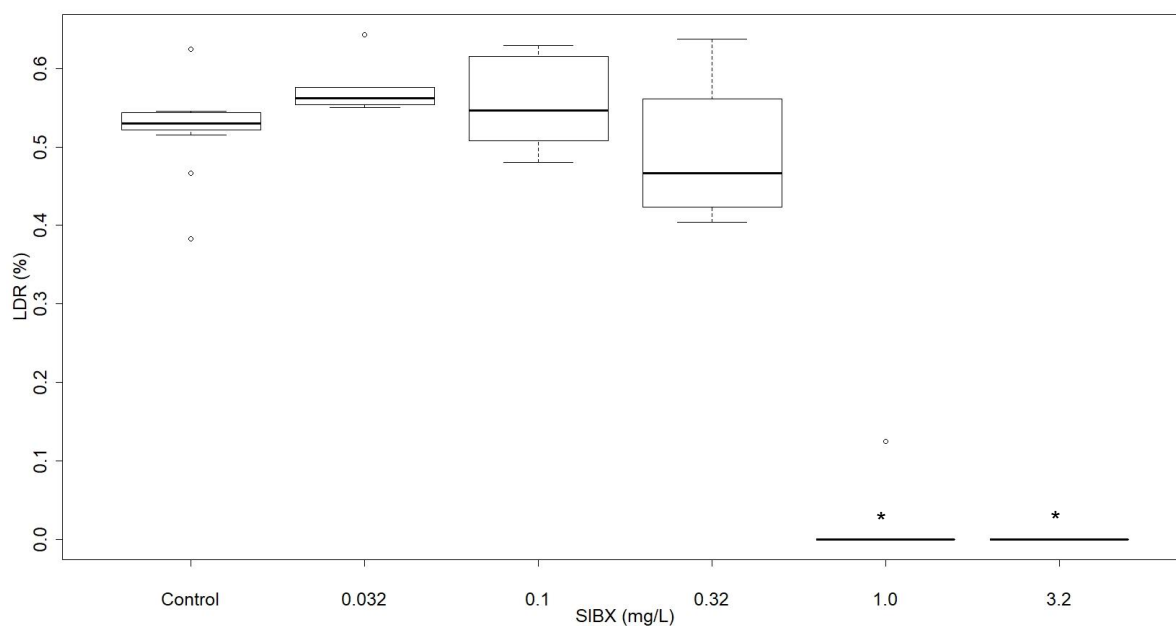


Figure 6.3 Larval development ratio in LDR test with SIBX. An asterisk in the boxplot indicates a significant difference between the test group and the control group ($p < 0.05$).

6.2 Fish, Early-life Stage Toxicity Test on embryo and sac-fry stages with *Cyprinodon variegatus*

The primary data from the toxicity test with *Cyprinodon variegatus* are presented in Appendix . The results of this test are summarized Table 6.5. All validity criteria prescribed in the OECD 210 guideline /2/ were fulfilled.

Furthermore, the biological results are presented as box-and-whisker plots for the endpoints hatching and survival in Figures 6.4 and 6.5. 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.

The toxicity test with *Cyprinodon variegatus* showed no significant effects of the test item on any of the endpoints (Table 6.5). The NOEC were, therefore, equivalent to the highest tested concentration of 10 mg/L, whereas the LOEC were estimated above 10 mg/L.

Adherence of food particles etc. were observed on Day 6 and 7, but this did not seem to affect the survival of the fish larvae.

As no significant differences were observed, the data were not computed to establish a dose-response curve for the hatching success. The EC10 and EC50 for all the endpoints were therefore estimated to be higher than 10 mg/L.

Table 6.5 Results of the *Cyprinodon variegatus* toxicity test after exposure to SIBX.

Endpoint	NOEC (mg/L)	LOEC (mg/L)	EC/LC10 (mg/L)	EC/LC50 (mg/L)
Hatching success	10	>10	>10	>10
Total mortality	10	>10	>10	>10
Body length	10	>10	>10	>10
Body weight	10	>10	>10	>10

Table 6.6 Hatching success of *Cyprinodon variegatus* after exposure to SIBX.

Concentration (mg/L)	Hatching success mean after 5 days (%)	Standard deviation
0	74	10
0.1	73	5
0.32	79	9
1	58	10
3.2	70	12
10.0	80	10

Table 6.7 Mortality of *Cyprinodon variegatus* embryos and fish after exposure to SIBX.

Concentration (mg/L)	Mortality, mean	Standard deviation
0	17.5	6.5
0.1	26.3	4.8
0.32	15.0	4.1
1	30.0	8.2
3.2	12.5	6.5
10.0	7.5	6.5

Table 6.8 Body length of *Cyprinodon variegatus* fish after exposure to SIBX.

Concentration (mg/L)	Body length, mean (mm)	Standard deviation
0	12.9	1.5
0.1	12.9	1.3
0.32	11.9	1.8
1	12.8	1.2
3.2	12.3	1.4
10.0	12.4	1.6

Table 6.9 Body weight of *Cyprinodon variegatus* fish after exposure to SIBX.

Concentration (mg/L)	Body weight, mean (mg)	Standard deviation
0	27.4	13.6
0.1	30.8	14.0
0.32	22.8	15.1
1	25.3	8.9
3.2	22.1	7.9
10.0	23.3	9.4

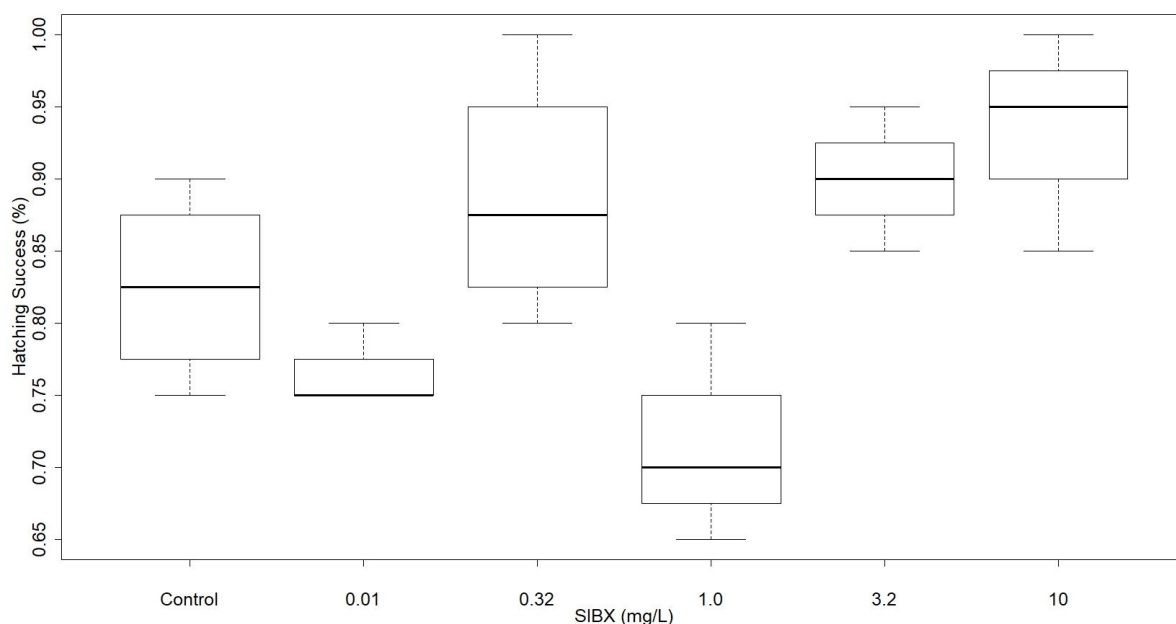


Figure 6.4 Hatching success in OECD 210 test with SIBX. A hatching success of 1.0 corresponds to 100% hatching.

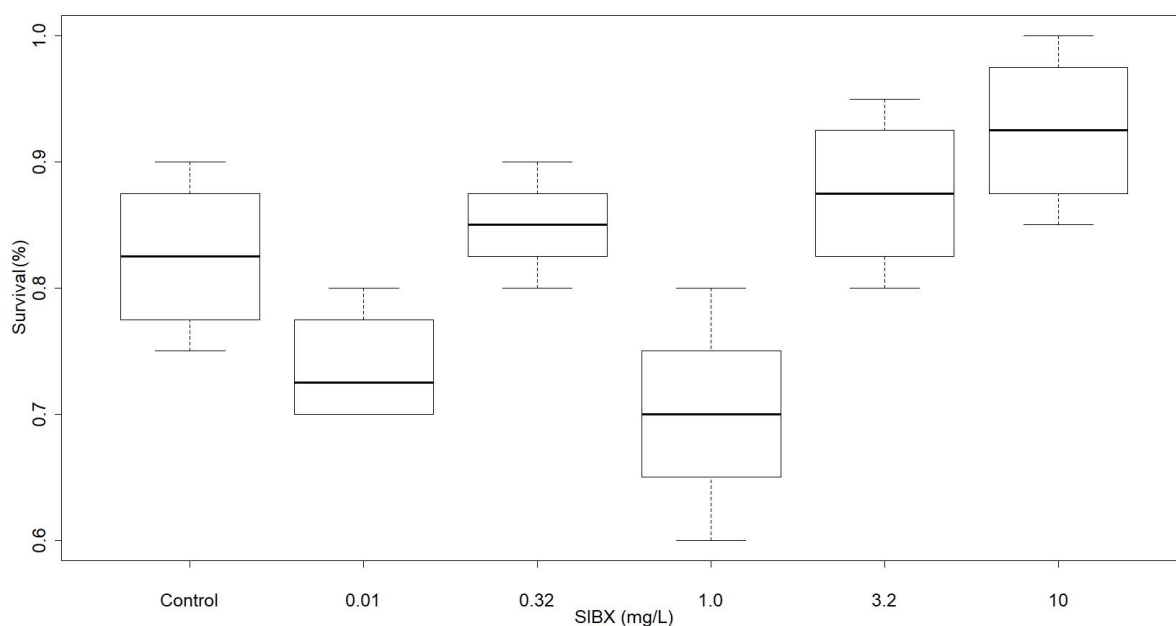


Figure 6.5 Survival in OECD 210 test with SIBX. A survival of 1.0 corresponds to 100% survival.

7 Statistics

The statistical analyses were performed using the open source software R. The LOEC values (lowest observed effect concentration) and the NOEC values (no observed effect concentration) were calculated by use of Dunnett's test. When a significant effect was observed associated with a dose response, a Probit analysis was conducted to estimate the EC/LC10 or EC/LC50.

8 Validity criteria

For both studies, all validity criteria were fulfilled. The criteria and the results are presented in Annex A for the *Acartia tonsa* test and in Annex B, for the *Cyprinodon variegatus* test.

9 References

- /1/ ISO 16778:2015. "Water quality - Calanoid copepod early-life stage test with *Acartia tonsa*". First edition. 2015.06.15.
- /2/ OECD Guideline for Testing of Chemicals No. 210: Fish, Early-life Stage Toxicity Test. 2013.07.26.

APPENDICES

APPENDIX A

Raw data: Early-life stage test with *Acartia tonsa*

A Raw data: Early-life stage test with *Acartia tonsa* with SIBX

A.1.1 Calculations

Hatching success, larval development ratio and early life stage mortality were tabulated according to the SIBX concentrations and exposure periods.

- Hatching success (HS):

$$HS = \sum \frac{egg_{t0,r} - Unhatchedegg_{te,r}}{egg_{t0,r}} \quad (A.1)$$

Where:

- R: number of replicates used for each test group.
- Eggs t0, r: Initial number of eggs at t0 in each replicate (r).
- Unhatched eggs te, r: number of un-hatched eggs at the endpoint (te), in each replicate (r).
- Larval Development Ratio (LDR):

$$LDR = \left(\sum_R \frac{Copepodites_{te,r}}{Nauplii_{te,r} - Copepodites_{te,r}} \right) \times \frac{1}{R} \quad (A.2)$$

Where:

- R: number of replicates used for each test group.
- Nauplii te, r: number of nauplii in each replicate at the endpoint (te), in each replicate (r).
- Copepodites te, r: number of copepodites at the endpoint (te), in each replicate (r).
- Early life stage mortality (ELS)

$$ELS = \left(\sum_R 1 - \frac{Nauplii_{te,r} - Copepodites_{te,r}}{Egg_{t0,r} - Unhatchedeggs_{te,r}} \right) \times \frac{1}{R} \quad (A.3)$$

Where:

- R: number of replicates used for each test group.
- Nauplii te, r: number of nauplii at the endpoint (te), in each replicate (r).
- Copepodites te, r: number of copepodites at the endpoint (te), in each replicate (r).
- Eggs t0, r: Number of eggs at t0, in each replicate (r).
- Unhatched eggs te, r: number of unhatched eggs at the endpoint (te), in each replicate (r).

- Inhibition effect

When an effect of the test item was observed on HS, LDR and ELS, the respective means of the HS, LDR and ELS were used to calculate the percentage inhibition (I) for each individual test flask according to the equation:

$$I = \frac{(\mu_c - \mu_i) \times 100}{\mu_c} \quad (A.4)$$

Where:

- I: percentage of HS/LDR/ELS inhibition for test flask i
- μ_i : HS/LDR/ELS for test flask i
- μ_c : the mean HS/LDR/ELS for the control

The averages of HS, LDR and ELS were tested using an analysis of variance to find NOEC/LOEC. The percentage inhibition (I) for each test bottle was tested using a Probit method to find EC/LC10, EC/LC50.

Table A. 1 Raw data of the number and larvae stages of organisms generated in the early-life stage test with SIBX. Test period from 2019.11.22 to 2019.11.28.

Concentration	Replicate	Number at the test start:			Number at the end of the test:		
		Eggs	Nauplii	Total	Eggs unhatched	Nauplii	Copepodites
Control	A	68	0	68	2	24	40
	B	71	0	71	1	27	32
	C	79	1	80	1	31	35
	D	87	0	87	4	30	36
	E	64	0	64	0	31	33
	F	73	0	73	1	32	28
	G	78	0	78	1	23	26
	H	83	0	83	4	28	33
	I	74	0	74	1	37	23
	J	73	0	73	1	21	35
	K	80	0	80	0	33	37
	L	77	0	77	0	32	36
0.032 mg/L	A	71	0	71	3	14	18
	B	78	0	78	1	25	34
	C ¹⁾	62	0	62	-	-	-
	D	70	0	70	1	20	36
	E	90	0	90	1	37	46
	F	77	0	77	0	27	33
0.1 mg/L	A	84	0	84	1	40	37
	B	84	0	84	1	27	38
	C	66	0	66	2	20	32
	D	86	0	86	3	33	34
	E	73	0	73	1	20	34
	F	83	0	83	1	31	32
0.32 mg/L	A	77	0	77	0	31	21
	B	89	0	89	0	32	41
	C	83	0	83	1	30	27
	D	85	0	85	1	25	44
	E	67	0	67	1	30	22
	F	60	0	60	0	27	23
1.0 mg/L	A	81	0	81	2	14	2
	B	80	0	80	2	19	0

Concentration	Replicate	Number at the test start:			Number at the end of the test:		
		Eggs	Nauplii	Total	Eggs unhatched	Nauplii	Copepodites
	C	90	0	90	2	6	0
	D	64	0	64	0	17	0
	E	71	0	71	1	8	0
	F	80	0	80	1	6	0
3.2 mg/L	A	76	0	76	3	0	0
	B	85	0	85	3	1	0
	C	72	0	72	1	1	0
	D	79	0	79	3	0	0
	E	80	0	80	2	0	0
	F	80	0	80	2	0	0

1) The replicate was lost

A.1.2 Physical parameters

Table A. 2 Physical parameters – start of the test, day 0: 2019.11.22.

SIBX (mg/L)	pH	Salinity ‰	Dissolved oxygen %
Control	8.0	31.4	100
0.032	8.0	31.5	100
0.1	8.0	31.4	100
0.32	8.0	31.4	100
1.0	8.0	31.5	100
3.2	8.0	31.5	100

Table A. 3 Physical parameters – before addition of test solutions, day 3: 2019.11.25.

SIBX (mg/L)	pH	Salinity ‰	Dissolved oxygen %
Control	8.3	32.0	100
0.032	8.3	32.1	100
0.1	8.3	32.3	100
0.32	8.3	32.2	100
1.0	8.3	32.0	100
3.2	8.3	32.1	100

Table A. 4 Physical parameters – after addition of test solutions, day 3: 2019.11.25.

SIBX (mg/L)	pH	Salinity ‰	Dissolved oxygen %
Control	8.1	31.8	100
0.032	8.1	31.9	100
0.1	8.1	32.0	100
0.32	8.1	31.9	100
1.0	8.1	31.8	100
3.2	8.1	31.8	100

Table A. 5 Physical parameters – end of the test, day 6: 2019.11.28.

SIBX (mg/L)	pH	Salinity ‰	Dissolved oxygen %
Control	8.4	31.8	100
0.032	8.4	32.0	100
0.1	8.4	32.1	100
0.32	8.4	32.3	100
1.0	8.4	32.2	100
3.2	8.4	32.1	100

Table A. 6 Physical parameters – temperature monitoring from 2019.11.22 to 2019.11.28.

Mean °C	Standard deviation °C	Minimum °C	Maximum °C
19.4	0.1	19.2	19.6

A.1.3 Statistical analysis – Determination of effect concentrations after exposure to SIBX

A.1.3.1 Determination of NOEC and LOEC for hatching success

Table A. 7 Experimental data of hatching success (%)

Replicate No.	Control	0.032 mg/L	0.1 mg/L	0.32 mg/L	1.0 mg/L	3.2 mg/L
1	97.1	95.8	98.8	100	97.5	96.1
2	98.6	98.7	98.8	100	97.5	96.5
3	98.7	¹⁾	97.0	98.8	97.8	98.6
4	95.4	98.6	96.5	98.8	100	96.2
5	100	98.9	98.6	98.5	98.6	97.5
6	98.6	100	98.8	100	98.8	97.5
7	98.7					
8	95.2					
9	98.6					
10	98.6					
11	100					
12	10					
Count	12	5	6	6	6	6
Mean	98.3	98.4	98.1	99.4	98.4	97.1

¹⁾ The replicate was lost

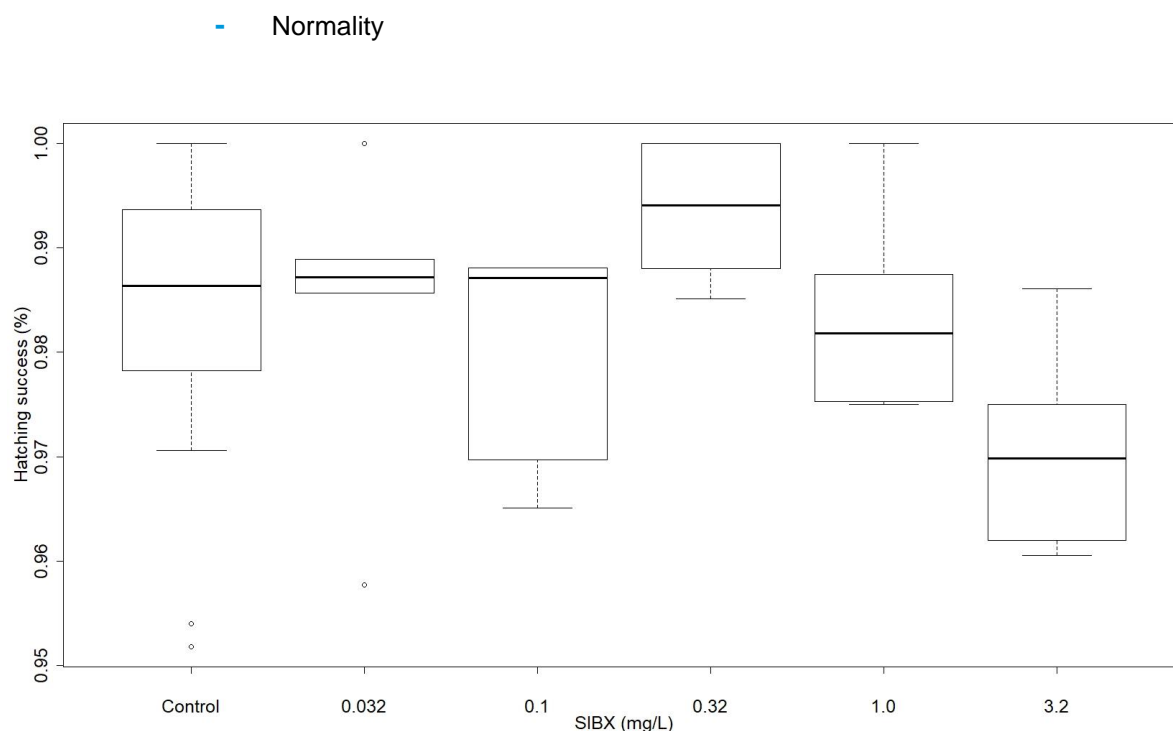


Figure A. 1 Boxplots of every test group for the hatching success.

The boxplot patterns show the data distribution (Figure A. 1). A normal distribution in each test group will be assumed in the following study.

- Dunnett's test

Dunnett's test tests the null hypothesis that the averages of every test groups 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⁾
SIBX-0.032-mg/L - SIBX-0-mg/L = 0	0.001	0.007	0.134	1.0	
SIBX-0.1-mg/L - SIBX-0-mg/L = 0	-0.002	0.006	-0.331	0.998	
SIBX-0.32-mg/L - SIBX-0-mg/L = 0	0.011	0.006	1.659	0.382	
SIBX-1-mg/L - SIBX-0-mg/L = 0	0.001	0.006	0.093	1.0	
SIBX-3.2-mg/L - SIBX-0-mg/L = 0	-0.012	0.006	-1.955	0.231	

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 group with SIBX is significantly different from the laboratory control was rejected ($t < 0$ and $P < 0.05$) (Table A. 8).

Table A. 9 Hatching success (HS) endpoint, estimations of NOEC and LOEC, Dunnett's test.

Endpoint	NOEC	LOEC	Unit
Hatching success	3.2	>3.2	mg/L

A.1.3.2 Results of the Probit analysis with SIBX

As no significant effect was observed among the test groups, the data to estimate the ECX were not computed.

Table A. 10 Hatching success endpoint, estimations of EC10 and EC50, Probit analysis.

Endpoint	EC10 (%)	EC50 (%)
Hatching success	>3.2	>3.2

A.1.3.3 Determination of NOEC and LOEC for the early life stage survival with SIBX

Table A. 11 Experimental data of early life stage survival (%)

Replicate No.	Control	0.032 mg/L	0.1 mg/L	0.32 mg/L	1.0 mg/L	3.2 mg/L
1	97.0	47.1	92.8	67.5	20.3	0
2	84.3	76.6	78.3	82.0	24.4	1.2
3	83.5	NA ¹⁾	81.3	69.5	6.8	1.4
4	79.5	81.2	80.7	82.1	26.6	0
5	100.0	93.3	75.0	78.8	11.4	0
6	83.3	77.9	76.8	83.3	7.6	0
7	63.6					
8	77.2					
9	82.2					
10	77.8					
11	87.5					
12	88.3					
Count	12	5	6	6	6	6
mean	83.7	75.2	80.8	77.2	16.2	0.4

1) The replicate was lost

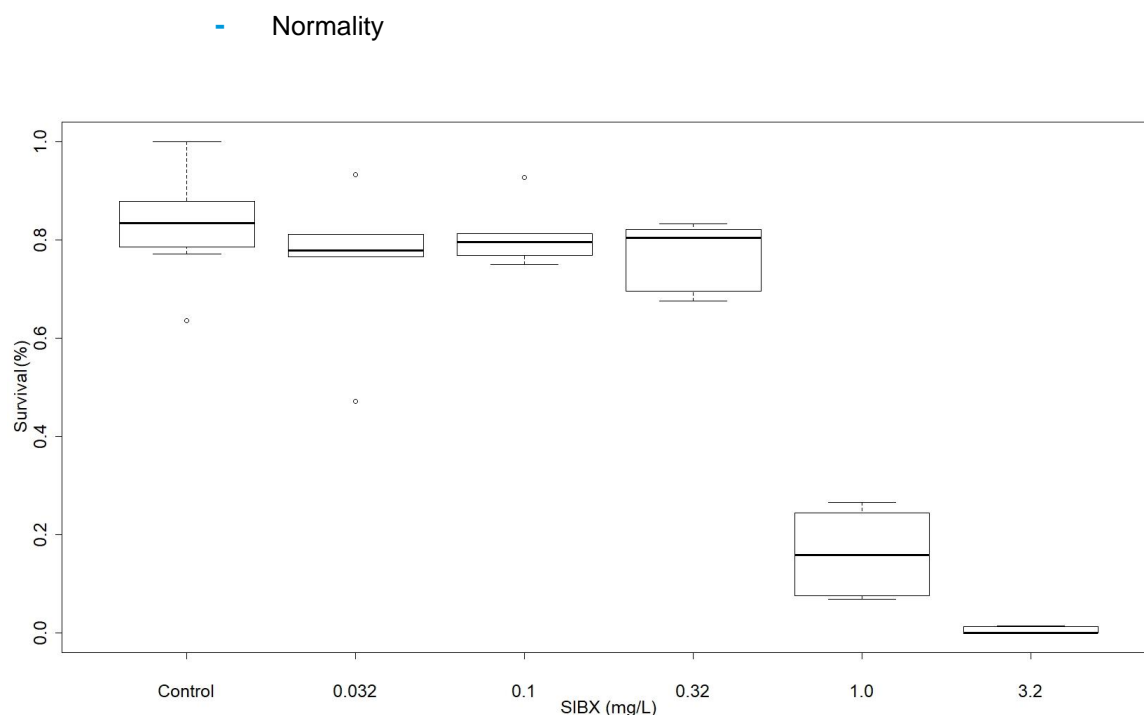


Figure A.2 Boxplots of every test group for the early life stage mortality represented as survival.

The boxplot patterns show the data distribution (Figure A.2). A normal distribution in each test group was assumed in the following study.

- Dunnett's test

Dunnett's test tests the null hypothesis that the averages of every test groups are not different from the control group (Table A. 12).

Table A. 12 Multiple comparisons of means: Dunnett's contrasts.

Mean comparisons	Estimate	Std. Error	t value	Pr(> t)	Significance ¹⁾
SIBX-0.032-mg/L - SIBX-0-mg/L = 0	-0.08	0.05	-1.7	0.34	
SIBX-0.1-mg/L - SIBX-0-mg/L = 0	-0.03	0.05	-0.63	0.97	
SIBX-0.32-mg/L - SIBX-0-mg/L = 0	-0.06	0.05	-1.4	0.55	
SIBX-1-mg/L - SIBX-0-mg/L = 0	-0.68	0.05	-15	<0.001	***
SIBX-3.2-mg/L - SIBX-0-mg/L = 0	-0.83	0.05	-18	<0.001	***

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 SIBX is significantly higher than the laboratory control was verified ($t < 0$ and $P < 0.05$) at 1 mg/L and above (Table A. 12).

Table A. 13 Early-life stage mortality endpoint. estimations of NOEC and LOEC, Dunnett's test.

Endpoint	NOEC	LOEC	Unit
Early life stage mortality	0.32	1.0	mg/L

A.1.3.4 Results of the Probit analysis with SIBX

A.1.3.5 Inhibition of the early life stage survival of *Acartia tonsa* with SIBX

Table A. 14 Control values – Early-life stage survival after 6 days of exposure

Control	Early-life stage survival ¹⁾ (%)	Inhibition (%)
Control 1	97.0	-15.9
Control 2	84.3	-0.7
Control 3	83.5	0.2
Control 4	79.5	5.0
Control 5	100	-19.5
Control 6	83.3	0.4
Control 7	63.6	24.0
Control 8	97.0	7.7
Control 9	82.2	1.8
Control 10	77.8	7.1
Control 11	87.5	-4.6
Control 12	88.3	-5.5
Control mean	83.7	0

1) Four significant figures of the Early life stage mortality were used in the calculation of the inhibition and for the Probit analysis

Table A. 15 Experimental data – inhibition of the early-life stage survival after 6 days of exposure.

Concentration (mg/L)	Early-life stage survival ¹⁾ (%)	Inhibition (%)
0.032	47.1	43.8
0.032	76.6	8.4
0.032	NA ²⁾	NA
0.032	81.2	3.0
0.032	93.3	-11.4
0.032	77.9	6.9
0.1	92.8	-10.9
0.1	78.3	6.4
0.1	81.3	2.9
0.1	80.7	3.5
0.1	75.0	10.4

Concentration (mg/L)	Early-life stage survival ¹⁾ (%)	Inhibition (%)
0.1	76.8	8.2
0.32	67.5	19.3
0.32	82.0	2.0
0.32	69.5	16.9
0.32	82.1	1.8
0.32	78.8	5.9
0.32	83.3	0.4
1	20.3	75.8
1	24.4	70.9
1	6.8	91.9
1	26.6	68.3
1	11.4	86.3
1	7.6	90.9
3.2	0	100
3.2	1.2	98.5
3.2	1.4	98.3
3.2	0	100
3.2	0	100
3.2	0	100

- 1) Four significant figures of the early life stage mortality were used in the calculation of the inhibition and for the Probit analysis
- 2) The replicate was lost

A.1.3.6 Results of the Probit analysis with SIBX

Table A. 16 LCx estimates, Probit analysis with R after 6 days.

LCx	Concentration (mg/L)	Std. Error	Lower (mg/L)	Upper (mg/L)
LC10	0.34	0.03	0.27	0.41
LC50	0.58	0.05	0.49	0.68

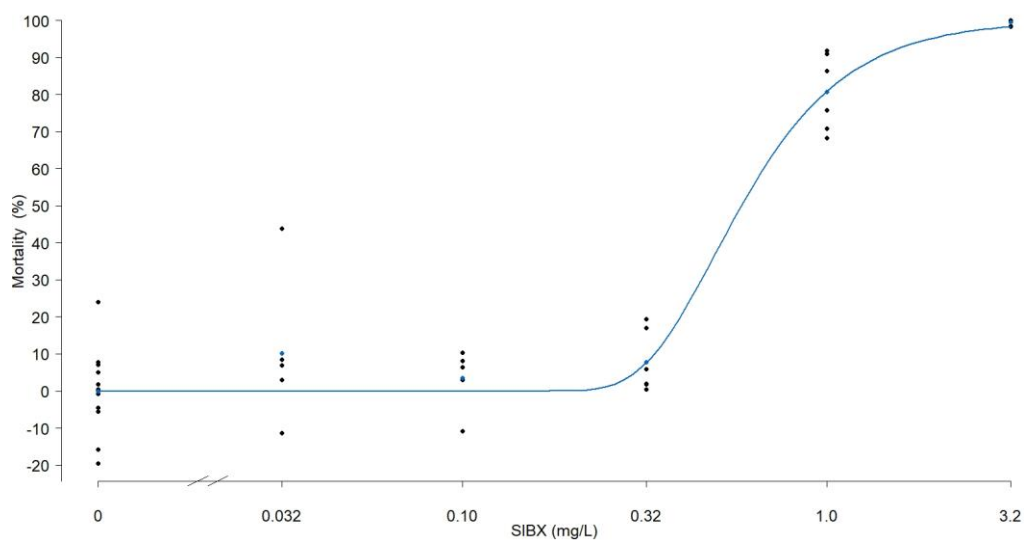


Figure A.3 Effect of the SIBX on the early life stage mortality after 6 days of exposure. In blue, the dose response curve established by use of the free software R. The blue points correspond to the average inhibition per concentrations. The black points correspond to the inhibition per replicates.

Table A. 17 Early life stage mortality endpoint, estimations of LC10 and LC50

Endpoint	LC10 (mg/L)	LC50 (mg/L)
Early life stage mortality	0.34 (0.27 - 0.41)	0.58 (0.49 - 0.68)

A.1.3.7 Determination of NOEC and LOEC for the larval development ratio with SIBX

Table A. 18 Experimental data of larval development ratio (%).

Replicate No.	Control	0.032 mg/L	0.1 mg/L	0.32 mg/L	1.0 mg/L	3.2 mg/L
1	62.5	56.3	48.1	40.4	12.5	0
2	54.2	57.6	58.5	56.2	0	0
3	53.0	NA ¹⁾	61.5	47.4	0	0
4	54.5	64.3	50.7	63.8	0	0
5	51.6	55.4	63.0	42.3	0	0
6	46.7	55.0	50.8	46.0	0	0
7	53.1					
8	54.1					
9	38.3					
10	62.5					
11	52.9					
12	52.9					
Count	12	5	6	6	6	2
Mean	53.0	57.7	55.4	49.3	2.1	0

¹⁾ The replicate was lost

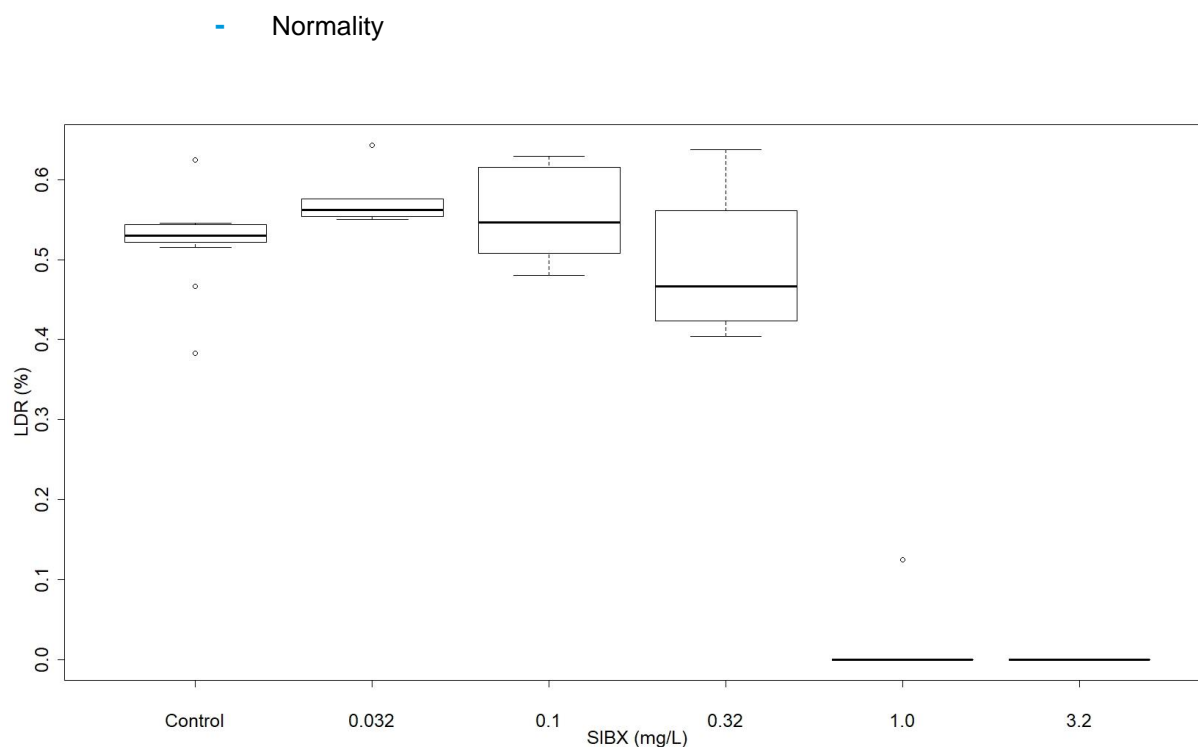


Figure A.4 Boxplots of every test group for the larval development ratio

The boxplot patterns show the data distribution (Figure A.4). A normal distribution in each test group was assumed in the following study.

- Dunnett's test

Dunnett's test tests the null hypothesis that the averages of every test groups are not different from the control group (Table A. 19).

Table A. 19 Multiple comparisons of means: Dunnett's contrasts.

Mean comparisons	Estimate	Std. Error	t value	Pr(> t)	Significance ¹⁾
SIBX-0.032-mg/L - SIBX-0-mg/L = 0	0.05	0.03	1.4	0.56	
SIBX-0.1-mg/L - SIBX-0-mg/L = 0	0.02	0.03	0.76	0.93	
SIBX-0.32-mg/L - SIBX-0-mg/L = 0	-0.04	0.03	-1.2	0.72	
SIBX-1-mg/L - SIBX-0-mg/L = 0	-0.51	0.03	-16	<0.000	***
SIBX-3.2-mg/L - SIBX-0-mg/L = 0	-0.53	0.05	-11	<0.000	***

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 group with SIBX is significantly lower than the laboratory control was verified ($t < 0$ and $P < 0.05$) at 1 mg/L and above (Table A. 19).

Table A. 20 Larval development ratio endpoint, estimations of NOEC and LOEC, Dunnett's test.

Endpoint	NOEC	LOEC	Unit
Larval development ratio	0.32	1.0	mg/L

A.1.3.8 Results of the Probit analysis with SIBX

A.1.3.9 Inhibition of the larval development ratio of *Acartia tonsa* with SIBX

Table A. 21 Control values – Larval development ratio (%) after 6 days of exposure

Laboratory control	Larval development ratio ¹⁾ (%)	Inhibition (%)
Control 1	62.5	-17.9
Control 2	54.2	-2.3
Control 3	53.0	0.0
Control 4	54.5	-2.9
Control 5	51.6	2.8
Control 6	46.7	12.0
Control 7	53.1	-0.1
Control 8	54.1	-2.0
Control 9	38.3	27.7
Control 10	62.5	-17.9
Control 11	52.9	0.3
Control 12	52.9	0.2
Control mean	53.0	0.0

1) Four significant figures of the Larval development ratio were used in the calculation of the inhibition and for the Probit analysis

Table A. 22 Experimental data – inhibition of the larval development ratio after 6 days of exposure.

Concentration (mg/L)	Larval development ratio ¹⁾ (%)	Inhibition (%)
0.032	56.3	-6.1
0.032	57.6	-8.7
0.032	NA ²⁾	NA
0.032	64.3	-21.2
0.032	55.4	-4.5
0.032	55.0	-3.7
0.1	48.1	9.4
0.1	58.5	-10.2
0.1	61.5	-16.0

Concentration (mg/L)	Larval development ratio ¹⁾ (%)	Inhibition (%)
0.1	50.7	4.3
0.1	63.0	-18.7
0.1	50.8	4.2
0.32	40.4	23.8
0.32	56.2	-5.9
0.32	47.4	10.7
0.32	63.8	-20.3
0.32	42.3	20.2
0.32	46.0	13.3
1	12.5	76.4
1	0.0	100
1	0.0	100
1	0.0	100
1	0.0	100
1	0.0	100
1	0.0	100
3.2	0.0	100
3.2	0.0	100
3.2	0.0	100
3.2	0.0	100
3.2	0.0	100
3.2	0.0	100

1) Four significant figures of the Larval development ratio were used in the calculation of the inhibition and for the Probit analysis

2) The replicate was lost

A.1.3.10 Results of the Probit analysis with SIBX

Table A. 23 ECx estimates, Probit analysis with R after 6 days.

ECx	Concentration (mg/L)	Std. Error	Lower (mg/L)	Upper (mg/L)
EC10	0.36	0.07	0.23	0.50
EC50	0.63	0.07	0.50	0.77

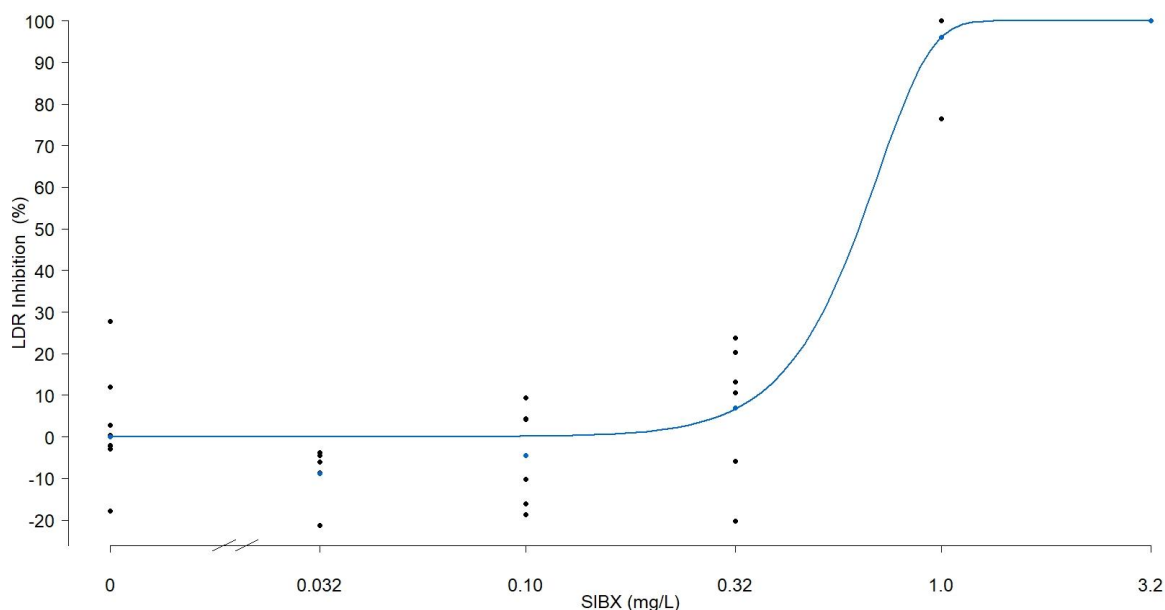


Figure A.5 Effect of the SIBX on the Larval development ratio after 6 days of exposure. In blue, the dose response curve established by use of the free software R. The blue points correspond to the average inhibition per concentrations. The black points correspond to the inhibition per replicates.

Table A. 24 Larval development ratio endpoint, estimations of EC10 and EC50, Probit analysis.

Endpoint	EC10 (mg/L)	EC50 (mg/L)
Larval development ratio	0.36 (0.23 - 0.50)	0.63 (0.50 - 0.77)

A.1.4 Validity criteria

Table A. 25 Validity criteria of the test according to the ISO 16778 /1/ in the larval development test with *Acartia tonsa*.

Criteria	Target value	Fulfilled
Early-life stage mortality in the control	$\leq 30\%$ (observed value: 16%)	Yes
Hatching success in the control	$\geq 75\%$ (observed value: 98%)	Yes
Larval development ratio in the control	$60\% \pm 20\%$ (observed value: 53%)	Yes
Temperature variation from the control start (Ti) value	$T_i \text{ } ^\circ\text{C} \pm 1 \text{ } ^\circ\text{C}$ (observed value: $19.2 \text{ } ^\circ\text{C} \pm 19.6 \text{ } ^\circ\text{C}$)	Yes
Control pH increase during test	< 1.0 (observed value: 0.4)	Yes
Dissolved oxygen concentration	$> 70\%$ throughout test (observed value: $\geq 100\%$)	Yes
Salinity variation from the control start (Si) value	$Si\text{‰} \pm 10\%$ (observed value: $31\text{‰} \pm 3\text{‰}$)	Yes

APPENDIX B

Raw data: Fish, early-life stage toxicity test

B Raw data: Fish, early-life stage toxicity test

Table B. 1 Raw data of the number of eggs, larvae and fish generated daily in the test period 2019.11.14 to 2019.12.16.

Date		2019.11.14								Accumulated day(s)								0			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		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	0	0	0	0	0	0	0
0.1	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.32	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.2	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Date		2019.11.15								Accumulated day(s)								1			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		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	0	0	0	0	0	0	0
0.1	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.32	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.2	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Date		2019.11.16								Accumulated day(s)								2			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		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	0	0	0	0	0	0	0
0.1	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.32	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.2	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Date		2019.11.17								Accumulated day(s)								3			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	20	17	20	18	0	0	0	0	0	3	0	2	0	0	0	0	0	0	0	0
0.1	80	19	20	20	20	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0.32	80	20	20	18	20	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
1	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.2	80	19	20	20	20	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
10	80	20	20	20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Date		2019.11.18								Accumulated day(s)								4			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	10	11	15	8	0	0	0	0	10	9	5	12	0	0	0	0	0	0	0	0
0.1	80	13	12	15	10	0	0	0	0	7	8	5	10	0	0	0	0	0	0	0	0
0.32	80	12	13	10	12	0	0	0	0	8	7	10	8	0	0	0	0	0	0	0	0
1	80	11	14	13	13	0	0	0	0	9	6	7	7	0	0	0	0	0	0	0	0
3.2	80	11	17	11	7	0	0	0	0	9	3	9	13	0	0	0	0	0	0	0	0
10	80	12	6	8	6	0	0	0	0	8	14	12	14	0	0	0	0	0	0	0	0

Date		2019.11.19								Accumulated day(s)								5			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	3	5	8	5	0	0	0	0	17	15	12	15	0	0	0	0	0	0	0	0
0.1	80	5	5	7	5	0	0	0	0	15	15	13	15	0	0	0	0	0	0	0	0
0.32	80	3	7	3	4	0	0	0	0	17	13	17	16	0	0	0	0	0	0	0	0
1	80	7	11	9	7	0	0	0	0	13	9	11	13	0	0	0	0	0	0	0	0
3.2	80	4	9	7	4	0	0	0	0	16	11	13	16	0	0	0	0	0	0	0	0
10	80	7	3	3	3	0	0	0	0	13	17	17	17	0	0	0	0	0	0	0	0

Date		2019.11.20								Accumulated day(s)								6			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	2	3	5	4	1	0	0	0	17	17	15	16	0	0	0	0	0	0	0	0
0.1	80	5	5	3	5	0	0	1	0	14	15	16	15	1	0	0	0	0	0	0	0
0.32	80	2	5	1	0	1	0	1	0	17	15	18	19	0	0	0	1	0	0	0	0
1	80	6	9	5	3	0	0	1	1	14	10	14	16	0	1	0	0	0	0	0	0
3.2	80	3	2	1	0	0	1	1	1	17	17	17	19	0	0	1	0	0	0	0	0
10	80	1	1	1	0	0	2	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.11.21								Accumulated day(s)								7			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	1	3	5	4	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	5	4	3	5	0	1	0	0	14	15	16	15	0	0	0	0	0	0	0	0
0.32	80	2	4	1	0	1	1	1	0	17	16	18	18	0	0	0	1	0	0	0	0
1	80	5	6	4	3	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	3	2	1	0	0	0	0	0	17	17	16	19	0	0	1	0	0	0	0	0
10	80	1	1	1	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.11.22								Accumulated day(s)								8			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	1	3	5	4	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	5	4	3	5	14	15	16	15	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	2	4	1	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	5	6	4	3	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	2	2	1	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	1	1	1	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.11.23								Accumulated day(s)								9			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	15	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.11.24								Accumulated day(s)								10			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	15	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.11.25								Accumulated day(s)								11			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	1	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.11.26								Accumulated day(s)								12			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.11.27								Accumulated day(s)								13			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.11.28								Accumulated day(s)								14			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.11.29								Accumulated day(s)								15			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.11.30								Accumulated day(s)								16			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.01								Accumulated day(s)								17			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.02								Accumulated day(s)								18			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.03								Accumulated day(s)								19			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.04								Accumulated day(s)								20			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.05								Accumulated day(s)								21			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.06								Accumulated day(s)								22			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.07								Accumulated day(s)								23			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	18	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.08								Accumulated day(s)								24			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	17	0	0	0	1	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.09								Accumulated day(s)								25			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	17	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.10								Accumulated day(s)								26			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	17	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.11								Accumulated day(s)								27			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	17	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.12								Accumulated day(s)								28			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	17	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.13								Accumulated day(s)								29			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	17	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.14								Accumulated day(s)								30			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	17	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.15								Accumulated day(s)								31			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	17	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	19	20	0	0	0	0	0	0	0	0

Date		2019.12.16								Accumulated day(s)								32			
Concentration (mg/L)	Initial eggs per test group					Daily number of:															
		Eggs				Eggs left unhatched				Live larvae/fish				Dead larvae/fish				Malformed larvae/fish			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Control	80	0	0	0	0	0	0	0	0	18	17	15	16	0	0	0	0	0	0	0	0
0.1	80	0	0	0	0	0	0	0	0	14	15	16	14	0	0	0	0	0	0	0	0
0.32	80	0	0	0	0	0	0	0	0	17	16	18	17	0	0	0	0	0	0	0	0
1	80	0	0	0	0	0	0	0	0	14	12	14	16	0	0	0	0	0	0	0	0
3.2	80	0	0	0	0	0	0	0	0	18	17	16	19	0	0	0	0	0	0	0	0
10	80	0	0	0	0	0	0	0	0	19	17	18	20	0	0	1	0	0	0	0	0

B.1.1 Physical parameters

Table B. 2 Physical parameters measured at the test start, at renewal and at the end of the test.

Concentration mg/L	Start day 0			
	A - pH	A - Temp. °C	A - Oxygen %	A - Salinity ‰
Control	8.0	24.0	100	31.3
0.1	8.0	24.0	100	31.3
0.32	8.0	24.0	100	31.4
1	8.0	23.8	100	31.5
3.2	8.0	24.1	100	31.5
10	8.0	24.3	100	31.5

Concentration mg/L	Day 1 before renewal - pH				Day 1 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	8.2	8.1	8.1	8.1	8.0	8.1	8.1	8.1
0.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1
0.32	8.2	8.2	8.2	8.1	8.1	8.1	8.1	8.1
1	8.2	8.2	8.1	8.1	8.1	8.1	8.1	8.1
3.2	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1
10	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1

Concentration mg/L	Day 1 before renewal - Temp. °C				Day 1 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	23.9	23.9	23.9	23.9	23.6	23.6	23.5	23.5
0.1	23.9	23.9	23.9	23.8	23.5	23.8	23.9	23.7
0.32	23.9	23.9	23.9	23.9	23.7	23.9	23.9	24.0
1	23.8	23.9	24.0	24.0	24.1	24.1	24.0	23.8
3.2	23.9	24.0	24.0	24.0	23.9	23.9	24.0	24.0
10	23.9	24.0	24.0	24.0	24.0	23.8	24.0	24.0

Concentration mg/L	Day 1 before renewal - Oxygen %				Day 1 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	99.0	99.0	99.0	99.0	100	100	100	100
0.1	100	100	100	100	100	100	100	100
0.32	100	100	100	99.0	100	100	100	100
1	100	100	99.0	98.0	100	100	100	100
3.2	100	100	100	99.0	100	100	100	100
10	99.0	99.0	99.0	99.0	100	100	100	100

Concentration mg/L	Day 1 before renewal - Salinity ‰				Day 1 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	31.5	31.6	31.6	31.6	31.4	31.6	31.6	31.5
0.1	31.6	31.8	31.6	31.7	31.8	31.6	31.6	31.6
0.32	31.7	31.7	31.6	31.8	31.7	31.6	31.6	31.5
1	31.8	31.8	31.6	31.6	31.5	31.4	31.5	31.7
3.2	31.7	31.8	31.6	31.6	31.7	31.6	31.6	31.5
10	31.7	31.6	31.6	31.6	31.6	31.6	31.6	31.6

Concentration mg/L	Day 4 before renewal - pH				Day 4 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	8.2	8.1	8.1	8.1	8.0	8.0	8.0	8.0
0.1	8.1	8.1	8.1	8.1	8.0	8.0	8.0	8.1
0.32	8.1	8.2	8.1	8.1	8.0	8.0	8.0	8.0
1	8.2	8.2	8.2	8.2	8.0	8.0	8.0	8.0
3.2	8.2	8.2	8.2	8.2	8.0	8.0	8.0	8.0
10	8.2	8.2	8.2	8.1	8.1	8.1	8.0	8.1

Concentration mg/L	Day 4 before renewal - Temp. °C				Day 4 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	23.9	23.7	23.7	23.9	24.1	24.0	23.9	23.8
0.1	23.9	24.0	23.9	23.7	23.8	23.8	23.8	23.7
0.32	23.9	23.9	24.0	23.9	23.8	23.8	23.7	23.7
1	23.8	24.0	24.0	24.1	23.8	23.8	23.7	23.7
3.2	24.0	24.1	24.1	24.1	23.7	23.7	23.6	23.6
10	24.2	24.1	24.1	24.1	23.7	23.7	23.6	23.5

Concentration mg/L	Day 4 before renewal - Oxygen %				Day 4 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	100	100	99.0	99.0	100	100	100	100
0.1	98.0	98.0	98.0	99.0	100	100	100	100
0.32	99.0	98.0	98.0	98.0	100	100	100	100
1	99.0	98.0	98.0	98.0	100	100	100	100
3.2	98.0	98.0	98.0	98.0	100	100	100	100
10	97.0	97.0	97.0	96.0	100	100	100	100

Concentration mg/L	Day 4 before renewal - Salinity ‰				Day 4 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	31.9	32.2	32.2	32.0	31.2	31.3	31.3	31.4
0.1	32.0	32.0	32.0	32.1	31.6	31.5	31.5	31.5
0.32	32.1	32.1	31.9	32.0	31.5	31.5	31.5	31.5
1	32.2	32.0	32.0	31.8	31.6	31.5	31.5	31.5
3.2	32.2	32.0	31.9	31.9	31.6	31.5	31.6	31.5
10	31.8	31.9	31.9	31.9	31.5	31.5	31.6	31.6

Concentration mg/L	Day 6 before renewal - pH				Day 6 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
0.1	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
0.32	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
1	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
3.2	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
10	8.0	8.0	8.0	8.0	8.0	8.1	8.1	8.1

Concentration mg/L	Day 6 before renewal - Temp. °C				Day 6 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	23.8	23.9	23.9	23.9	24.1	24.0	23.9	23.9
0.1	23.8	23.9	23.9	23.9	23.9	23.9	23.9	23.8
0.32	23.8	24.0	23.9	23.9	24.0	23.9	23.8	23.8
1	23.8	24.0	24.0	23.8	23.8	23.8	23.7	23.7
3.2	23.9	24.0	24.0	23.9	23.7	23.7	23.7	23.7
10	24.0	24.0	24.0	23.9	23.9	23.8	23.8	23.8

Concentration mg/L	Day 6 before renewal - Oxygen %				Day 6 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	100	94.0	95.0	94.0	100	100	100	100
0.1	94.0	94.0	93.0	91.0	100	100	100	100
0.32	97.0	94.0	93.0	93.0	100	100	100	100
1	94.0	93.0	92.0	92.0	100	100	100	100
3.2	94.0	93.0	93.0	93.0	100	100	100	100
10	98.0	94.0	92.0	91.0	100	100	100	100

Concentration mg/L	Day 6 before renewal - Salinity ‰				Day 6 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	31.8	31.9	31.8	31.8	31.4	31.6	31.6	31.6
0.1	32.1	32.0	32.0	32.0	31.6	31.6	31.6	31.6
0.32	32.1	31.9	32.0	32.0	31.5	31.5	31.6	31.6
1	32.0	32.0	32.0	32.0	31.6	31.6	31.6	31.6
3.2	32.0	32.0	31.9	31.9	31.6	31.6	31.6	31.6
10	32.0	31.9	31.9	32.0	31.5	31.6	31.6	31.6

Concentration mg/L	Day 8 before renewal - pH				Day 8 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	8.0	7.9	7.9	7.9	7.9	7.9	7.9	7.9
0.1	7.9	8.0	8.0	8.0	8.0	8.0	8.0	8.0
0.32	7.9	7.9	7.9	7.9	8.0	8.0	8.0	8.0
1	7.9	7.9	7.9	7.9	8.0	8.0	8.0	8.0
3.2	7.9	7.9	7.9	7.9	8.0	8.0	8.0	8.0
10	7.9	7.9	7.9	7.9	8.0	8.0	8.0	8.0

Concentration mg/L	Day 8 before renewal - Temp. °C				Day 8 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	24.4	24.2	24.2	24.1	23.9	23.9	23.9	23.9
0.1	24.2	24.2	24.1	24.0	23.9	23.9	23.9	23.9
0.32	24.3	24.3	24.2	24.1	23.8	23.9	23.9	23.9
1	24.3	24.3	24.2	24.1	23.9	23.9	23.9	24.0
3.2	24.3	24.3	24.2	24.1	24.0	24.0	24.0	23.9
10	24.3	24.3	24.3	24.2	23.9	23.9	23.9	23.9

Concentration mg/L	Day 8 before renewal - Oxygen %				Day 8 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	100.0	100.0	97.0	96.0	100	100	100	100
0.1	95.0	96.0	96.0	97.0	100	100	100	100
0.32	100.0	100.0	100.0	97.0	100	100	100	100
1	97.0	95.0	94.0	93.0	100	100	100	100
3.2	100.0	99.0	97.0	96.0	100	100	100	100
10	98.0	95.0	93.0	93.0	100	100	100	100

Concentration mg/L	Day 8 before renewal - Salinity ‰				Day 8 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	31.7	31.7	31.7	31.7	31.5	31.6	31.6	31.6
0.1	31.6	31.7	31.7	31.7	31.6	31.5	31.6	31.6
0.32	31.6	31.6	31.6	31.6	31.7	31.6	31.6	31.6
1	31.6	31.7	31.6	31.7	31.6	31.6	31.6	31.6
3.2	31.7	31.6	31.7	31.7	31.6	31.5	31.6	31.5
10	31.6	31.6	31.7	31.7	31.6	31.6	31.5	31.5

Concentration mg/L	Day 11 before renewal - pH				Day 11 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	7.9	7.8	7.9	7.9	7.8	7.9	7.9	7.9
0.1	7.9	7.9	7.9	7.9	7.8	7.9	7.8	7.9
0.32	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9
1	7.9	7.9	8.0	8.0	7.9	7.9	7.9	7.8
3.2	7.9	7.9	7.9	7.9	7.9	7.8	7.8	7.9
10	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.8

Concentration mg/L	Day 11 before renewal - Temp. °C				Day 11 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	24.0	24.3	24.4	24.3	24.5	24.4	24.3	24.1
0.1	24.2	24.3	24.3	24.3	24.2	24.3	24.3	24.1
0.32	24.4	24.5	24.5	24.5	24.1	24.2	24.3	24.3
1	24.4	24.4	24.4	24.5	24.3	24.4	24.3	24.2
3.2	24.4	24.5	24.4	24.5	24.2	24.4	24.4	24.3
10	24.4	24.4	24.5	24.6	24.2	24.2	24.2	24.2

Concentration mg/L	Day 11 before renewal - Oxygen %				Day 11 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	96.0	92.0	93.0	91.0	99.0	98.0	98.0	98.0
0.1	95.0	96.0	95.0	95.0	98.0	99.0	98.0	99.0
0.32	96.0	92.0	93.0	92.0	99.0	99.0	98.0	99.0
1	94.0	95.0	94.0	96.0	99.0	99.0	99.0	100
3.2	97.0	95.0	95.0	96.0	100	100	100	100
10	95.0	95.0	95.0	95.0	100	100	100	99.0

Concentration mg/L	Day 11 before renewal - Salinity ‰				Day 11 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	32.2	32.1	32.0	32.0	31.1	31.2	31.2	31.3
0.1	32.1	32.3	32.3	32.0	31.2	31.2	31.1	31.2
0.32	32.3	32.3	32.2	32.2	31.2	31.2	31.2	31.2
1	32.3	32.2	32.0	32.5	31.2	31.1	31.1	31.1
3.2	32.2	32.3	32.5	32.2	31.1	31.1	31.1	31.1
10	32.3	32.2	32.1	32.4	31.2	31.2	31.2	31.2

Concentration mg/L	Day 13 before renewal - pH				Day 13 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	8.1	8.0	8.0	7.9	7.8	7.8	7.8	7.8
0.1	8.0	8.0	7.9	7.9	7.8	7.8	7.8	7.8
0.32	7.9	7.9	8.0	8.0	7.9	7.9	7.8	7.8
1	8.0	8.0	8.0	8.0	7.9	7.9	7.9	7.9
3.2	8.0	8.0	8.0	8.0	7.9	7.9	7.8	7.9
10	8.0	8.0	8.0	8.0	7.8	7.8	7.8	7.8

Concentration mg/L	Day 13 before renewal - Temp. °C				Day 13 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	24.0	24.0	24.0	24.0	24.3	24.4	24.3	24.3
0.1	24.0	24.0	24.0	24.0	24.2	24.2	24.3	24.2
0.32	24.0	24.1	24.1	24.3	24.2	24.2	24.2	24.3
1	24.3	24.4	24.4	24.4	24.2	24.2	24.2	24.3
3.2	24.4	24.4	24.4	24.4	24.2	24.2	24.2	24.2
10	24.4	24.4	24.4	24.4	24.2	24.1	24.2	24.2

Concentration mg/L	Day 13 before renewal - Oxygen %				Day 13 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	90.0	83.0	86.0	91.0	94.0	95.0	94.0	95.0
0.1	92.0	92.0	92.0	93.0	96.0	96.0	95.0	96.0
0.32	94.0	94.0	94.0	95.0	97.0	98.0	97.0	97.0
1	96.0	96.0	96.0	96.0	98.0	98.0	98.0	98.0
3.2	96.0	95.0	95.0	95.0	99.0	99.0	99.0	99.0
10	95.0	93.0	91.0	91.0	98.0	99.0	99.0	100

Concentration mg/L	Day 13 before renewal - Salinity ‰				Day 13 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	31.3	31.4	31.3	31.4	31.0	31.1	31.1	31.1
0.1	31.3	31.4	31.2	31.3	31.1	31.0	31.0	31.0
0.32	31.4	31.2	31.2	31.3	31.0	31.0	31.0	31.0
1	31.3	31.1	31.3	31.3	31.0	31.0	31.0	31.0
3.2	31.3	31.3	31.3	31.2	31.0	31.0	31.0	31.0
10	31.3	31.3	31.4	31.3	31.0	31.1	31.0	31.0

Concentration mg/L	Day 15 before renewal - pH				Day 15 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	7.9	7.9	8.0	7.9	7.9	7.9	7.9	7.9
0.1	8.0	8.0	8.0	8.0	7.9	7.9	7.9	7.9
0.32	7.9	7.9	7.9	8.0	7.9	8.0	8.0	8.0
1	8.0	8.0	8.0	8.0	7.9	7.9	7.9	7.9
3.2	8.0	8.0	8.0	8.0	7.9	7.9	7.9	7.9
10	7.9	7.9	7.9	8.0	7.9	7.9	7.9	7.9

Concentration mg/L	Day 15 before renewal - Temp. °C				Day 15 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	24.5	24.5	24.4	24.5	24.3	24.4	24.3	24.4
0.1	24.4	24.4	24.4	24.4	24.3	24.3	24.4	24.4
0.32	24.4	24.4	24.5	24.5	24.4	24.4	24.4	24.4
1	24.5	24.5	24.5	24.5	24.3	24.4	24.3	24.2
3.2	24.5	24.5	24.6	24.6	24.2	24.2	24.2	24.3
10	24.5	24.6	24.6	24.6	24.2	24.2	24.2	24.2

Concentration mg/L	Day 15 before renewal - Oxygen %				Day 15 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	90.0	88.0	87.0	87.0	93.0	94.0	95.0	95.0
0.1	87.0	89.0	91.0	92.0	96.0	96.0	95.0	96.0
0.32	90.0	87.0	86.0	87.0	96.0	98.0	99.0	99.0
1	89.0	90.0	91.0	92.0	99.0	99.0	99.0	99.0
3.2	92.0	91.0	90.0	91.0	99.0	100	99.0	99.0
10	90.0	88.0	87.0	87.0	99.0	99.0	99.0	99.0

Concentration mg/L	Day 15 before renewal - Salinity ‰				Day 15 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	31.3	31.4	31.4	31.3	31.6	31.7	31.7	31.7
0.1	31.4	31.3	31.3	31.3	31.1	31.0	31.0	31.0
0.32	31.3	31.2	31.2	31.3	31.5	31.5	31.4	31.5
1	31.3	31.3	31.3	31.2	31.1	31.1	31.1	31.1
3.2	31.3	31.2	31.2	31.2	31.1	31.0	31.0	31.0
10	31.3	31.4	31.3	31.3	30.9	30.9	30.9	30.9

Concentration mg/L	Day 18 before renewal - pH				Day 18 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	7.6	7.7	7.6	7.6	7.9	7.9	7.9	7.9
0.1	7.7	7.6	7.7	7.7	7.9	7.8	7.8	7.8
0.32	7.7	7.6	7.6	7.7	7.8	7.8	7.8	7.8
1	7.6	7.7	7.7	7.7	7.8	7.8	7.8	7.8
3.2	7.7	7.7	7.7	7.7	7.8	7.8	7.8	7.8
10	7.6	7.6	7.6	7.7	7.9	7.9	7.9	7.9

Concentration mg/L	Day 18 before renewal - Temp. °C				Day 18 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	24.3	24.3	24.3	24.3	24.2	24.2	24.2	24.2
0.1	24.3	24.3	24.3	24.2	24.2	24.1	24.1	24.1
0.32	24.2	24.3	24.3	24.3	24.0	24.1	24.1	24.1
1	24.2	24.3	24.3	24.2	24.1	24.1	24.0	24.0
3.2	24.3	24.3	24.3	24.3	24.0	24.0	24.0	24.0
10	24.3	24.4	24.4	24.5	24.0	24.0	24.0	24.0

Concentration mg/L	Day 18 before renewal - Oxygen %				Day 18 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	84.0	75.0	80.0	82.0	100	98.0	98.0	98.0
0.1	83.0	83.0	80.0	81.0	99.0	100	100	100
0.32	84.0	82.0	81.0	83.0	100	100	100	100
1	83.0	83.0	85.0	83.0	100	100	100	100
3.2	82.0	80.0	79.0	81.0	100	100	100	100
10	78.0	77.0	78.0	80.0	100	100	100	100

Concentration mg/L	Day 18 before renewal - Salinity ‰				Day 18 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	32.0	32.2	32.0	32.2	31.6	31.7	31.6	31.6
0.1	31.5	31.5	31.7	31.5	31.1	31.0	31.0	31.0
0.32	31.9	32.1	31.9	31.9	31.0	31.0	31.0	31.1
1	31.6	31.7	31.7	31.5	31.1	31.0	31.0	31.0
3.2	31.6	31.5	31.7	31.4	30.8	31.1	31.1	31.0
10	31.3	31.5	31.4	31.4	30.9	31.1	31.2	31.1

Concentration mg/L	Day 20 before renewal - pH				Day 20 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	7.7	7.7	7.8	7.8	7.8	7.9	7.8	7.8
0.1	7.9	7.9	7.8	7.9	7.8	7.8	7.9	7.8
0.32	7.9	7.9	7.9	7.8	7.9	8.0	7.9	7.8
1	7.9	7.9	7.9	7.8	7.9	7.9	7.8	7.9
3.2	7.8	7.8	7.8	7.9	7.8	7.8	7.9	7.9
10	7.8	7.8	8.0	7.8	7.8	7.8	7.8	7.9

Concentration mg/L	Day 20 before renewal - Temp. °C				Day 20 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	23.1	23.1	23.1	23.1	22.5	22.5	22.4	22.4
0.1	23.2	23.0	23.0	23.0	22.4	22.6	22.7	22.7
0.32	23.0	23.0	23.0	23.2	22.5	22.7	23.1	23.0
1	23.0	23.1	23.2	23.3	23.2	23.0	22.9	23.0
3.2	23.1	23.1	23.1	23.1	23.2	23.0	22.9	23.1
10	23.1	23.1	23.2	23.2	22.9	22.8	22.7	22.9

Concentration mg/L	Day 20 before renewal - Oxygen %				Day 20 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	60.0	54.0	63.0	66.0	100	100	100	100
0.1	78.0	82.0	81.0	84.0	100	100	100	100
0.32	84.0	82.0	83.0	82.0	100	100	99.0	100
1	85.0	86.0	82.0	79.0	100	100	100	100
3.2	78.0	78.0	78.0	82.0	100	100	100	100
10	81.0	77.0	73.0	73.0	100	100	100	100

Concentration mg/L	Day 20 before renewal - Salinity ‰				Day 20 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	31.7	31.7	31.7	31.7	31.0	31.0	30.9	30.9
0.1	31.2	31.1	31.1	31.1	30.9	30.9	30.9	30.9
0.32	31.1	31.1	31.1	31.0	30.9	31.0	31.0	30.9
1	31.1	31.1	31.1	31.1	30.9	30.9	31.0	30.9
3.2	31.1	31.1	31.1	31.1	30.9	30.9	30.9	30.9
10	31.1	31.1	31.2	31.1	30.9	31.0	31.0	30.9

Concentration mg/L	Day 22 before renewal - pH				Day 22 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	7.9	7.9	8.0	8.0	7.9	7.9	7.9	7.9
0.1	8.0	7.9	8.0	8.0	7.9	7.9	7.9	7.9
0.32	8.1	8.1	8.0	7.9	7.9	7.9	7.9	7.9
1	8.1	8.2	7.9	8.1	7.9	7.9	7.9	7.9
3.2	8.1	8.0	8.2	8.1	7.9	7.9	7.9	7.9
10	8.0	8.1	8.0	8.0	7.9	7.9	7.9	7.9

Concentration mg/L	Day 22 before renewal - Temp. °C				Day 22 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	24.7	24.8	24.9	24.9	24.9	24.9	24.9	24.9
0.1	24.8	24.9	24.9	24.9	24.9	24.9	24.9	25.0
0.32	24.8	24.7	24.7	24.9	25.0	25.0	24.9	24.9
1	24.8	24.8	24.8	24.9	24.9	24.9	24.8	24.8
3.2	24.7	24.7	24.9	24.8	24.7	24.8	24.8	24.7
10	24.7	24.7	24.9	25.0	24.7	24.8	24.9	24.0

Concentration mg/L	Day 22 before renewal - Oxygen %				Day 22 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	91.0	95.0	94.0	94.0	100	100	100	100
0.1	100	93.0	96.0	98.0	100	100	100	99.0
0.32	100	100	86.0	92.0	100	100	100	100
1	99.0	100	92.0	97.0	100	100	100	100
3.2	97.0	90.0	96.0	100	100	100	100	100
10	96.0	97.0	98.0	99.0	99.0	99.0	99.0	99.0

Concentration mg/L	Day 22 before renewal - Salinity ‰				Day 22 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	31.0	31.1	31.0	31.0	30.9	30.9	30.9	31.0
0.1	31.0	31.0	31.1	31.1	30.9	30.9	31.0	31.0
0.32	31.0	31.2	31.1	31.2	30.8	30.9	31.0	30.9
1	31.0	31.0	31.0	31.0	30.8	30.9	30.9	31.0
3.2	31.0	31.0	31.2	31.0	30.9	30.9	30.9	30.9
10	31.0	31.0	31.0	31.0	30.8	30.9	30.9	30.9

Concentration mg/L	Day 25 before renewal - pH				Day 25 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	7.9	8.0	8.0	8.0	7.8	7.8	7.8	7.8
0.1	8.0	8.0	8.0	8.0	7.9	7.8	7.8	7.8
0.32	8.1	8.1	8.1	8.1	7.8	7.8	7.9	7.8
1	8.1	8.1	8.1	8.1	7.8	7.8	7.8	7.8
3.2	8.1	8.1	8.1	8.1	7.8	7.8	7.8	7.8
10	7.8	8.0	8.0	8.0	7.8	7.8	7.8	7.8

Concentration mg/L	Day 25 before renewal - Temp. °C				Day 25 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	24.0	24.7	25.0	25.0	25.0	25.0	25.0	25.1
0.1	25.1	25.3	25.4	25.3	24.7	24.8	24.9	24.9
0.32	25.3	25.1	25.1	24.9	24.9	25.0	24.9	24.9
1	24.6	24.9	24.9	25.1	24.9	25.0	24.9	24.8
3.2	25.0	24.9	25.1	25.1	24.8	24.7	24.5	24.4
10	24.9	25.2	25.1	25.3	24.6	24.6	24.2	24.1

Concentration mg/L	Day 25 before renewal - Oxygen %				Day 25 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	92.0	94.0	98.0	98.0	100	100	100	100
0.1	97.0	96.0	98.0	98.0	100	100	100	100
0.32	98.0	98.0	99.0	99.0	100	100	100	100
1	99.0	98.0	99.0	98.0	100	100	100	100
3.2	99.0	99.0	98.0	99.0	100	100	100	100
10	66.0	90.0	95.0	96.0	100	100	100	100

Concentration mg/L	Day 25 before renewal - Salinity ‰				Day 25 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	31.1	31.1	31.1	31.2	30.9	30.9	31.0	31.0
0.1	31.0	31.0	31.1	31.1	31.0	31.0	31.0	31.0
0.32	31.0	31.2	31.2	31.3	30.9	31.0	31.0	31.0
1	31.0	31.2	31.1	31.1	30.9	31.0	31.0	31.0
3.2	31.0	31.1	31.1	31.2	30.9	31.0	31.0	31.0
10	30.9	31.3	31.1	31.1	30.9	31.0	31.0	31.0

Concentration mg/L	Day 27 before renewal - pH				Day 27 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	8.0	8.0	8.0	8.0	7.8	7.8	7.8	7.8
0.1	7.9	7.9	8.0	8.0	7.8	7.8	7.8	7.8
0.32	8.0	8.0	8.0	8.0	7.8	7.8	7.9	7.9
1	8.1	8.1	8.1	8.1	7.8	7.9	7.9	7.8
3.2	8.1	8.1	8.0	7.9	7.9	7.9	7.9	7.8
10	8.1	8.0	7.9	8.0	7.9	7.9	7.8	7.8

Concentration mg/L	Day 27 before renewal - Temp. °C				Day 27 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	24.5	24.5	24.5	24.5	24.4	24.3	24.5	24.5
0.1	24.6	24.7	24.8	24.9	24.5	24.8	24.9	24.8
0.32	24.9	24.9	24.8	24.7	25.0	25.0	25.0	25.0
1	24.8	24.6	24.5	24.6	24.8	24.8	24.7	24.7
3.2	24.5	24.4	24.6	24.7	24.8	24.8	24.8	24.7
10	24.7	24.9	25.1	25.2	24.8	24.8	24.7	24.6

Concentration mg/L	Day 27 before renewal - Oxygen %				Day 27 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	93.0	95.0	97.0	90.0	100	100	100	100
0.1	97.0	96.0	96.0	96.0	100	100	100	100
0.32	98.0	98.0	98.0	98.0	100	98.0	98.0	98.0
1	99.0	99.0	99.0	98.0	100	100	100	100
3.2	100	98.0	98.0	93.0	100	100	100	100
10	98.0	97.0	96.0	94.0	100	100	100	100

Concentration mg/L	Day 27 before renewal - Salinity ‰				Day 27 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	31.1	31.1	31.1	31.1	31.0	31.1	31.0	31.1
0.1	31.1	31.0	31.0	31.0	31.0	30.9	30.9	30.9
0.32	31.1	31.1	31.1	31.1	30.8	31.0	30.9	31.0
1	31.2	31.3	31.2	31.1	30.9	31.0	31.0	31.0
3.2	31.2	31.0	31.1	31.0	30.9	31.0	31.0	31.0
10	31.2	31.1	31.0	31.0	31.0	30.9	31.0	30.9

Concentration mg/L	Day 29 before renewal - pH				Day 29 after renewal - pH			
	A	B	C	D	A	B	C	D
Control	8.1	8.0	8.0	8.1	8.0	7.9	7.9	7.9
0.1	8.0	7.9	8.0	8.0	7.9	7.9	7.9	7.9
0.32	8.1	7.6	8.2	8.1	7.9	7.9	7.9	7.9
1	8.1	8.2	8.1	8.1	7.9	7.9	7.9	7.9
3.2	8.0	7.8	8.1	8.0	7.9	7.9	7.9	7.9
10	8.0	7.8	8.0	8.0	7.9	7.9	7.9	7.9

Concentration mg/L	Day 29 before renewal - Temp. °C				Day 29 after renewal - Temp. °C			
	A	B	C	D	A	B	C	D
Control	24.5	24.5	24.8	25.1	24.8	24.9	25.0	25.2
0.1	25.1	25.0	25.1	25.1	25.1	25.2	24.9	25.1
0.32	25.2	25.1	25.1	25.2	24.7	24.8	24.8	25.0
1	25.2	25.1	25.1	25.3	24.8	24.8	24.9	24.6
3.2	25.2	25.1	25.0	25.0	24.8	24.7	24.6	24.7
10	25.2	25.2	25.2	25.3	24.6	24.6	24.5	24.5

Concentration mg/L	Day 29 before renewal - Oxygen %				Day 29 after renewal - Oxygen %			
	A	B	C	D	A	B	C	D
Control	97.0	97.0	96.0	98.0	97.0	97.0	98.0	98.0
0.1	97.0	96.0	96.0	96.0	98.0	96.0	100	100
0.32	98.0	87.0	98.0	98.0	100	100	100	100
1	98.0	99.0	99.0	99.0	100	100	100	100
3.2	99.0	93.0	90.0	95.0	100	100	100	100
10	95.0	90.0	95.0	96.0	100	100	100	100

Concentration mg/L	Day 29 before renewal - Salinity ‰				Day 29 after renewal - Salinity ‰			
	A	B	C	D	A	B	C	D
Control	31.2	31.2	31.0	31.0	31.0	31.0	31.0	30.9
0.1	31.0	31.0	31.1	31.1	30.9	30.9	31.0	31.0
0.32	31.0	31.1	31.1	31.1	31.0	31.0	30.9	30.9
1	31.0	31.1	31.2	31.1	31.0	31.0	30.9	31.1
3.2	31.0	31.1	31.2	31.0	30.9	30.9	31.0	31.0
10	31.0	31.0	31.1	31.0	31.0	30.9	31.0	31.0

Concentration mg/L	End day 32 - pH			
	A	B	C	D
Control	8.2	8.0	8.1	8.1
0.1	7.9	7.9	8.0	8.0
0.32	7.9	8.1	8.1	8.1
1	8.0	8.1	8.0	8.0
3.2	8.0	8.1	8.1	8.0
10	8.0	8.0	7.9	7.9

Concentration mg/L	End day 32 - Temp. °C			
	A	B	C	D
Control	23.9	24.0	24.0	24.3
0.1	24.3	24.4	24.3	24.3
0.32	24.1	24.0	24.0	24.2
1	24.0	24.0	24.0	24.1
3.2	24.0	24.0	24.0	24.0
10	24.0	24.0	24.0	24.0

Concentration mg/L	End day 32 – Oxygen %			
	A	B	C	D
Control	99.0	97.0	99.0	99.0
0.1	98.0	97.0	100	99.0
0.32	99.0	100	100	99.0
1	99.0	100	99.0	98.0
3.2	99.0	100	100	99.0
10	100	99.0	98.0	97.0

Concentration mg/L	End day 32 – Salinity ‰			
	A	B	C	D
Control	31.4	31.2	31.2	31.5
0.1	31.0	31.0	31.4	31.1
0.32	31.3	31.7	31.9	31.3
1	31.3	31.4	31.6	31.2
3.2	31.1	31.3	31.4	31.2
10	31.2	31.5	31.1	31.3

B.1.2 Statistical analysis – Determination of effect concentration after exposure to SIBX

B.1.2.1 Embryonic development, hatching time and survival of embryo and larvae/fish

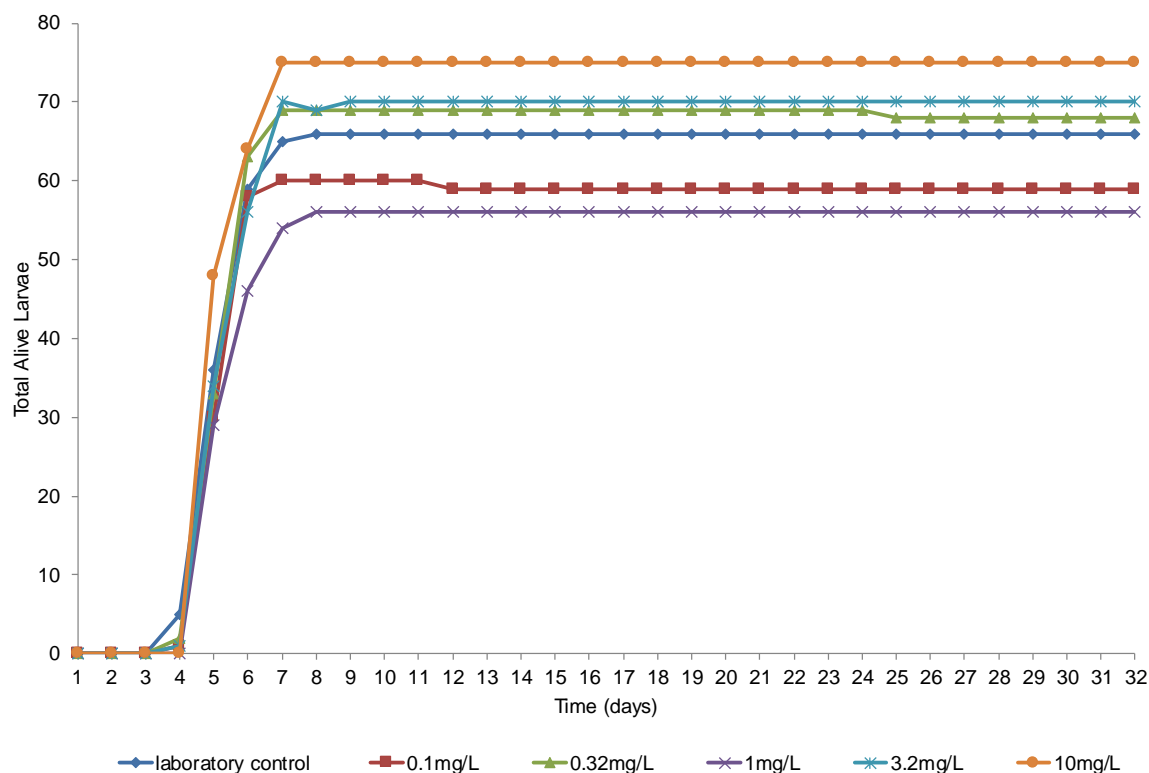


Figure B. 1 Embryonic development time and number of hatched larvae/fish alive of *Cyprinodon variegatus* observed against the time with different concentrations of SIBX.

B.1.2.2 Determination of NOEC and LOEC for the hatching success

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

Replicate No.	0 mg/L	0.1 mg/L	0.32 mg/L	1 mg/L	3.2 mg/L	10 mg/L
1	90	75	85	70	90	95
2	85	75	80	65	85	85
3	75	80	90	70	90	95
4	80	75	100	80	95	100
Count	4	4	4	4	4	4
Mean	83	76	89	71	90	94

- Normality

The data distribution was not established because only three replicates were available per test group for these two endpoints. A normal distribution in each test group was assumed in the following study.

- Dunnett's test

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

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

Mean Comparisons	Estimate	Std. Error	t value	Pr(> t)	Significance ¹⁾
SIBX-0.1-mg/L - SIBX-0-mg/L = 0	-0.063	0.042	-1.47	0.46	
SIBX-0.32-mg/L - SIBX-0-mg/L = 0	0.063	0.042	1.47	0.46	
SIBX-1-mg/L - SIBX-0-mg/L = 0	-0.11	0.042	-2.65	0.063	.
SIBX-3.2-mg/L - 0 SIBX-0-mg/L = 0	0.075	0.042	1.77	0.30	
SIBX-10.0-mg/L - SIBX-0-mg/L = 0	0.11	0.042	2.65	0.063	.

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 SIBX is significantly different from the laboratory control was rejected ($t > 0$ and $P > 0.05$) at all the tested concentrations (Table B. 5).

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

Endpoint	NOEC	LOEC	Unit
Hatching success	10	>10	mg/L

B.1.2.3 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 B. 6 Hatching success endpoint, estimations of EC10 and EC50.

Endpoint	EC10 (mg/L)	EC50 (mg/L)
Hatching success	>10	>10

B.1.2.4 Determination of NOEC and LOEC for the total mortality

Table B. 7 Experimental data the total mortality (as survival, %).

Replicate No.	0 mg/L	0.1 mg/L	0.32 mg/L	1 mg/L	3.2 mg/L	10 mg/L
1	90	70	85	70	90	95
2	85	75	80	60	85	85
3	75	80	90	70	80	90
4	80	70	85	80	95	100
Count	4	4	4	4	4	4
Mean	83	74	85	70	88	93

- Normality

The data distribution was not established because only three replicates were available per test group for these two endpoints. A normal distribution in each test group was assumed in the following study.

- Dunnett's test

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

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

Mean comparisons	Estimate	Std. Error	t value	Pr(> t)	Significance ¹⁾
SIBX-0.1-mg/L - SIBX-0-mg/L = 0	-0.088	0.044	-1.99	0.21	
SIBX-0.32-mg/L - SIBX-0-mg/L = 0	0.025	0.044	0.57	0.97	
SIBX-1-mg/L - SIBX-0-mg/L = 0	-0.13	0.044	-2.85	0.042	*
SIBX-3.2-mg/L - SIBX-0-mg/L = 0	0.050	0.044	1.14	0.68	
SIBX-10.0-mg/L - SIBX-0-mg/L = 0	0.10	0.044	2.28	0.13	

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 group with SIBX is significantly lower than the laboratory control group was verified ($t < 0$ and $P < 0.05$) at 1 mg/L. However, no other significant effects were observed and therefore, no dose response relationship was present (Table B. 8)

Table B. 9 Total mortality endpoint: estimations of NOEC and LOEC, Dunnett's test.

Endpoint	NOEC	LOEC	Unit
Total mortality	10	>10	mg/L

B.1.2.5 Results of the Probit analysis

As no difference was observed among the test groups, the data to estimate the LCX on the total mortality were not computed.

Table B. 10 Total mortality endpoint, estimations of LC10 and LC50.

Endpoint	LC10 (mg/L)	LC50 (mg/L)
Total mortality	>10	>10

B.1.2.6 Determination of NOEC and LOEC for the body length

Table B. 11 Experimental data of the body length (mm).

Replicate No.	Length (mm)					
	Control	0.1 mg/L	0.32 mg/L	1 mg/L	3.2 mg/L	10 mg/L
1	12.0	13.0	13.0	13.0	10.0	14.5
2	14.0	13.5	13.5	11.0	12.5	12.5
3	15.0	14.5	12.0	14.0	12.5	13.0
4	12.5	14.0	12.0	14.0	14.0	16.0
5	13.0	13.0	12.0	13.0	13.0	11.0
6	13.5	12.5	9.5	12.0	13.5	10.0
7	14.0	14.0	12.5	12.0	12.5	15.0
8	14.0	12.5	12.0	11.5	12.0	12.0
9	12.0	13.0	13.5	12.0	13.0	12.0
10	12.0	13.0	14.0	13.0	12.0	11.0
11	14.0	12.5	10.0	12.0	11.0	12.0
12	13.5	12.0	10.5	11.5	12.5	12.0
13	13.5	14.0	12.5	12.5	13.0	13.0
14	12.0	12.5	10.5	13.5	15.0	14.0
15	11.5	16.0	11.0	15.5	8.0	12.0
16	12.0	14.5	9.5	13.0	9.5	11.0
17	10.0	14.0	6.5	13.5	12.5	11.0
18	10.5	12.0	15.0	15.0	10.5	9.5
19	15.5	12.0	17.0	13.0	12.0	9.0
20	15.0	12.0	14.5	15.0	10.5	15.0
21	11.0	15.0	11.5	10.0	10.5	15.0
22	14.5	12.5	12.0	12.0	14.0	13.0
23	14.0	15.5	11.0	10.5	16.5	13.0
24	13.5	14.0	12.5	12.5	13.0	14.0
25	12.5	14.0	13.5	13.0	11.5	14.0
26	13.0	12.5	12.0	12.0	12.0	12.5
27	13.5	14.0	12.0	12.0	12.0	12.0
28	12.0	11.5	8.5	15.0	12.0	13.0
29	15.0	9.5	12.0	12.0	11.5	13.0
30	12.0	12.5	10.5	11.5	12.5	11.0
31	13.5	12.0	8.5	13.5	13.0	12.0
32	14.0	12.5	9.5	11.5	12.0	13.0
33	12.0	11.5	10.5	13.5	13.5	12.0
34	12.5	13.0	12.0	13.5	11.0	11.0
35	12.5	11.0	12.5	12.0	12.0	11.5
36	15.5	14.0	11.5	13.5	13.0	12.0
37	13.0	15.0	12.0	13.5	12.5	13.5
38	8.5	11.0	15.5	12.5	15.0	14.0
39	14.0	12.0	12.0	13.0	13.0	15.0
40	13.0	14.0	11.0	12.0	13.5	12.0

Replicate No.	Length (mm)					
	Control	0.1 mg/L	0.32 mg/L	1 mg/L	3.2 mg/L	10 mg/L
41	10.0	12.0	10.5	12.5	13.0	11.0
42	13.5	13.0	12.0	12.0	13.0	12.0
43	13.0	12.0	11.0	11.5	13.0	11.5
44	12.5	12.0	11.5	13.0	14.0	11.0
45	14.5	10.5	11.5	12.0	12.0	13.0
46	13.0	12.0	12.5	15.5	12.0	12.0
47	10.5	13.5	11.5	13.0	13.0	12.0
48	14.0	12.5	11.0	15.5	12.0	13.5
49	12.0	12.0	11.0	14.0	12.0	12.5
50	13.0	13.5	9.5	14.0	12.5	11.0
51	13.5	12.5	9.5	11.0	11.0	14.0
52	14.0	15.0	15.0	11.5	9.5	10.0
53	12.0	12.0	14.0	13.5	13.5	11.0
54	9.0	12.0	12.0	12.0	15.0	11.0
55	14.0	13.5	12.5	13.0	12.0	14.0
56	12.0	13.5	12.0	13.0	13.0	13.0
57	16.0	12.0	12.5	-	11.0	16.0
58	12.5	12.5	13.0	-	12.5	15.5
59	12.0	15.5	10.0	-	12.0	12.5
60	11.0	-	11.5	-	12.0	14.0
61	13.0	-	12.0	-	12.0	15.0
62	12.0	-	13.0	-	9.5	12.0
63	13.0	-	13.5	-	11.5	14.0
64	12.0	-	12.5	-	12.0	13.5
65	13.0	-	13.0	-	14.0	9.5
66	12.0	-	10.0	-	11.5	12.0
67	-	-	14.0	-	13.5	12.0
68	-	-	11.0	-	13.0	11.5
69	-	-	-	-	12.0	14.0
70	-	-	-	-	10.0	11.0
71	-	-	-	-	-	11.5
72	-	-	-	-	-	12.0
73	-	-	-	-	-	11.0
74	-	-	-	-	-	9.5
Count	66	59	68	56	70	74
Mean size	12.8	12.9	11.8	12.8	12.3	12.4
Stdev.	1.5	1.3	1.7	1.2	1.4	1.6

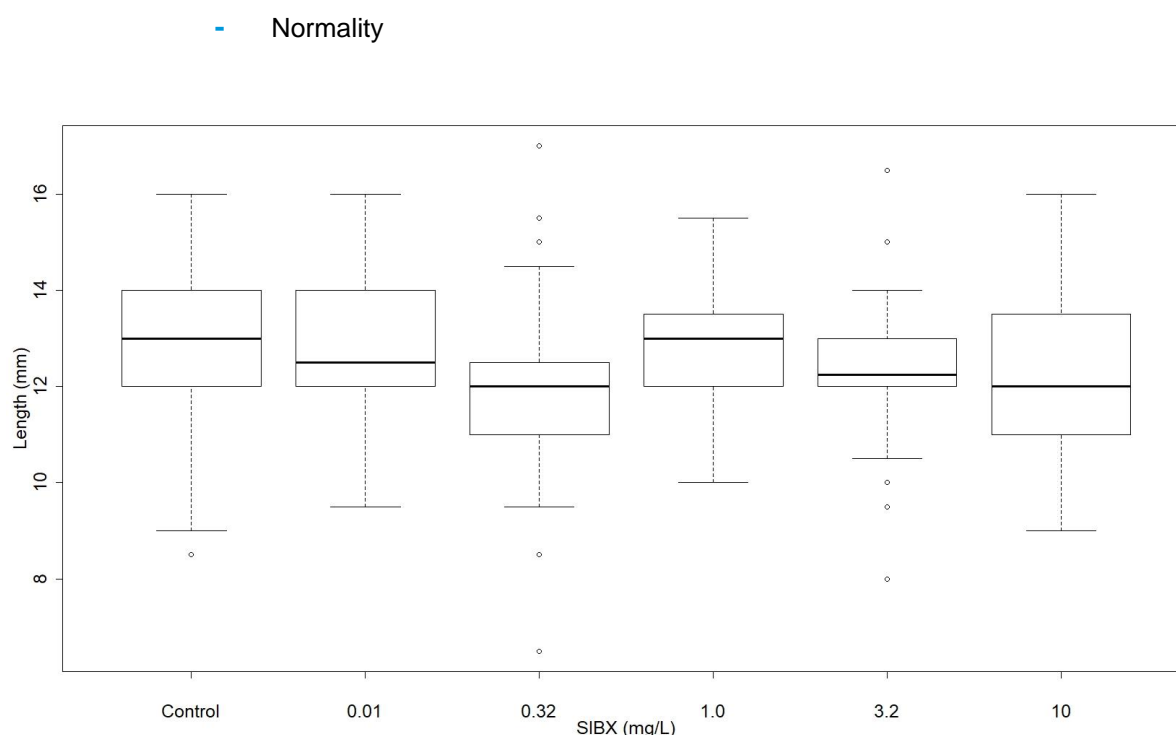


Figure B. 2 Boxplots of every test groups for the body length.

The boxplot patterns show the data distribution (Figure B. 2). A normal distribution in each test group was assumed in the following study.

- Dunnett's test

Dunnett's test tests the null hypothesis that the averages of every test group are not different from the control group (Table B. 12).

Table B. 12 Multiple comparisons of means: Dunnett's contrasts.

Mean comparisons	Estimate	Std. Error	t value	Pr(> t)	Significance ¹⁾
SIBX-0.1-mg/L - SIBX-0-mg/L = 0	0.11	0.27	0.43	0.99	
SIBX-0.32-mg/L - SIBX-0-mg/L = 0	-0.99	0.27	-3.86	<0.001	***
SIBX-1-mg/L - SIBX-0-mg/L = 0	-0.032	0.27	-0.12	1	
SIBX-3.2-mg/L - SIBX-0-mg/L = 0	-0.53	0.25	-2.07	0.15	
SIBX-10.0-mg/L - SIBX-0-mg/L = 0	-0.37	0.25	-1.49	0.43	

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 group with SIBX is significantly lower than the laboratory control group was verified ($t < 0$ and $P < 0.05$) at 0.32 mg/L. However, no other significant effects were observed and therefore, no dose response relationship was present (Table B. 12).

Table B. 13 Body length endpoint: estimations of NOEC and LOEC, Dunnett's test.

Endpoint	NOEC	LOEC	Unit
Body length	10	>10	mg/L

B.1.2.7 Results of the Probit analysis

As no dose response was observed among the test groups, the data to estimate the ECX on the total mortality were not computed.

Table B. 14 Body length endpoint, estimations of EC10 and EC50.

Endpoint	EC10 (mg/L)	EC50 (mg/L)
Body length	>10	>10

B.1.2.8 Determination of NOEC and LOEC for the body weight

Table B. 15 Experimental data of the body weight (mg).

Replicate No.	Weight (mg)					
	Control	0.1 mg/L	0.32 mg/L	1 mg/L	3.2 mg/L	10 mg/L
1	18.7	30.3	28.2	27.0	13.2	33.8
2	35.1	33.3	31.4	19.4	23.0	21.8
3	42.9	36.7	25.0	32.9	23.1	23.8
4	21.7	30.7	17.9	31.7	35.1	48.1
5	28.6	24.4	21.5	31.9	21.9	12.8
6	25.6	19.3	9.1	18.9	27.1	11.0
7	30.7	31.8	27.5	22.1	21.8	38.1
8	33.5	17.9	22.3	16.1	19.4	18.5
9	20.8	30.8	31.1	18.8	26.7	19.2
10	16.5	22.7	34.2	24.6	23.1	13.5
11	31.1	25.7	15.1	20.8	16.8	21.4
12	31.1	22.6	15.2	15.4	22.5	17.7
13	30.3	31.3	29.6	20.3	23.8	29.1
14	23.6	19.6	14.4	27.5	41.1	30.1
15	18.4	44.5	14.1	48.4	5.0	16.2
16	17.9	37.7	11.5	27.8	8.9	13.8
17	9.4	32.2	5.5	31.1	22.4	16.5
18	14.3	18.8	38.6	51.4	13.3	8.8
19	41.4	18.5	60.8	27.6	22.3	7.6
20	35.2	19.4	34.8	43.0	14.7	40.3
21	16.7	39.1	14.6	10.1	14.5	36.3
22	35.7	19.9	20.5	21.9	33.4	30.5
23	32.4	50.2	13.6	13.6	50.7	22.5
24	26.8	26.1	22.7	20.7	21.7	30.3
25	19.9	33.5	26.6	26.9	16.4	29.8
26	23.7	22.7	22.0	17.0	18.3	21.9
27	29.6	28.7	15.6	15.9	16.5	21.6
28	16.7	13.1	6.7	39.5	18.3	21.1
29	36.1	8.5	18.4	22.0	18.5	30.1
30	14.3	22.5	10.5	18.9	21.9	13.3
31	30.9	18.7	6.5	28.5	23.9	20.2
32	35.2	19.4	7.9	16.4	19.0	24.6
33	19.4	17.1	12.8	27.8	32.0	21.1
34	18.2	25.7	20.0	28.1	13.5	17.2
35	16.0	14.7	23.0	17.9	19.4	21.1
36	42.9	37.3	18.1	30.7	26.7	21.7
37	25.6	39.9	18.2	30.3	23.4	30.4
38	8.7	13.2	55.1	22.4	36.9	31.2
39	30.6	19.1	20.5	25.6	25.3	44.9
40	25.1	41.3	15.2	24.4	26.6	22.4

Replicate No.	Weight (mg)					
	Control	0.1 mg/L	0.32 mg/L	1 mg/L	3.2 mg/L	10 mg/L
41	12.1	24.6	13.7	20.5	29.0	15.8
42	31.2	30.2	19.5	22.5	25.4	17.6
43	30.3	24.0	15.7	16.3	26.2	15.9
44	20.0	19.9	18.1	23.2	34.0	16.7
45	35.6	12.9	16.5	17.5	18.6	22.5
46	27.6	23.1	20.0	46.2	19.3	20.8
47	16.6	30.2	14.7	27.0	25.7	15.6
48	29.2	25.9	15.0	45.8	22.1	29.4
49	20.1	21.9	15.4	27.4	20.3	25.5
50	27.6	31.0	8.1	27.3	20.7	15.0
51	26.3	20.4	7.9	14.7	17.0	30.1
52	27.1	45.9	43.3	16.1	8.7	10.7
53	18.5	22.8	31.2	28.4	31.5	15.6
54	6.2	21.2	17.1	20.3	38.1	18.2
55	35.2	30.9	19.5	24.1	22.6	28.8
56	18.5	29.0	19.2	24.7	23.3	26.8
57	47.8	21.5	20.5	-	14.1	49.5
58	22.4	24.2	25.9	-	20.5	44.1
59	22.9	48.2	12.4	-	16.4	25.3
60	10.7	-	15.4	-	23.1	35.0
61	27.0	-	19.4	-	20.8	38.2
62	20.1	-	22.4	-	10.5	17.2
63	28.8	-	28.3	-	14.9	31.6
64	21.1	-	21.6	-	18.4	30.0
65	31.6	-	30.4	-	31.0	9.3
66	20.8	-	11.7	-	14.0	23.9
67	-	-	35.7	-	28.3	19.3
68	-	-	14.3	-	22.3	17.7
69	-	-	-	-	17.8	30.1
70	-	-	-	-	12.3	13.5
71	-	-	-	-	-	16.7
72	-	-	-	-	-	18.4
73	-	-	-	-	-	15.4
74	-	-	-	-	-	10.4
Count	66	59	68	56	70	74
Mean size	25.2	26.5	20.7	25.3	22.1	23.3
Stdev.	8.8	9.2	10.4	8.9	7.9	9.4

- Normality

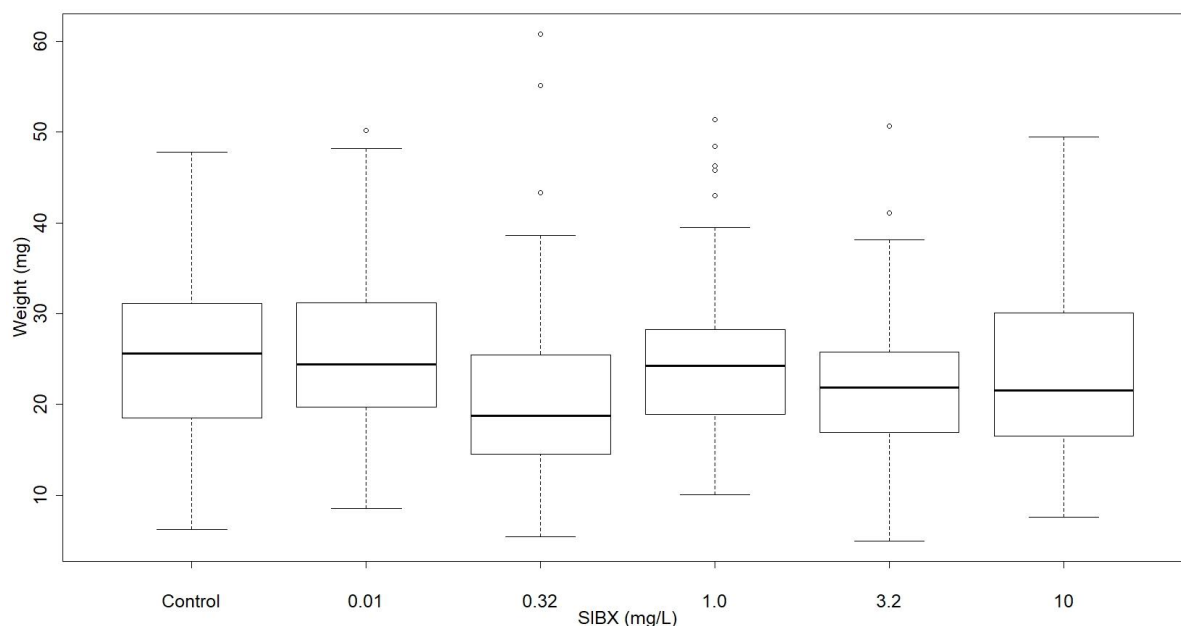


Figure B. 3 Boxplots of every test group for the body weight.

The boxplot patterns show the data distribution (Figure B. 3). A normal distribution in each test group was assumed in the following study.

- Dunnett's test

Dunnett's test tests the null hypothesis that the averages of every test groups are not different from the control group (Table B. 16).

Table B. 16 Multiple comparisons of means: Dunnett's contrasts.

Mean comparisons	Estimate	Std. Error	t value	Pr(> t)	Significance ¹⁾
SIBX-0.1-mg/L - SIBX-0-mg/L = 0	1.30	1.64	0.806	0.89	
SIBX-0.32-mg/L - SIBX-0-mg/L = 0	-4.53	1.58	-2.87	0.019	*
SIBX-1-mg/L - SIBX-0-mg/L = 0	0.066	1.66	0.04	1.00	
SIBX-3.2-mg/L - SIBX-0-mg/L = 0	-3.13	1.57	-2.00	0.17	
SIBX-10.0-mg/L - SIBX-0-mg/L = 0	-1.94	1.55	-1.26	0.59	

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 group with SIBX is significantly lower than the laboratory control group was verified ($t < 0$ and $P < 0.05$) at 0.32 mg/L. However, no other significant effects were observed and therefore, no dose response relationship was present (Table B. 16).

Table B. 17 Body weight endpoint: estimations of NOEC and LOEC, Dunnett's test.

Endpoint	NOEC	LOEC	Unit
Body weight	10	>10	mg/L

B.1.2.9 Results of the Probit analysis

As no dose response was observed among the test groups, the data to estimate the ECX on the total mortality were not computed.

Table B. 18 Body length endpoint, estimations of EC10 and EC50.

Endpoint	EC10 (mg/L)	EC50 (mg/L)
Body weight	>10	>10

B.1.3 Validity criteria

Table B. 19 Validity criteria of the test according to the OECD 210 in the development test with *Cyprinodon variegatus*.

Criteria	Target Value	Fulfilled
Survival of hatched larvae in the control group	> 80% (observed: 82%)	Yes
Hatching success in the control group	> 75% (observed: 83%)	Yes
Survival of hatched larvae in the control group	> 80% (observed: 82%)	Yes
Dissolved oxygen concentration	> 60% throughout test (minimum observed: 54%)	No ¹⁾
Water temperature differing between test chambers or between successive days and within 25 °C	25 °C ± 1.5 °C (observed: 24 ± 0.5)	Yes

- 1) The validity criterion was not fulfilled on day 20 in the lab control A and B (60% and 54%). Therefore, aeration was added to all test containers. During the test period the average dissolved oxygen concentration was 61%. The low oxygen concentration at one test day is not expected to have had influence on the test results.