

Short-term toxicity test with bromate on embryo and sac-fry stages of zebrafish (*Danio rerio*)

DHI Accredited report No.: 211



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



DANAK participates in the multilateral agreements for testing and calibration under the European co-operation for Accreditation (EA) and under the International Laboratory Accreditation Cooperation (ILAC) based on peer evaluation. The use of the accreditation mark on this test protocol documents that the service is provided as an accredited service under the company's DANAK accreditation.



Approved by

22-09-2022

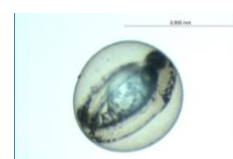
X 

Approved by

Signed by: Gitte Petersen

Short-term toxicity test with bromate on embryo and sac-fry stages of zebrafish (*Danio rerio*)

Prepared for NIRAS
Represented by Anders Sjølin



Zebra fish egg

Project manager	Anja Kamper
Quality supervisor	Gitte Ingelise Petersen

Project number	11827338
Approval date	22-09-2022
Revision	Revised final report / 11827338 / 22-09-2022
Classification	Confidential

CONTENTS

1	Introduction	2
2	Test item	2
3	Test method	2
3.1	Statistical analysis	4
4	Results	4
4.1	Hatching success	5
4.2	Mortality	6
6	References	7

APPENDICES

A	Raw data toxicity test on embryo and sac-fry stages with <i>Danio rerio</i>	11
B	Results from chemical analyses	26
C	Identification of test item (CoA)	33
D	Accreditation Certificate	37

1 Introduction

DHI has conducted an ecotoxicological toxicity test of sodium bromate on zebra fish embryo and sac-fry stages.

The test performed was a chronic test investigating the lethal and, to a limited extent, 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 Testing of Chemicals No. 212 "Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages" /1/.

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

2 Test item

The purpose of the test was to investigate the chronic toxicity of bromate (BrO_3^-), which will be formed if ozone treatment is installed at the treatment plant at Sjölanda (Malmö). DHI has therefore purchased 99.5% pure Sodium Bromate (NaBrO_3) from Sigma-Aldrich (Product No. 71325, Batch No. BCBX9200) for use in the test. The certificate of analysis and the safety data sheet is attached in Annex C.

Table 2.1 Information regarding the test item

Product Name:	Sodium Bromate
Purity	>= 99.5 % RT
Product Number:	71325
Batch Number:	BCBX9200
Brand:	Sigma-Aldrich
CAS Number:	7789-38-0
Formula:	NaBrO_3
Formula Weight:	150.89
Quality Release Date:	03 AUG 2018
Recommended Retest Date:	JAN 2023

3 Test method

The toxicity of bromate to embryo and sac-fry stages of *Danio rerio* (zebra fish) was examined in accordance with the OECD Guideline for the Testing of Chemicals No. 212 /1/.

The test concentrations were prepared as dilutions of a stock solution of the test item with laboratory medium. At every water exchange a new stock solution was prepared. The stock solution was prepared by dissolving 236 mg NaBrO_3 in 2000 ml ISO medium

(described in table 3.1). Adjustment of the pH was necessary on one occasion as the pH was 8.6 which is outside the required range of 7.8 ± 0.2 .

The test was carried out as a semi-static test in 175-mL glass beakers with 150 mL of test mixture. The test was started with newly fertilized eggs and was terminated just before the yolk-sac of any larvae was completely absorbed, or before mortality by starvation was observed in the control.

Three times a week, approx. 95% of each test solution was removed by siphoning and carefully replaced with the same amount of freshly prepared test solution. Shortly after hatching the larvae are attached to the walls of the aquaria for approx. 2 days. During this period the newly hatched larvae are extremely sensitive to disturbance and therefore no water exchange was performed.

The test conditions are summarized in Table 3.1.

Table 3.1 Test conditions for the short-term toxicity test on embryo and sac-fry stages with the fish *Danio rerio*.

Test guideline	OECD 212 /1/
Test organism	<i>Danio rerio</i> (Zebra fish)
Test organism source	Department of Biology at University of Southern Denmark
Test organism life stage	Fertilized eggs (0-8 hours post fertilization)
Test concentrations	Concentration of NaBrO ₃ : 0; 1.4; 4.4; 13; 39 and 118 mg/L corresponding to a concentration of bromate (BrO ₃ ⁻) of 0; 1.2; 3.7; 11; 33 and 100 mg/L
Test duration	5 days post hatch (8-10 days). The test is ended just before: • Yolk-sac has been completely absorbed in any of the larvae or • Mortality by starvation is observed in controls
Test container	175-mL glass beakers (150 mL test solution)
Replicates	• 3 × laboratory control • 3 × test concentration
Eggs per container and eggs per concentration	40 eggs per replicate. After 24 hours of exposure the number of eggs was reduced to 10 eggs per replicate and thus 30 eggs per concentration
Renewal of test solutions	The test solutions are renewed three times a week
Observations	• Cumulative mortality • Number of healthy larvae at the end of the test • Time to start of hatching and end of hatching (i.e. 90% hatching in each replicate) • Number of larvae hatching each day • Length of surviving animals at the end of the test • Number of larvae that are deformed or of abnormal appearance • Number of larvae exhibiting abnormal behaviour

Laboratory control medium	Medium as described in ISO 7346-2 standard /2/.
Food regime	No feeding is applied during the testing period
Photoperiod	12:12 hours
Temperature	25 °C ± 1 °C
Initial pH	In the laboratory control medium: 7.8 ± 0.2
Validity criteria	• Hatching success in the laboratory control: ≥ 80% • Survival of hatched larvae in the laboratory control: ≥ 90% • Dissolved oxygen concentration: ≥ 60% • Water temperature differing between test chambers or between successive days: ± 1.5 °C

The water temperature, pH, and dissolved oxygen were measured at the test start, before and after each water renewal and at the end of the test. The room temperature was measured continuously by a temperature logger.

Larvae and eggs were enumerated every day, abnormal behaviour and dead individuals were reported on daily basis until termination of the test.

Length of surviving animals at the end of the test was measured.

Samples for chemical analysis was withdrawn at every water renewal. Samples from one renewal and two concentrations were analysed.

3.1 Statistical analysis

All the analyses were run using the free software R /3/. The corresponding packages used for the different analysis are described in Section 5, References.

The LOEC/NOEC values were determined by use of the Dunnett's test /4/. 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 /5/. The variance was analysed with a Levene's test /6/.

4 Results

4.1 Results from chemical analysis

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.

The samples were collected in 60-mL PE bottles containing NaOH and EDTA for conservation and stored at $4 \pm 2.0^{\circ}\text{C}$. The samples were shipped cold to the analytical laboratory, ALS Scandinavia AB, Danderyd, Sweden.

Verification of the test item stability in the test system was verified by analysing the lowest and the highest test concentrations at day 6 (new solution) and the same solutions at day 8 (old solutions) (Table 4.1 and Annex B). The stability was satisfactory and therefore no more samples were analysed.

Table 4.1 Results from chemical analysis

Nominal concentration		Actual measured concentration of bromate		
NaBrO ₃ mg/L	BrO ₃ ⁻ mg/L	Day 6 (new)	Day 8 (old)	Average
1.4	1.2	1.31	1.30	1.30
118	100	102	104	103

4.2 Ecotoxicological results

The primary data are presented in Appendix A. The results of this test are summarized in Table 4.5. All validity criteria prescribed in the OECD 212 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 118 mg/L and >118 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 118 mg/L.

As all test concentrations had hatched (>90% hatching success) at day 3, no statistical evaluation on the time to hatch endpoint could be computed.

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

Concentration (nominal)		Hatching success mean after 4 days	Standard deviation
NaBrO ₃ mg/L	BrO ₃ ⁻ mg/L		
0	0	100%	0%
1.4	1.2	97%	6%
4.4	3.7	93%	6%
13	11	97%	6%
39	33	97%	6%
118	100	97%	6%

4.2.2 Mortality

No significant effects on the endpoint mortality were observed (Table 4.3). NOEC and LOEC values are thus estimated to be 118 mg/L and >118 mg/L, respectively.

As no significant effect or any dose-response relationship was observed, the EC10 and EC50 for the endpoint mortality were estimated to be higher than 118 mg/L.

Table 4.3 Mortality of *Danio rerio* after exposure to sodium bromate.

Concentration		Mortality, embryos and larvae, mean	Standard deviation
NaBrO ₃ mg/L	BrO ₃ ⁻ mg/L		
0	0	0.0%	0.0%
1.4	1.2	6.7%	5.8%
4.4	3.7	6.7%	5.8%
13	11	3.3%	5.8%
39	33	3.3%	5.8%
118	100	3.3%	5.8%

4.2.3 Body length

No significant effects on the body length were observed (Table 4.3). NOEC and LOEC values are thus estimated to be 118 mg/L and >118 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 higher than 118 mg/L.

Table 4.4 Body length of *Danio rerio* larvae after exposure to sodium bromate.

Concentration		Body length (mm)	Standard deviation
NaBrO ₃ mg/L	BrO ₃ ⁻ mg/L		
0	0	39.4	1.0
1.4	1.2	39.3	1.0
4.4	3.7	39.2	0.9
13	11	39.4	0.7
39	33	39.7	0.8
118	100	39.3	0.7

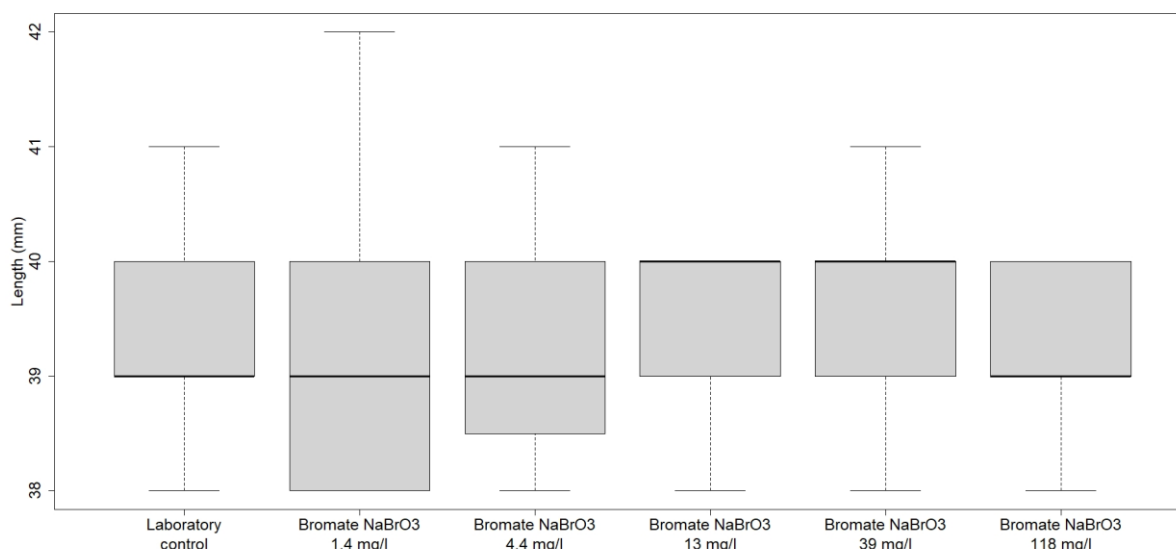


Figure 4.1 Body length of *Danio rerio* embryos and larvae after exposure to sodium bromate. The box itself represents the middle 50% of the data. The box edges are the 25th and 75th percentiles. An asterisk in the plot indicates a significant difference between the test group and the control group

5 Conclusion

The temperature for two replicates in the control group and one replicate from the exposure group of 1.4 mg/L differed more than 1.5°C from day 0 to day 2. As the difference was observed over 2 days, the validity criteria of differing less than 1.5°C between successive days is expected to be fulfilled. All other validity criteria were fulfilled and no effects on the evaluated endpoints were observed. Therefore, all effect concentrations were above the highest tested nominal concentration of 118 mg NaBrO₃/L corresponding to a nominal concentration of bromate of 100 mg/L.

Table 4.5 Results of the *Danio rerio* toxicity test after exposure to sodium bromate

Endpoint	NOEC (mg/L)		LOEC (mg/L)		EC/LC10		EC/LC50	
	NaBrO ₃	BrO ₃ ⁻	NaBrO ₃	BrO ₃ ⁻	NaBrO ₃	BrO ₃ ⁻	NaBrO ₃	BrO ₃ ⁻
Hatching success and time to hatch	118	100	>118	>100	>118	>100	>118	>100
Mortality	118	100	>118	>100	>118	>100	>118	>100
Body length	118	100	>118	>100	>118	>100	>118	>100

6 References

- /1/ OECD Guideline for Testing of Chemicals No. 212 (1998): "Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages". Adopted 1998.09.21.

- /2/ ISO 7346-2 (1996): "Water Quality - Determination of the acute lethal toxicity of substances to a freshwater fish [Brachy *Danio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)]. Part 2: Semi static method".
- /3/ 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/>.
- /4/ Frank Bretz, Torsten Hothorn and Peter Westfall (2010) (Dunnett's test), Multiple Comparisons Using R, CRC Press, Boca Raton.
- /5/ Fox, J. (2005) The R Commander: A Basic Statistics Graphical User Interface to R. Journal of Statistical Software, 14(9): 1–42
- /6/ Fox, J. and Weisberg, S. (2011) *An R Companion to Applied Regression*, Second Edition, Sage.

APPENDICES

APPENDIX A

Raw data and statistical evaluation

A Raw data toxicity test on embryo and sac-fry stages with *Danio rerio*

A.1.1 Primary data on short-term toxicity test

Table A.1 Raw data of the number and larvae stages of organisms generated in the short-term toxicity test with NaBrO₃. Test period 2022/02/15 to 2022/02/23.

Date		15 February, 2022						Accumulated day(s)						0		
NaBrO ₃ Concentration (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	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0
1.4	30	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0
4.4	30	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0
13	30	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0
39	30	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0
118	30	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0

Date		16 February, 2022						Accumulated day(s)						1		
NaBrO ₃ Concentration (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	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0
1.4	30	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0
4.4	30	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0
13	30	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0
39	30	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0
118	30	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0

Date		17 February, 2022						Accumulated day(s)						2		
NaBrO ₃ Concentration (mg/L)	Initial eggs per test group	Daily number o :														
		Eggs			Eggs that will never hatch today			Live larvae			Dead larvae			Malformed larvae		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	6	7	6	0	0	0	4	3	4	0	0	0	0	0	0
1.4	30	6	7	4	0	0	0	4	3	6	0	0	0	0	0	0
4.4	30	3	6	6	0	1	1	7	3	3	0	0	0	0	0	0
13	30	5	9	8	1	0	0	4	1	2	0	0	0	0	0	0
39	30	1	5	6	1	0	0	8	5	4	0	0	0	0	0	0
118	30	8	9	10	0	0	0	2	1	0	0	0	0	0	0	0



Date		18 February, 2022						Accumulated day(s)						3		
NaBrO ₃ Concentration (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	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	10	10	10	0	0	0	0	0	0
1.4	30	0	1	0	0	0	0	10	9	9	0	0	1 ¹⁾	0	0	0
4.4	30	0	0	1	0	0	0	10	9	8	0	0	0	0	0	0
13	30	0	0	0	0	0	0	9	10	10	0	0	0	0	0	0
39	30	0	0	0	0	0	0	9	10	10	0	0	0	0	0	0
118	30	0	0	1	0	0	0	10	10	9	0	0	0	0	0	0

1) The larva was found dead and was removed from the container

Date		19 February, 2022						Accumulated day(s)						4		
NaBrO ₃ Concentration (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	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	10	10	10	0	0	0	0	0	0
1.4	30	0	0	0	0	1	0	10	9	9	0	0	0	0	0	0
4.4	30	0	0	0	0	0	0	10	9	9	0	0	0	0	0	0
13	30	0	0	0	0	0	0	9	10	10	0	0	0	0	0	0
39	30	0	0	0	0	0	0	9	10	10	0	0	0	0	0	0
118	30	0	0	0	0	0	1	10	10	9	0	0	0	0	0	0

Date		20 February, 2022						Accumulated day(s)						5		
NaBrO ₃ Concentration (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	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	10	10	10	0	0	0	0	0	0
1.4	30	0	0	0	0	0	0	10	9	9	0	0	0	0	0	0
4.4	30	0	0	0	0	0	0	10	9	9	0	0	0	0	0	0
13	30	0	0	0	0	0	0	9	10	10	0	0	0	0	0	0
39	30	0	0	0	0	0	0	9	10	10	0	0	0	0	0	0
118	30	0	0	0	0	0	0	10	10	9	0	0	0	0	0	0

Date		21 February, 2022						Accumulated day(s)						6		
NaBrO ₃ Concentration (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	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	10	10	10	0	0	0	0	0	0
1.4	30	0	0	0	0	0	0	10	9	9	0	0	0	0	0	0
4.4	30	0	0	0	0	0	0	10	9	9	0	0	0	0	0	0
13	30	0	0	0	0	0	0	9	10	10	0	0	0	0	0	0
39	30	0	0	0	0	0	0	9	10	10	0	0	0	0	0	0
118	30	0	0	0	0	0	0	10	10	9	0	0	0	0	0	0

Date		22 February, 2022						Accumulated day(s)						7		
NaBrO ₃ Concentration (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	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	10	10	10	0	0	0	0	0	0
1.4	30	0	0	0	0	0	0	10	9	9	0	0	0	0	0	0
4.4	30	0	0	0	0	0	0	10	9	9	0	0	0	0	0	0
13	30	0	0	0	0	0	0	9	10	10	0	0	0	0	0	0
39	30	0	0	0	0	0	0	9	10	10	0	0	0	0	0	0
118	30	0	0	0	0	0	0	10	10	9	0	0	0	0	0	0

Date		23 February, 2022						Accumulated day(s)						8		
NaBrO ₃ Concentration (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	A	B	C	A	B	C	A	B	C	A	B	C
Laboratory control	30	0	0	0	0	0	0	10	10	10	0	0	0	0	0	0
1.4	30	0	0	0	0	0	0	10	9	9	0	0	0	0	0	0
4.4	30	0	0	0	0	0	0	10	9	9	0	0	0	0	0	0
13	30	0	0	0	0	0	0	9	10	10	0	0	0	0	0	0
39	30	0	0	0	0	0	0	9	10	10	0	0	0	0	0	0
118	30	0	0	0	0	0	0	10	10	9	0	0	0	0	0	0

A.1.2 Physical parameters

Table A.2 Measurements of temperature, dissolved oxygen and pH generated in the short-term toxicity test with NaBrO₃. Test period 2022/02/15 to 2022/02/23.

Date	15 February, 2022			Remark			Start of the test		
NaBrO ₃ Concentration (mg/L)	Temperature (°C)			Oxygen (% sat)			pH		
	A	B	C	A	B	C	A	B	C
Laboratory control	26.3	-	-	100	-	-	7.9	-	-
1.4	26.3	-	-	100	-	-	7.8	-	-
4.4	26.3	-	-	100	-	-	7.9	-	-
13	26.2	-	-	100	-	-	7.9	-	-
39	25.7	-	-	100	-	-	7.9	-	-
118	25.6	-	-	100	-	-	7.9	-	-

Date	17 February, 2022			Remark			Before medium renewal		
NaBrO ₃ Concentration (mg/L)	Temperature (°C)			Oxygen (% sat)			pH		
	A	B	C	A	B	C	A	B	C
Laboratory control	24.5	24.8	25.1	100	100	100	7.9	7.9	7.9
1.4	24.2	25.3	25.4	100	100	100	7.9	7.9	7.9
4.4	25.5	25.5	25.5	100	100	100	7.9	7.9	7.9
13	25.6	25.6	25.6	100	100	100	7.9	7.9	7.9
39	25.7	25.7	25.7	100	100	100	7.9	7.9	8.0
118	25.6	25.6	25.6	100	100	100	8.0	7.9	8.0

Date	17 February, 2022			Remark			After medium renewal		
NaBrO ₃ Concentration (mg/L)	Temperature (°C)			Oxygen (% sat)			pH		
	A	B	C	A	B	C	A	B	C
Laboratory control	25.5	25.3	25.3	100	100	100	7.7	7.7	7.8
1.4	25.2	25.2	25.3	100	100	100	7.7	7.7	7.7
4.4	25.2	25.3	25.2	100	100	100	7.7	7.7	7.7
13	25.3	25.4	25.3	100	100	100	7.7	7.7	7.6
39	25.1	25.2	25.3	100	100	100	7.6	7.6	7.6
118	25.1	25.1	25.1	100	100	100	7.6	7.6	7.6

Date	21 February, 2022			Remark			Before medium renewal		
NaBrO ₃ Concentration (mg/L)	Temperature (°C)			Oxygen (% sat)			pH		
	A	B	C	A	B	C	A	B	C
Laboratory control	25.5	25.5	25.6	100	100	100	8.0	8.0	8.0
1.4	25.6	25.7	25.8	100	100	100	8.0	8.0	8.0
4.4	25.8	25.9	26.0	100	100	100	8.0	8.0	8.0
13	26.0	26.0	26.0	100	100	100	8.0	8.0	8.0
39	26.0	26.0	26.0	100	100	100	8.0	8.0	8.0
118	26.0	26.0	26.0	100	100	100	8.0	8.0	8.0

Date	21 February, 2022			Remark			After medium renewal		
NaBrO ₃ Concentration (mg/L)	Temperature (°C)			Oxygen (% sat)			pH		
	A	B	C	A	B	C	A	B	C
Laboratory control	25.0	25.0	25.0	100	100	100	7.7	7.7	7.7
1.4	25.0	25.1	25.2	100	100	100	7.7	7.7	7.7
4.4	25.2	25.2	25.1	100	100	100	7.7	7.7	7.7
13	25.2	25.1	25.1	100	100	100	7.8	7.8	7.8
39	25.0	25.1	25.2	100	100	100	7.8	7.8	7.8
118	25.3	25.2	25.1	100	100	100	7.8	7.8	7.8

Date	23 February, 2022			Remark			End of the test		
NaBrO ₃ Concentration (mg/L)	Temperature (°C)			Oxygen (% sat)			pH		
	A	B	C	A	B	C	A	B	C
Laboratory control	25.2	25.4	25.6	100	100	100	7.7	7.8	7.8
1.4	25.8	26.1	26.1	100	100	100	7.8	8.0	7.8
4.4	26.2	26.2	26.2	100	100	100	7.9	7.9	7.9
13	26.2	26.2	26.3	100	100	100	7.9	7.9	7.9
39	26.3	26.2	26.3	100	100	100	7.9	7.9	8.0
118	26.4	26.3	26.4	100	100	100	8.0	8.0	8.0

A.1.3 Statistical analysis

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

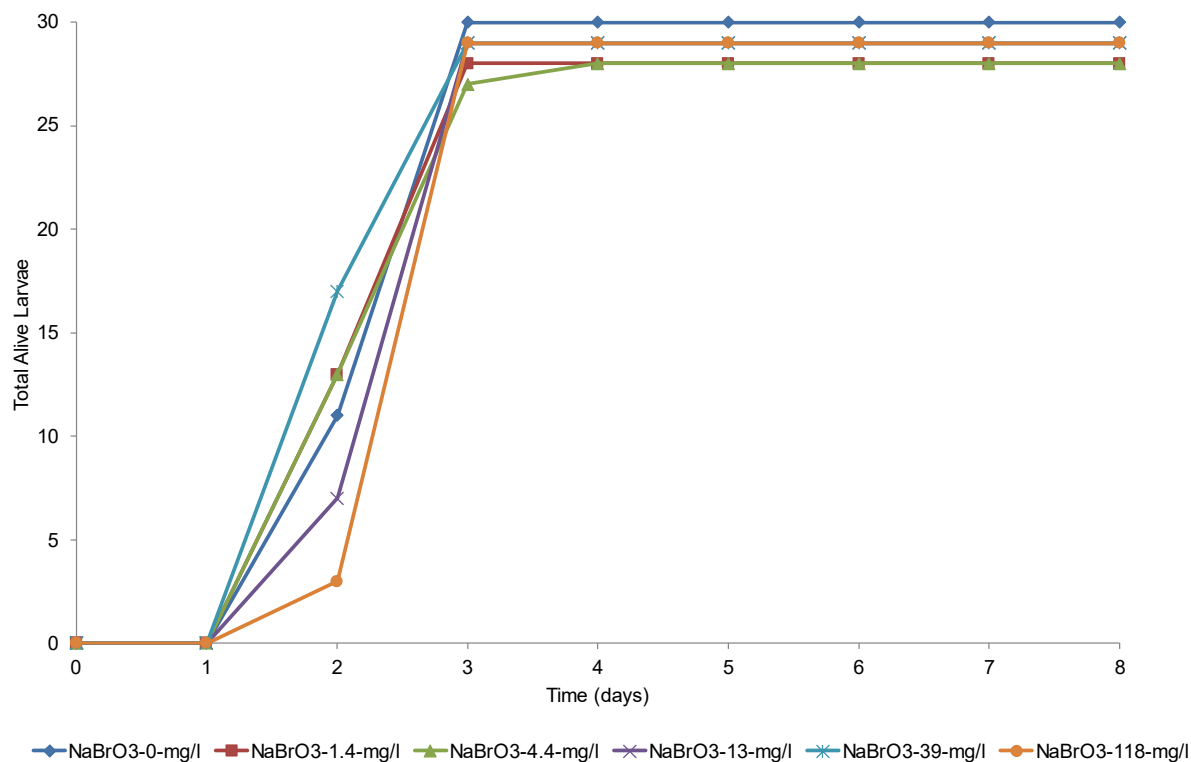


Figure A.1 Embryonic development time and number of hatched larvae alive of *Danio rerio* observed against the time with different concentration of the test item.

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	1.4 mg NaBrO3/L	4.4 mg NaBrO3/L	13 mg NaBrO3/L	39 mg NaBrO3/L	118 mg NaBrO3/L
1	100	100	100	90	90	100
2	100	90	90	100	100	100
3	100	100	90	100	100	90
Count	3	3	3	3	3	3
Mean	100	97	93	97	97	97

- 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⁾
Bromate_NaBrO3-1.4-mg/l - Bromate_NaBrO3-0-mg/l = 0	3.333	5.09	0.655	0.943	-
Bromate_NaBrO3-4.4-mg/l - Bromate_NaBrO3-0-mg/l = 0	10.000	5.09	1.964	0.238	-
Bromate_NaBrO3-13-mg/l - Bromate_NaBrO3-0-mg/l = 0	3.333	5.09	0.655	0.943	-
Bromate_NaBrO3-39-mg/l - Bromate_NaBrO3-0-mg/l = 0	3.333	5.09	0.655	0.943	-
Bromate_NaBrO3-118-mg/l - Bromate_NaBrO3-0-mg/l = 0	3.333	5.09	0.655	0.943	-

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

Endpoint	NOEC	LOEC	Unit
Hatching success	118	>118	mg NaBrO3/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	>118	>118	mg NaBrO3/L

A.1.3.4 Determination of NOEC and LOEC for the mortality

Table A.7 Experimental data for mortality (%)

Replicate No.	Laboratory control	1.4 mg NaBrO ₃ /L	4.4 mg NaBrO ₃ /L	13 mg NaBrO ₃ /L	39 mg NaBrO ₃ /L	118 mg NaBrO ₃ /L
1	0	0	0	10	10	0
2	0	0	10	0	0	0
3	0	10	10	0	0	10
Count	3	3	3	3	3	3
Mean	0	3.33	6.67	3.33	3.33	3.33

As no dose-relationship was found, no further statistical evaluation was performed for the endpoint mortality.

Table A.8 Mortality endpoint: estimations of NOEC and LOEC.

Endpoint	NOEC	LOEC	Unit
Mortality	118	>118	mg NaBrO ₃ /L

A.1.3.5 Results of the Probit analysis

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

Table A.9 Mortality endpoint, estimations of LC10 and LC50

Endpoint	LC10	LC50	Unit
Mortality	>118	>118	mg NaBrO ₃ /L

A.1.3.6 Determination of NOEC and LOEC for the body length with NaBrO₃

Table A.10 Experimental data of the body length (mm) in the laboratory control group and the NaBrO₃ exposure groups.

Replicate No.	Laboratory control	NaBrO ₃ 1.4 mg/L	NaBrO ₃ 4.4 mg/L	NaBrO ₃ 13 mg/L	NaBrO ₃ 39 mg/L	NaBrO ₃ 118 mg/L
1	38.00	40.00	41.00	40.00	40.00	39.00
2	38.00	39.00	39.00	39.00	40.00	39.00
3	38.00	39.00	40.00	40.00	39.00	40.00
4	39.00	38.00	38.00	40.00	40.00	40.00
5	39.00	42.00	40.00	40.00	41.00	40.00
6	39.00	39.00	39.00	40.00	40.00	39.00
7	40.00	38.00	39.00	39.00	40.00	39.00
8	39.00	39.00	41.00	39.00	38.00	39.00
9	40.00	39.00	39.00	40.00	39.00	40.00
10	40.00	39.00	38.00	40.00	39.00	40.00
11	39.00	40.00	40.00	40.00	40.00	40.00
12	39.00	39.00	39.00	38.00	40.00	40.00
13	38.00	38.00	38.00	40.00	40.00	40.00
14	39.00	38.00	40.00	40.00	40.00	39.00
15	40.00	40.00	39.00	39.00	40.00	40.00
16	41.00	38.00	38.00	40.00	41.00	39.00
17	39.00	38.00	39.00	39.00	38.00	39.00
18	40.00	38.00	38.00	39.00	39.00	39.00
19	40.00	40.00	40.00	38.00	40.00	40.00
20	38.00	40.00	40.00	39.00	40.00	40.00
21	41.00	40.00	40.00	39.00	40.00	39.00
22	38.00	40.00	38.00	40.00	41.00	40.00
23	39.00	40.00	39.00	40.00	39.00	38.00
24	40.00	39.00	39.00	40.00	40.00	39.00
25	41.00	38.00	40.00	39.00	39.00	38.00
26	40.00	41.00	38.00	38.00	40.00	39.00

Replicate No.	Laboratory control	NaBrO ₃ 1.4 mg/L	NaBrO ₃ 4.4 mg/L	NaBrO ₃ 13 mg/L	NaBrO ₃ 39 mg/L	NaBrO ₃ 118 mg/L
27	39.00	40.00	39.00	40.00	40.00	38.00
28	40.00	40.00	39.00	38.00	40.00	40.00
29	41.00	-	-	39.00	39.00	39.00
30	39.00	-	-	-	-	-
Count	30	28	28	29	29	29
Mean	39.4	39.3	39.2	39.4	39.7	39.3

- Normality

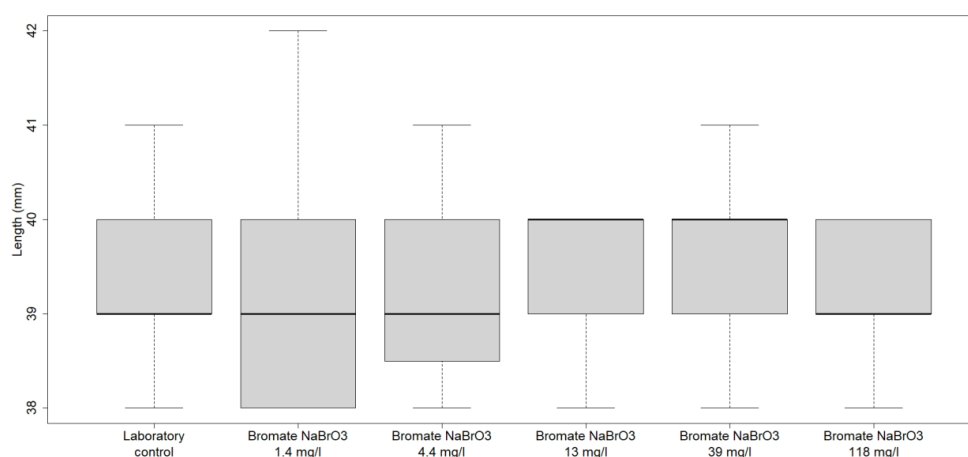


Figure A.2 Boxplots of every test group for the body length.

The boxplot patterns show the data distribution (Figure A.2).

- 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.11).

Table A.11 Multiple comparisons of means: Dunnett's contrasts.

Mean comparisons	Estimate	Std. Error	t value	Pr(> t)	Significance ¹⁾
Bromate_NaBrO3-1.4-mg/l - Bromate_NaBrO3-0-mg/l = 0	0.30	0.57	0.521	0.980	-
Bromate_NaBrO3-4.4-mg/l - Bromate_NaBrO3-0-mg/l = 0	0.48	0.57	0.839	0.873	-
Bromate_NaBrO3-13-mg/l - Bromate_NaBrO3-0-mg/l = 0	-0.03	0.56	-0.057	1.000	-

Bromate_NaBrO3-39-mg/l - Bromate_NaBrO3-0-mg/l =0	-0.91	0.56	-1.609	0.359	-
Bromate_NaBrO3-118-mg/l - Bromate_NaBrO3-0-mg/l =0	0.06	0.56	0.098	1.000	-

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.12 Body length endpoint: estimations of NOEC and LOEC, Dunnett's test.

Endpoint	NOEC	LOEC	Unit
Body length	118	>118	mg NaBrO3/L

A.1.3.7 Results of the Probit analysis

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

Table A.13 Body length endpoint, estimations of EC10 and EC50.

Endpoint	EC10	EC50	Unit
Body length	>118	>118	mg NaBrO3/L

A.1.4 Validity criteria

Table A.14 Validity criteria of the test according to the OECD 212 /1/ in the development test with *Danio rerio*.

Criteria in the control	Target Value	Fulfilled
Survival of hatched larvae	> 90% (observed: 100%)	Yes
Hatching success	> 80% (observed: 100%)	Yes
Dissolved oxygen concentration	> 60% throughout test (minimum observed: 100%)	Yes
Temperature	25 °C ± 1.0 °C (observed: 26 ± 0.5)	Yes

APPENDIX B– Chemical analysis

Results from chemical analyses

B Results from chemical analyses



Analyscertifikat

Ordernummer	: ST2206093	Sida	: 1 av 5
Kund	: DHI A/S	Projekt	: Project 11827338
Kontaktperson	: Anja Kamper	Beställningsnummer	: Anders Sjölin/VA syd
Adress	: Agern allé 5	Provtagare	: JAB
	: 2970 Hørsholm	Provtagningspunkt	: ----
	: Danmark	Ankomstdatum, prover	: 2022-03-03 10:40
E-post	: aka@dhigroup.com	Analys påbörjad	: 2022-03-04
Telefon	: ----	Utfärdad	: 2022-03-09 08:06
C-O-C-nummer	: ----	Antal ankomna prover	: 4
(eller			
Orderblankett-num			
mer)			
Offertnummer	: ST2021SE-NIR-SWE0001 (OF210090)	Antal analyserade prover	: 4

Generell kommentar

Denna rapport får endast återges i sin helhet, om inte utfärdande laboratorium i förväg skriftligen godkänt annat. Resultatet gäller endast materialet såsom det har mottagits, identifierats och testats. Laboratoriet tar inget ansvar för information i denna rapport som har lämnats av kunden, eller resultat som kan ha påverkats av sådan information. Beträffande laboratoriets ansvar i samband med uppdrag, se vår webbplats www.alsglobal.se

Signatur	Position
Niels-Kristian Terkildsen	Laboratoriechef

Niels-Kristian Terkildsen

Laboratorium	: ALS Scandinavia AB	hemsida	: www.alsglobal.se
Adress	: Rinkebyvägen 19C	E-post	: info.ta@alsglobal.com
	: 182 36 Danderyd	Telefon	: +46 8 5277 5200
	: Sverige		

Sida : 2 av 5
 Ordernummer : ST2206093
 Kund : DHI A/S



Analysresultat

Matris: HAVSVATTEN		Provbeteckning		DHI 11827338 ÖT-20-1250 1.4 mg/L A New					
		Laboratoriets provnummer		sampling 21-02-2022					
		Provtagningsdatum / tid		ST2206093-001					
				2022-02-21					
Parameter		Resultat	MU	Enhet	LOR	Analyspaket	Metod	Utf.	
Oorganiska parametrar									
bromater		1310	± 263	µg/L	5.0	Bromat	W-OXY-IC	PR	

Sida : 3 av 5
 Ordernummer : ST2206093
 Kund : DHI A/S



Matris: HAVSVATTEN		Provbeteckning		DHI 11827338 ÖT-20-1250 118 mg/L A New				
		Laboratoriets provnummer		sampling 21-02-2022				
		Provtagningsdatum / tid		ST2206093-002				
				2022-02-21				
Parameter	Resultat	MU	Enhet	LOR	Analyspaket	Metod	Utf.	
Oorganiska parametrar								
bromater	102000	± 20400	µg/L	5.0	Bromat	W-OXY-IC	PR	

Sida : 4 av 5
 Ordernummer : ST2206093
 Kund : DHI A/S



Matris: HAVSVATTEN		Provbeteckning		DHI 11827338 ÖT-20-1250 1.4 mg/L A Old					
		Laboratoriets provnummer		Sampling 23-02-2022					
		Provtagningsdatum / tid		ST2206093-003					
				2022-02-23					
Parameter		Resultat	MU	Enhet	LOR	Analyspaket	Metod		Utf.
Oorganiska parametrar									
bromater		1300	± 260	µg/L	5.0	Bromat	W-OXY-IC		PR

Sida : 5 av 5
 Ordernummer : ST2206093
 Kund : DHI A/S



Matris: HAVSVATTEN		Provbezeichnung		DHI 11827338 ÖT-20-1250 118 mg/L A Old				
		Laboratoriets provnummer		sampling 23-02-2022				
		Provtagningsdatum / tid		ST2206093-004				
				2022-02-23				
Parameter	Resultat	MU	Enhet	LOR	Analyspaket	Metod	Utf.	
Oorganiska parametrar								
bromater	104000	± 20700	µg/L	5.0	Bromat	W-OXY-IC	PR	

Metodsammanfattningar

Analysmetoder	Metod
W-OXY-IC	Bestämning av löst bromat, klorat och klorit enligt metod baserad på CSN EN ISO 15061, CSN EN ISO 10304-4. Mätning utförd med jonkromatografi. Bestämning av summan klorat och klorit genom beräkning från uppmätta värden.

Nyckel: LOR = Den rapporteringsgräns (LOR) som anges är standard för respektive parameter i metoden. Rapporteringsgränsen kan påverkas vid t.ex. spädning p.g.a. matrisstörningar, begränsad provmängd eller låg torrsubstanshalt.

MU = Mätosäkerhet

* = Asterisk efter resultatet visar på ej ackrediterat test, gäller både egna lab och underleverantör

Mätosäkerhet:

Mätosäkerheten anges som en utvidgad osäkerhet (enligt definitionen i "Evaluation of measurement data- Guide to the expression of uncertainty in measurement", JCGM 100:2008 Corrected version 2010) beräknad med täckningsfaktor lika med 2 vilket ger en konfidensnivå på ungefär 95%.

Mätosäkerhet anges endast för detekterade ämnen med halter över rapporteringsgränsen.

Mätosäkerhet från underleverantör anges oftast som en utvidgad osäkerhet beräknad med täckningsfaktor 2. För ytterligare information kontakta laboratoriet.

Utförande laboratorium (teknisk enhet inom ALS Scandinavia eller anlitat laboratorium (underleverantör)).

	Utf.
PR	Analys utförd av ALS Czech Republic s.r.o Prag, Na Harfe 336/9 Prag Tjeckien 190 00 Ackrediterad av: CAI Ackrediteringsnummer: 1163

APPENDIX C – CoA

Identification of test item (CoA)

C Identification of test item (CoA)

SIGMA-ALDRICH®

3050 Spruce Street, Saint Louis, MO 63103 USA
 Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name: SODIUM BROMATE
 puriss. p.a., >= 99.5 % RT
Product Number: 71325
Batch Number: BCBX9200
Brand: Sigma-Aldrich
CAS Number: 7789-38-0
Formula: NaBrO₃
Formula Weight: 150.89
Quality Release Date: 03 AUG 2018
Recommended Retest Date: JAN 2023

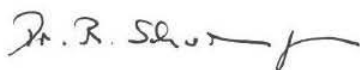
TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	COLORLESS OR WHITE	WHITE
APPEARANCE (FORM)	POWDER OR CRYSTALS	CRYSTALS
REDOX TITRATION	99.5 - 101.0 %	100.0 %
REDOXTITRATION (METHOD)	IODOMETRIC	IODOMETRIC
SOLUBILITY (COLOR)	COLORLESS	COLORLESS
SOLUBILITY (TURBIDITY)	CLEAR	CLEAR
SOLUBILITY (METHOD)	0.5G IN 10ML WATER	0.5G IN 10ML WATER
PH	5.0 - 9.0 (50 MG/ML H ₂ O, 25C)	6.3
METAL TRACE ANALYSIS (ICP)	CORRESPONDS TO REQUIREMENTS	PASSED
CALCIUM (ICP)	≤ 10 MG/KG	< 10 MG/KG
CADMIUM (ICP)	≤ 5 MG/KG	< 5 MG/KG
COBALT (ICP)	≤ 5 MG/KG	< 5 MG/KG
CHROMIUM (ICP)	≤ 5 MG/KG	< 5 MG/KG
COPPER (ICP)	≤ 5 MG/KG	< 5 MG/KG
IRON (ICP)	≤ 5 MG/KG	< 5 MG/KG
POTASSIUM (ICP)	≤ 100 MG/KG	< 100 MG/KG
MAGNESIUM (ICP)	≤ 5 MG/KG	< 5 MG/KG
MANGANESE (ICP)	≤ 5 MG/KG	< 5 MG/KG
NICKEL (ICP)	≤ 5 MG/KG	< 5 MG/KG
LEAD (ICP)	≤ 5 MG/KG	< 5 MG/KG
ZINC (ICP)	≤ 5 MG/KG	< 5 MG/KG

SIGMA-ALDRICH®

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

BROMIDE (BR)	≤ 500 MG/KG	< 500 MG/KG
TOTAL NITROGEN	≤ 50 MG/KG	45 MG/KG
SULFATE (SO₄)	≤ 500 MG/KG	< 500 MG/KG



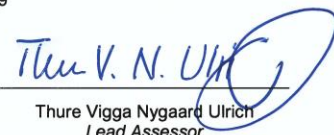
Dr. Reinhold Schwenninger
Quality Assurance
Buchs, Switzerland

Sigma-Aldrich warrants that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

APPENDIX D

Accreditation Certificate

D Accreditation Certificate

 Member of EA MLA											
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										
<table><tr><td>DANAK</td><td>Dyregårdsvej 5 B</td><td>Tlf: +45 77 33 95 00</td><td>Bank: Reg. nr. 2191</td><td>danak@danak.dk</td></tr><tr><td>Den Danske Akkrediteringsfond</td><td>2740 Skovlunde</td><td>CVR-nr. 26 89 93 89</td><td>Kontonr: 8967 583627</td><td>www.danak.dk</td></tr></table>		DANAK	Dyregårdsvej 5 B	Tlf: +45 77 33 95 00	Bank: Reg. nr. 2191	danak@danak.dk	Den Danske Akkrediteringsfond	2740 Skovlunde	CVR-nr. 26 89 93 89	Kontonr: 8967 583627	www.danak.dk
DANAK	Dyregårdsvej 5 B	Tlf: +45 77 33 95 00	Bank: Reg. nr. 2191	danak@danak.dk							
Den Danske Akkrediteringsfond	2740 Skovlunde	CVR-nr. 26 89 93 89	Kontonr: 8967 583627	www.danak.dk							

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