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Gelsenkirchen, 13.08.2024

Product "SORB® XT Stain Solution Pro ECO"
here: Determination of aquatic toxicity

Your order of 27.06.2024; Mr Torsten Narawitz

Dear ladies and gentlemen,

As part of the above-mentioned order placement, Allegro Capital, Logistics Services & More GmbH commissioned us to analyse the cleaning agent with the designation "**SORB® XT Stain Solution Pro ECO**", which was sent to our company by post on 8 July 2024, for toxicity to fish (96 h LC_{50}) in accordance with OECD Guideline 236 or EC No. 440/2008 C.49*.

The test results are described below with a brief outline of the test method used and the test conditions selected.

Test results

Determination of acute toxicity to zebrafish embryos

The acute toxicity of chemicals and test substances to the embryonic development of the zebrafish (Danio rerio, Hamilton-Buchanan) is determined according to OECD Guideline 236 (OECD Guidelines for the Testing of Chemicals, Test No. 236: Fish Embryo Acute Toxicity (FET) Test) or according to the analogue method C.49 from Regulation (EC) No. 440/2008.

Freshly fertilised zebrafish eggs are exposed to the test solution for a period of 96 hours. Up to four apical observations are recorded at 24-hour intervals as lethality indicators: Coagulation of the fertilised eggs, lack of somite formation, lack of separation of the tail bud from the yolk sac and lack of heartbeat. At the end of the exposure period, the acute toxicity is determined based on a positive result in one of the four recorded apical observations and the LC50 is calculated.

Examination procedure

Sample receipt:	08/07/2024
Registration:	08/07/2024
Internal test number:	A2024 - 17982
Standard operating procedure:	SOP 9.34-A1 (002/12.2020)
Start of the audit:	29/07/2024
End of the test:	09/08/2024
Preparation of the test report:	13/08/2024

Test procedure

Test system

The tests were carried out with eggs of the zebrafish (Danio rerio, Hamilton 1822). The test organisms are bred in the institute's own rearing facility.

A laboratory room is available for carrying out this procedure, which is used exclusively for keeping the fish and incubating the fish eggs. Complete darkening to maintain a constant light/dark rhythm of 12:12 hours is guaranteed. The parent animals are kept in glass aquaria with a volume of at least 54 litres.

The water is checked at least once a week for the parameters nitrite (NO2-), nitrate (NO3-), pH value (pH), total hardness (GH) and carbonate hardness (KH) and the measurement results are documented accordingly.

The water temperature (26 ± 1 °C) is checked daily and the parent animals are visually inspected for signs of disease or other anomalies.

Implementation

In order to carry out the test, it is necessary to obtain and differentiate between the two.

Egg extraction

To obtain eggs, spawning trays with grids and dummy plants are placed in the parent fish's tank before the lighting is switched on. The trays can usually be removed 30 minutes after switching on the aquarium lighting.

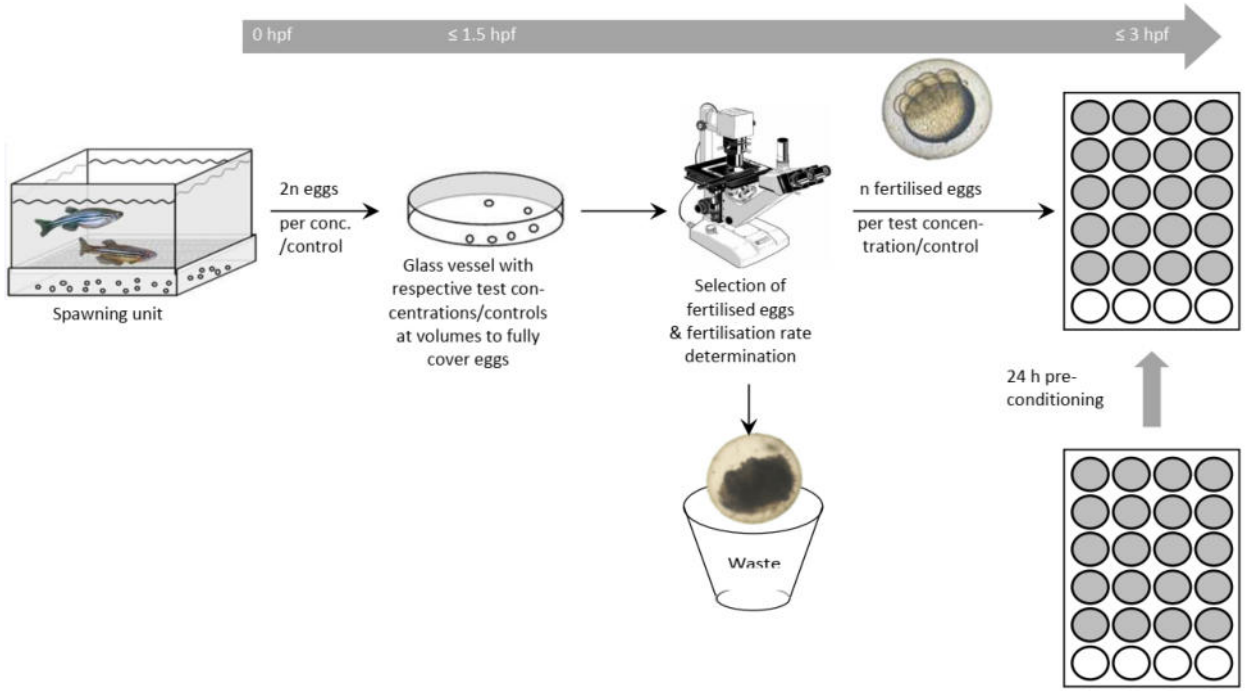


Figure 1: Scheme of the toxicity test on zebrafish according to OECD 236

Differentiation

Before differentiation, a suitable number of eggs (>20) are added to the respective dilution stages for pre-exposure. The differentiation of fertilised and unfertilised eggs must be carried out within the first 60 minutes after egg deposition. It is carried out under a stereomicroscope on a dark background. In fertilised eggs, the first cell division begins at 26°C after about 15 minutes. Fertilised eggs can be clearly recognised from the 4-cell stage.

As already described, the principle of the method is based on determining the disturbance of embryonic development when the test substances are added compared to a non-toxic control preparation.

The mean lethal concentration (LC_{50}) is determined mathematically or graphically over the course of 96 hours. The fertilised eggs of the zebrafish are exposed to the various dilution levels of the test substance or application solutions and their embryonic development in the various test preparations is assessed over the stated test duration of 96 hours.

The application solutions of the test item were prepared as follows:

For the main test, exact weights of 10 mg/l, 20 mg/l, 40 mg/l, 80 mg/l and 100 mg/l of the original material of the test item "**SORB® XT Stain Solution Pro ECO**" were suspended in 1 litre of storage water each and stirred at room temperature with a magnetic stirrer. The control preparation was also carried out with storage water. Test doses of 500 mg/l and 1000 mg/l were also investigated in an orientating preliminary study.

The samples to be analysed are tempered to approx. 26°C before the test is carried out. The pH value is determined and adjusted to pH 7 ±0.2 if necessary. This is done with 0.1 mol/l NaOH or 0.1 mol/l HCl, avoiding exceeding the neutral point.

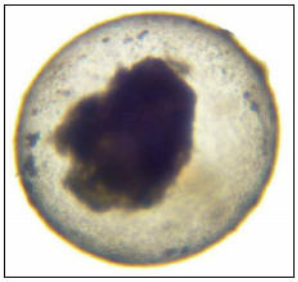
As a rule, dilution steps are prepared from the sample material using standard dilution water. The dilution levels should be selected so that the LC_{50} can be determined. Ideally, no lethality (NOEC / LC0) can be detected at the highest dilution level and complete lethality (LC_{100}) at the lowest dilution level.

A 3,4-dichloroaniline solution (4 mg/l) is used as a positive control, in the sense of a complete lethal effect. The untreated standard dilution water is used as a negative control.

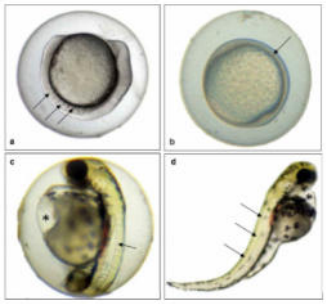
The cell culture plates used with the fertilised fish eggs are incubated in an incubator at a temperature of 26 ±1 °C and a light/dark cycle of 12:12 hours for a period of 96 hours.

The fish eggs are analysed after 24, 48, 72 and 96 hours for the development of the embryos with regard to the following lethal criteria:

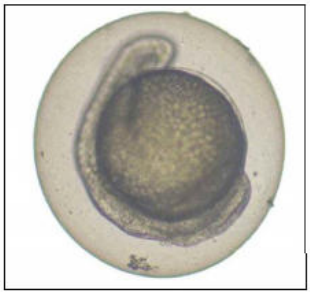
- Coagulation:



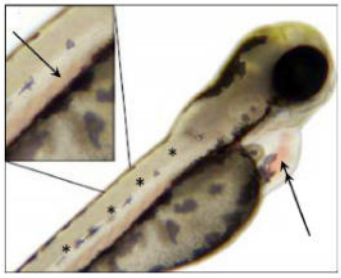
- No formation of somites:



- No detachment of the tail from the yolk:



- No heartbeat (from 48h):



Results

The results of the test according to OECD 236 for the test sample "**SORB® XT Stain Solution Pro ECO**" are listed below:

Concentration of the original substance in mg/l	**Survival rate in %**
10	100
20	100
40	95
80	75
100	5
500 (from pre-test)	0
1000 (from pre-test)	0
Negative control	90
Positive control	25

Table 1: Results FET - survival rates

Concentration of the original substance in mg/l	10	20	40	80	100
Number of dead embryos in %	0	0	5	25	95

Table 2: Results FET - lethality rates

The following mean effective and lethal concentrations for the "**SORB® XT Stain Solution Pro ECO**" test sample can be determined from the test results:

LC_0 / NOEC	(96 h)	=	20	mg/l
LC_{50}	**(96 h)**	**=**	**80**	**mg/l**
LC_{100}	(96 h)	=	500	mg/l

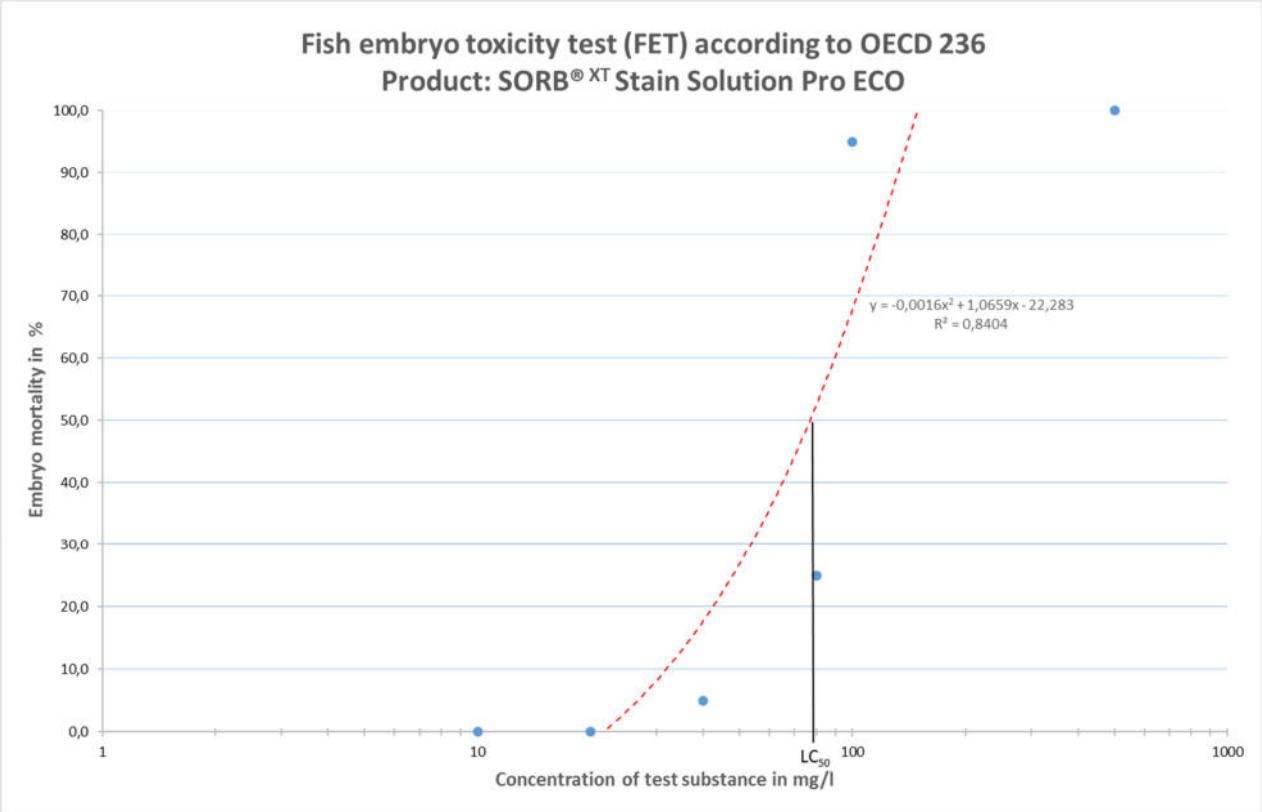


Figure 2: Graphical analysis of the test results

As NOEC (No Observed Effect Concentration) - taking into account the negative control carried out in parallel - the test concentration of 20 mg/l used here can be reported. A concentration of 80 mg/l was determined as the LC_{50} (statistically calculated concentration of a substance that is expected to cause death in 50 % of the exposed animals within the study period).

Examination of the criteria for the validity of the test results

The validity criteria of the OECD guideline are deemed to be fulfilled if

- in the negative control an embryo lethality of ≤10% of the embryos used and
- a mortality rate of at least 30 % of the embryos in the positive control

can be determined.

In the test carried out here, the negative control showed a lethality rate of 0% and the positive control a proportion of dead embryos of 50%, so that the test carried out can be assessed as valid and the test results submitted here can be declared as conforming to the standard.

Discussion of the test results

The test results show that the experimentally determined **LC_{50}** value for the toxicity of the test substance "**SORB® XT Stain Solution Pro ECO**" to fish embryos is approx. **80 mg/l** and that a **NOEC** (no observed effect concentration) of **20 mg/l** was documented.

Literature

OECD Guideline for Testing of Chemicals, Test Guideline 236: Fish Embryo Acute Toxicity (FET) Test. Adopted on 26. July 2013.

OECD 2019: Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Environmental, Health and Safety Publications, Series on Testing and Assessment No. 23. , ENV/JM/MONO(2000)6/REV1, 16 May 2019 OECD.

Summary

As part of the above-mentioned order, Allegro Capital, Logistics Services & More GmbH commissioned us to analyse the cleaning agent "SORB® XT Stain Solution Pro ECO" with regard to its acute fish toxicity.

Method	NOEC	Effective size / unit	Toxicity values / test data
Fish embryo test – FET – OECD 236	**20 mg/l**	**LC_{50}**	**80 mg/l**

Table 3: Summary of the test results

As a result of the tests, an average lethal concentration (LC_{50}) of 80 mg/l was determined for the product "**SORB® XT Stain Solution Pro ECO**" according to OECD 236.

Best regards
The Director of the Institute
p.p.

Dipl.-Umweltwiss. Sebastian Bien
Deputy Head of Department
Environment and Consumer Protection

This document is digitally released.