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Gelsenkirchen, 06.03.2024

Product "410 Aquarium, transparent" (batch no. 161002)
hier: Determination of aquatic toxicity

Your order dated 17.01.2024; Mrs Lena Brandstätter

Dear ladies and gentlemen,

As part of the aforementioned order placement, Ramsauer GmbH & Co KG commissioned us to subject the sealant with the designation "410 Aquarium, transparent (batch no. 161002)" on a silicone basis (acetate system), which was sent to our company by post on 22.01.2024 in standard 310 ml cartridges, to ecotoxicological tests.

Due to the potential use of the sealing system, e.g. in terrarium or aquarium construction, material contact with living waters cannot be ruled out. The present study was therefore intended to clarify in the laboratory whether conceivable substance transfers from the reacted product "410 Aquarium, transparent" into the surrounding water phase could possibly have a relevant impact on water quality.

The criteria specified in Annex 1 of the CLP Regulation, Chapter 4.1.3. "Classification of mixtures" are decisive for the assessment and classification of substances and mixtures as hazardous to the aquatic environment.

In addition to the classification criteria for acute aquatic toxicity, the need for classification of chronic aquatic toxicity can also be checked using the criteria mentioned in chapter 4.1.3.3.2.

It can be quoted from the specifications there that the experimentally determined LC/EC/IC50 values for the effect concentrations on the aquatic model organisms fish, daphnia and algae must be > 100 mg/l or above the water solubility and the NOEC (No Observed Effect Concentration) must be above 1 mg/l, so that a classification as hazardous to the aquatic environment is not necessary.

On this basis, it was agreed to determine test data on aquatic toxicity in accordance with OECD Guideline 236 (Fish Embryo Acute Toxicity (FET) Test, 26 July 2013) for the basic assessment of the product properties in order to be able to make a product-related statement on possible aquatic toxicity.

Since the product to be analysed here is a hardening solid with an extremely low overall solubility in water, the above-mentioned toxicity test could only be carried out indirectly. To analyse poorly soluble products, the preparation of leachates or test eluates is therefore used as a sample preparation step. The solutions obtained in this way represent a "water-accomodated fraction" (WAF) as defined in the OECD Guidance Document on Aqueous-Phase Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD, 2019), which contains the maximum water-soluble portions of the test sample.

The studies on aquatic test organisms (fish eggs) summarised here were therefore carried out on leaching eluates prepared in accordance with the above OECD Guidance Document based on DIN 38414 ("S4") by means of continuous stirring over a period of 72 hours in the following solid/water ratios:

- 100 mg / 1.0 Liter
- 1 000 mg / 1.0 Liter
- 10 000 mg / 1.0 Liter
- 100 000 mg / 1.0 Liter

To prepare the eluate, the sealant was applied to an inert glass surface using a manual pressure press and left to rest under constant laboratory conditions (20 ± 2 °C) for a curing period of 7 days. The ecotoxicological tests described below were carried out on the eluates obtained from exact weights of the reacted material:

- Toxicity to fish embryos according to OECD 236

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

A chemical analysis of the migrates produced in the course of the sample preparation described above was initially dispensed with.

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.

Procedure of the examination

Sample receipt:	22.01.2024
Registration:	23.01.2024
Internal test number:	A2024 – 1447 bis – 1450
Standard operating procedure:	SOP 9.34-A1 (002/12.2020)
Start of the audit:	12.02.2024
End of the test:	16.02.2024
Preparation of the test report:	06.03.2024

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.
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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.

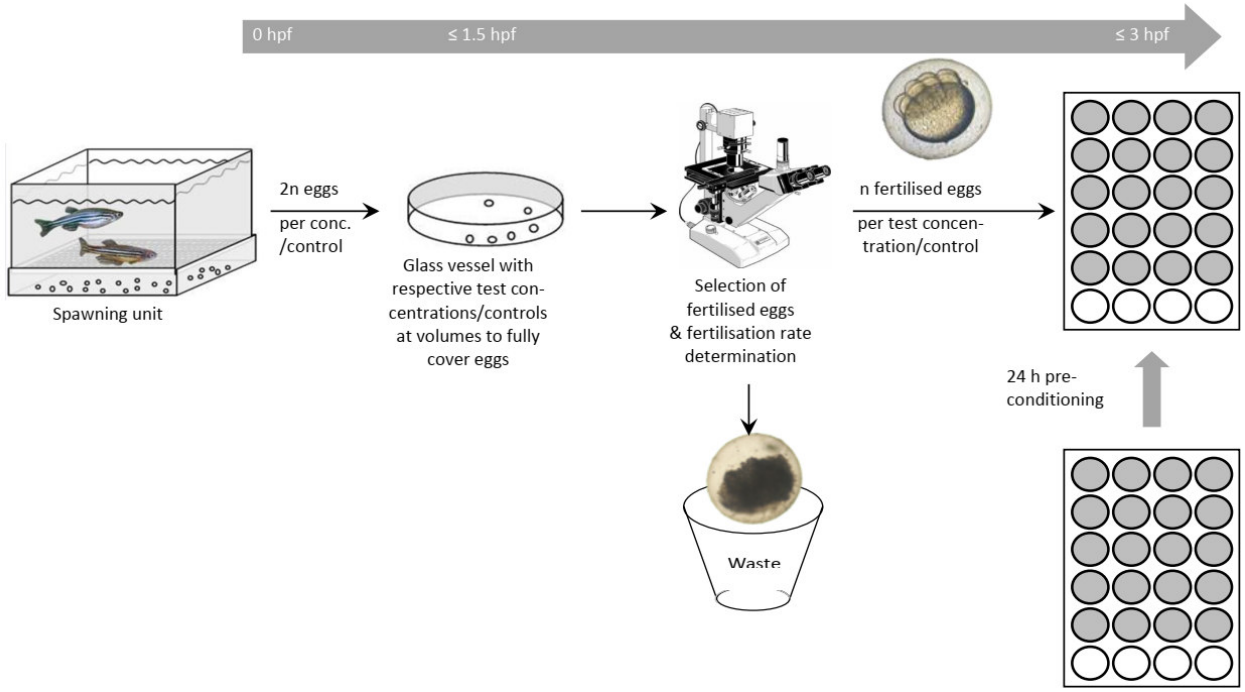


Figure 1: Scheme of the toxicity test on zebrafish according to OECD 236

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 (LC50) 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:

Exact weights of 100 mg/l, 1 000 mg/l, 10 000 mg/l and 100 000 mg/l of the cured original material of the test item "410 Aquarium, transparent" were suspended in 1 litre of holding water each and stirred for 72 hours at room temperature using a magnetic stirrer. The resulting solutions represent a "water-accomodated fraction" (WAF) as defined in the OECD Guidance Document on Aqueous-Phase Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD, 2019). The application solutions prepared by sedimentation of the undissolved material over 24 hours each contain the maximum water-soluble proportions of "410 Aquarium, transparent" per litre. The control preparation was also carried out with storage water.

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.

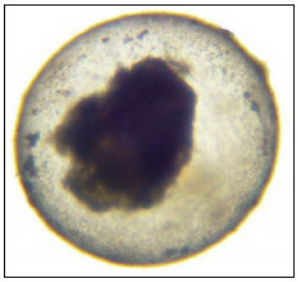
As a rule, dilution steps are prepared from the sample material using standard dilution water. The dilution levels should be selected so that the LC50 can be determined. Ideally, no lethality (NOEC / LC0) can be detected at the highest dilution level and complete lethality (LC100) at the lowest dilution 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.

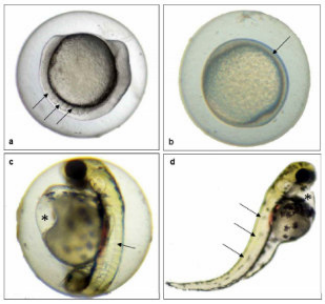
The cell culture plates used with the fertilised fish eggs are incubated in an incubator at a temperature of 26 ±1 °C and a light/dark cycle of 12:12 hours for a period of 96 hours.

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:

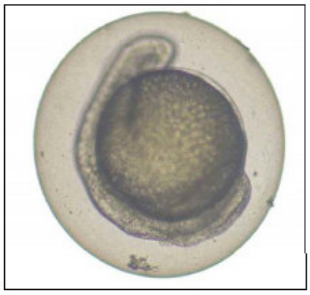
- Coagulation:



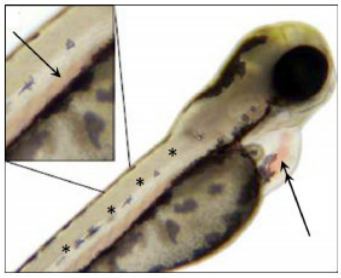
- No formation of somites:



- 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 "410 Aquarium, transparent" after 96 hours are listed below:

Concentration of the original substance in mg/l	Survival rate in %
100	95
1 000	90
10 000	100
100 000	100
Negative control	100
Positive control	10

Table 1: Results FET - survival rates

Concentration of the original substance in mg/l	100	1 000	10 000	100 000
Number of dead embryos in %	< 10	< 10	< 10	< 10

Table 2: Results FET - lethality rates

The following mean active and lethal concentrations for the test sample "**410 Aquarium, transparent**" can be determined from the test result:

LC_0 / **NOEC**	(96 h)	=		**100 000**	**mg/l**
LC_{50}	(96 h)	=	>	100 000	mg/l
LC_{100}	(96 h)	=	>	100 000	mg/l

The maximum test concentration of **100 g/l** (100,000 mg/l) used here can be reported as the **NOEC** (No Observed Effect Concentration), taking into account the negative control carried out in parallel and the statistical significance.

The results of the accompanying measurements of the pH values, electrical conductivity and oxygen content in the highest-dose test batch of "410 Aquarium, transparent" with 100,000 mg/l and in the negative control (NK) are shown as examples in Table 3.

Parameter	Unit	Beginning of the test (T = 0)	End of the test (T = 96 Hours)
pH (100 g/l)		7.21	7.43
pH (NK)		7.89	7.80
O_2 (100 g/l)	mg/l	7.85	7.97
O_2 (NK)	mg/l	10.55	8.06
Electrical conductivity (100 g/l)	µS/cm	681	685
Electrical conductivity (NK)	µS/cm	692	691

Table 3: pH values, conductivity and oxygen levels in the test batch with 100 000 mg/l „410 Aquarium, transparent“ and in the control group - fish toxicity

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 90%, 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 **LC50** value for the toxicity of the test substance "**410 Aquarium, transparent**" to fish embryos is **above 100,000 mg/l** and that the maximum test concentration of **100,000 mg/l** used here could be identified as **NOEC** (no observed effect concentration).

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, Ramsauer GmbH & Co KG commissioned us to subject the sealing and adhesive system "**410 Aquarium, transparent**" to appropriate ecotoxicological tests to assess its "water compatibility".

Method	NOEC	Effective size / unit	Toxicity values / test data
Fish embryo test – FET – OECD 236	**100 000 mg/l**	**LC_{50}**	**> 100 000 mg/l**

Table 4: Summary of the test results

Based on the area of application (e.g. aquarium construction) and the classification criteria of substances and mixtures as acutely or chronically hazardous to the aquatic environment from Annex 1 of the CLP Regulation (EC No. 1272-2008), the toxicity to fish embryos was tested in the laboratory. The test data experimentally determined here (LC50 value) are well above the 50% effect concentrations for fish, daphnia and algae of > 100 mg/l specified in the cited regulation and the NOEC (no observed effect concentrations) derived from the test data are well above 1 mg/l, so that in our opinion it is not necessary to classify the product "**410 Aquarium, transparent**" as hazardous to the aquatic environment and it can be assumed that the use of the silicone-based adhesive in the aquatic environment does not have a relevant negative impact on the living environment.

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.