Sikaflex Pro-3 Purform: Acute Toxicity to Zebrafish (*Danio rerio*) Embryos in a 96-hour Static Limit Test

Title:	Sikaflex PRO-3 Purform: Acute Toxicity to Zebrafish (*Danio rerio*) Embryos in a 96-hour Static Limit Test
Purpose:	The purpose of this study was to evaluate the acute toxicity of the test item Sikaflex PRO-3 Purform to fish embryos. Zebrafish embryos (≤16 cell stage) were exposed to the only loading rate of nominal 7.6 g/L under defined conditions for 96 hours. The following endpoints were recorded as indicators of acute lethality in fish embryos: the coagulation of the embryo, lack of somite formation, the non-detachment of the tail-bud from the yolk sac, the lack of heartbeat. Additionally, the hatching rate was determined. This limit-test was performed in compliance with the test guideline OECD 236.
Guidelines:	Based on OECD Guideline for Testing of Chemicals, Section 2, No. 236, "Fish Embryo Acute Toxicity (FET) Test", adopted July 26, 2013
Study ID:	193741267

Materials and Methods

Test Item:	Sikaflex PRO-3 Purform; ibacon substance ID: 24433
Test Species:	Zebrafish embryos (*Danio rerio*), cell stage determined based on the whole egg batch: 2 to 16, Source: In-house breeding
Test Design:	The acute toxicity to zebrafish embryos was determined in a static, 96-hour test using three treatment groups: one test item loading rate and a positive control each containing 20 individuals and a negative control containing 24 individuals. The test item solution of a nominal loading rate of 7.6 g test item/L was prepared by weighing 30.4 g test item in a glass aquarium with a total volume of 6 litres. The test item was then thinly spread across the surface and cured for 7 days. Then, 4000 mL reconstituted test water was added and stirred for another 7 days. Additionally, an internal plate control with 4 individuals was tested containing the test water without the test item or reference item. The test was conducted in 24-well plates, each well containing approx. 2 mL test medium. 3 days before test start the plates were pre-coated and the test medium was replaced at test start, i.e. when one egg per well was exposed. The test embryos were observed at test start and after approximately 24, 48, 72 and 96 hours test duration for apical observations (coagulation, somite formation, detachment of tail bud), whereas hatching and heartbeat were only observed after 48, 72 and 96 hours.
Endpoints:	Estimation No Effect Loading rate (NOEL), Lowest Effect Loading rate (LOEL) and Lethal Loading rate with 50% effect (EL_{50}, indicated by one

	of the four apical end points) after 96 h of exposure.
Test Concentration:	Loading rate of 7.6 g test item/L, a positive control containing 4.0 mg/L 3,4-Dichloroaniline and a negative control.
Test Conditions:	Water temperature: 25.5 to 26.8 °C; pH value: 7.6 to 7.9; dissolved oxygen concentration: 94 to 105 % of the air saturation value; Conductivity: 517 to 537 µS/cm; water hardness 160 mg $CaCO_3$/L; photoperiod: exposure in darkness.

Results

Validity Criteria:	The overall fertilisation rate of all eggs collected was 90% (should be ≥70 %). Water temperature was maintained at 25.5 to 26.8°C (should be 26 ± 1 °C). The survival of negative control embryos was 100 % (should be ≥90 %). Negative control hatching rate was 100 % (should be ≥80 %). The dissolved oxygen concentration in the negative control and the test concentration at the end of the test was 94 % and 96 % of saturation, respectively (should be ≥80 %). The mortality in the positive control was 100 % (should be ≥30 %). Thus, all validity criteria were met.
Biological Test Results:	In the control, 100 % of fish embryos survived until test end compared to a survival of 95% in the nominal 7.6 g test item/L treatment. Hatching success in the control was 100 % compared to 95 % in the 7.6 g test item/L treatment. In the internal plate control of the test item group, hatching success was 100% after 96 hours. In the positive control, containing 4.0 mg 3,4-Dichloroaniline/L, 100 % of the embryos died. All biological results are listed in Table 1.

Table 1. Observed mortality and hatching success of the zebrafish embryos (*Danio rerio*) exposed to Joint sealant for 96 hours

Treatment group	96-hour		96-hour (internal plate control)	
	% Mortality	% Hatching	% Mortality	% Hatching
Control	0	100	-	-
Sikaflex PRO-3 Purform	5	95	0	100
Positive Control	100	0	0	100

Conclusion

In this screening test, a hypothetical maximum exposure scenario was tested exceeding regular application conditions. All test validity criteria were met. No effects on zebrafish embryos were observed in the only tested concentration of 7.6 mg test item/L based on nominal concentration.

Based on the test results the 96-hour EL_{50} of Sikaflex PRO-3 Purform for zebrafish embryos (*Danio rerio*) was determined to be > 7.6 mg test item/L based on nominal concentration. The NOEC was determined to be ≥ 7.6 mg test item/L also based on nominal concentration.

Method	Unit	EL_{50} Result	Start and End of Experiment
EL_{50} Determination according to OECD 236	[g test item/L]	> 7.6	11/07/25 – 01/08/25