Fraunhofer
IME

Expert opinion

Risk assessment of the use of Elastocoast 6551/103 for the fortification of water courses based on the results of acute and chronic aquatic toxicity studies

December 15, 2009

Dr. Christoph Schäfers

Fraunhofer IME
Auf dem Aberg 1
57392 Schmallenberg

Table of contents

1. Scope of this expert opinion

Elastocoast is intended to be used as fortification of the bank of watercourses or coastal shores. As it contents reactive compounds with known toxicity, there was need for an investigation of the influence of the practical use on water quality. For this, a large scale experimental simulation of the introduction of Elastocoast in water bodies with subsequent elution was performed (chapter 2.1). Water samples were taken at different times after introduction and investigated in mechanistic bioassays for endocrine or mutagenic potential (chapter 2.2) and in acute and chronic aquatic toxicity tests for the effect on aquatic communities (chapter 2.3).

Scope of this expert opinion is the evaluation of the study results regarding their predictive potential for the risk of the use of Elastocoast by comparing the effect data under experimental exposure with realistic worst case exposure scenarios (chapter 3) to derive safe use scenarios (chapter 4).

2. Study results

2.1 Large scale experimental simulation of the introduction of Elastocoast in water bodies and subsequent elution

At the Fraunhofer-Institute for Molecular Biology and Applied Ecology a semi-technical leaching study with stones coated with Elastocoast 6551/103 was conducted. 200 kg of basalt-stones were coated with 3600 g Elastocoast 6551/103 polyurethane resin and poured into 1000 L of water of 20 °C (Simon, 2009a, Estimation of emissions to the aqueous environment, non-GLP study report). The ratio of Elastocoast and stones and the coating as well as the introduction in the water body followed the protocols of in-field use. Untreated basalt-stones were applied as a control in a second test basin under equal test conditions. A circular flow system (1000 L/h) was established to simulate current and aging of the test item. The study was conducted for 21 days. Water samples were collected at day 1 (15 min after introduction), 7, 14, and day 21 for analysis of the sum-parameters DOC and primary aromatic amines in the undiluted eluate.

The simulation study setup represents worst case conditions when compared to realistic applications of Elastocoast, as water movement simulates flowing waters but recirculation does not allow dilution.

DOC concentration in the eluates of the treatment was comparable throughout the test duration (7 to 8 mg/L), whereas DOC concentrations in the water samples of the control were between 2 to 3 mg/L. Differences between treatment and vehicle control were 3.9, 3.5, 5.8, and 5.2 mg DOC/L after 15 min, 7, 14, and 21 days of leaching, respectively.

Total primary aromatic amines concentration in the eluates of the treatment started with 34 µg/L 15 min after introduction, showed a decrease within the first two weeks and a stabilisation at 22 µg/L during the last week. Total primary aromatic amines concentration in the water samples of the vehicle control were always below or at the Limit of Quantification (2.2 µg/L).

Results of DOC and total primary aromatic amines measurements demonstrate that the main emission occurs directly at introduction of the coated stones. After 14 days of elution more or less stable conditions seem to be adjusted without significant net change of measured parameters.

2.2 Mechanistic bioassays (non-GLP) for endocrine or mutagenic potential

The sample with the highest concentration of primary aromatic amines (15 min after introduction) was investigated for endocrine potential by yeast estrogen [ERa] and yeast androgen [AR] screening assays, performed by the BASF experimental toxicology and ecology laboratory, Ludwigshafen, Germany, according to the method by Routledge and Sumpter (1996) (Böhn 2009a and 2009b). As the steroid receptors expressed in yeast are of human origin, the tests are indicative of substances potentially causing receptor-mediated disruption (either agonistic or antagonistic by adding hormones) in mammals. Due to the evolutionary conservatism of the sexual endocrine system they are also able to indicate threats to other vertebrates, e.g. fish.

The sample was diluted by a factor of 2 with yeast culture medium. As the ratio of coated stones and water was 1:5 in the large scale elution experiment, the tested eluate concentration corresponded to a ratio of coated stones : water of 1:10.

Neither the sample taken from the test system 15 min after introduction of Elastocoast-coated stones, nor the sample from the control system showed any estrogenic, anti-estrogenic, androgenic or anti-androgenic effect potential.

In consequence, the no effect ratio of Elastocoast-coated stones : water for endocrine potential is equal to or higher than 1 : 10.

Samples taken 15 min, 7 d and 21 d after the start of elution were investigated for the mutagenic potential by using the umu-Test (ISO 13829, DIN 38412-3) with and without metabolic activation (S9-extract) (Hund-Rinke, 2009).

No mutagenicity was observed at any time even at the highest test concentration. The highest test concentration applying the undiluted water sample corresponds to 76.5 % water sample in the test.

In consequence, the no effect ratio of Elastocoast-coated stones : water for mutagenic potential is equal to or higher than 1 : 6.5.

2.3 Aquatic toxicity tests (GLP)

To evaluate the risk for aquatic communities of an application of Elastocoast as fortification for water courses, aquatic toxicity tests were performed with samples taken at different times of elution after introducing Elastocoast-coated stones in a large-scale simulation study (see 2.1). Following accepted principles of ecotoxicological hazard assessment, representatives of different trophic levels were tested according to GLP: Destruents (cell multiplication rate of a pure culture of *Pseudomonas putida* according to DIN EN ISO 10712), primary producers (green freshwater alga *Desmodesmus subspicatus,* growth inhibition test according to OECD TG 201), primary consumers (*Daphnia magna* immobilization test according to OECD TG 202) and secondary consumers (Fish embryo toxicity test with *Danio rerio* along the OECD draft guideline, 2006). For fish we decided to replace the fish acute toxicity test (OECD TG 203) by the fish embryo test not only for reasons of animal welfare, but also for reasons of relevance for the relevant habitat. Stones in the riparian zone are places of spawning and hatching of fish fry.

All short-term test were conducted with eluate samples taken from the simulation system 15 min, 7 d and 21 d after introduction of Elastocoast-coated stones. The organisms were tested in dilution series with several dilution steps. At test start on day 1 (15 min of elution), dilution series of 1:2 (G2), 1:3 (G3), 1:4 (G4), 1:6 (G6) were prepared and tested additionally to the undiluted sample (G1). For *D. magna,* the same procedure was conducted also on day 7. On day 7 and 21, the organisms were exposed only to undiluted water samples (for *D. magna*: day 21). The number of dilution steps at the respective testing date was chosen with concern to the effects at the former test dates.

Beside acute toxicity, also chronic exposure to the eluate was investigated. The duration of chronic exposure is dependent on the generation time of the test organisms. For bacteria and algae, the performed tests already comprise multiple generations. We focussed on chronic invertebrate tests and performed a *Daphnia magna* reproduction test according to OECD TG 211 and a *Chironomus riparius* sediment-water test to represent insect larvae as most important invertebrate group of water courses and to include potential exposure via reaction products adsorptive to sediment. Both tests were conducted with eluate at different dilution steps (undiluted = G1, 1:2 = G2, 1:3 = G3). The *Daphnia magna* reproduction test with a 21 d semi-static exposure to the test item with renewal of the test solutions three times a week was fed by samples from the large-scale elution test collected 15 min, 2, 4, 7, 9, 11, 14, 16, and 18 d after start of the elution. The *Chironomus riparius* sediment-water test received overlaying water at the different dilution steps with renewal of the test solutions at day 7 and 14 with freshly collected water samples.

The results of the tests are listed in table 1.

Table 1: Results of the aquatic toxicity tests with eluates of the large scale simulation (2.1)

Test organism	Guideline	Reference	NOEL	EL_{50}	Ratio of coated stones : water at NOEL
Pseudomonas putida	DIN EN ISO 10712	Simon (2009b)	≥80% of G1*	>80% of G1*	> 1 : 6.3
Desmodesmus subspicatus	OECD 201	Wenzel (2009)	≥90% of G1*	>90% of G1*	> 1 : 5.6
***D. magna im-mob.* 15 min**	OECD 202	Simon (2009c)	G2	99% of G1	1 : 10
7 d			≥ G1	> G1	> 1 : 5
21 d			≥ G1	> G1	> 1 : 5
***D. rerio* em-bryo test**	OECD (2006) (draft)	Teigeler (2009)	≥ G1	> G1	> 1 : 5
21 d *D. magna* reproduction	OECD 211	Simon (2009d)			
r, caused by survival			G2	> G1	1 : 10
Offspring, growth			≥ G1	> G1	> 1 : 5
Chironomus riparius	OECD 219	Simon (2009e)	≥ G1	> G1	> 1 : 5

* due to methodological reasons the "undiluted" G1 sample contained culture medium that reduced the % test item

With the exception of the Daphnia magna tests, no significant effect was observed on any of the endpoints in any of the tests with any samples from different sampling dates.

In Daphnia magna, consistent effects were observed in undiluted eluates in the acute and chronic test. Survival was reduced by 55% in the acute test (15 min sample) and 40% in the chronic test (until day 9, before reproduction), respectively, the latter resulting in a significant reduction of the intrinsic rate of increase r. In the acute test, undiluted eluate samples taken after 7 and 21 d caused less mortality of 20% and 15%, respectively, which was no longer statistically significant. At a dilution of 1 : 2 (G2), no effect was observed on any endpoint in any of the *Daphnia magna* tests.

In consequence, the no effect ratio of Elastocoast-coated stones : water for aquatic toxicity endpoints is 1 : 10.

For the identification of an effect threshold concentration, regression analysis can be applied to data of a series of dilution steps with inverse dilution dependent effects between 0 and at least approximately 50%. The EL_{10} (effect level that causes 10% effect) expressed as % of the undiluted eluate, can serve as effect threshold. The lowest EL_{10} values calculated from the *Daphnia* effect data were 61% of the undiluted eluate for mortality in the reproduction study and 84.6% of the undiluted eluate for immobility in the acute study, resulting in threshold effect ratios of Elastocoast-coated stones : water for aquatic toxicity endpoints of 1 : 8.2 and 1 : 5.9, respectively.

3. Realistic worst case scenario for exposure of aquatic communities to Elastocoast

A realistic worst case scenario for exposure to eluates of Elastocoast-coated stones should consider the aspects

- sufficient / minimum size of the cross-section of a water course for being used by navigation,
- low slope of the banks and maximum coverage of banks with Elastocoast-coated stones,
- no dilution by the flow regimen.

Thus, the realistic worst case scenario is represented by a static channel with a bank slope of 1 : 3 and full coverage of both banks with the recommended mass of Elastogran-coated stones (300 kg/m²).

The minimum depth of a channel used for navigation that needs bank fortification is set to 2 m. This means a bank area of 3*2m=6 m²/m channel length on each side, resulting in 0.3 t/m² * 12 m² = 3.6 t Elastocoast-coated stones/m channel length.

With these pre-requisites a channel at no effect level conditions has a mean width (at the middle of the bank) of 18 m (18 m*2 m*1 m = 36 m³). This means, a static channel of 2 m depth with banks (slope: 1 : 3) completely covered with Elastocoast-coated stones should not be smaller than 18 m.

Realistic channels used for navigation of Europe ships (1350 t) should have minimum depths of 4 m and minimum mean widths of approximately 40 m. Provided that such channels have banks with slopes of 1 : 3 completely covered with Elastocoast-coated stones, the ratio between Elastocoast-coated stones and water is 1 : 22 (0.3 t/m² * 24 m² = 7.2 t; 40 m*4 m*1 m = 160 m³).

4. Conclusions for the risk assessment

Before application of Elastocoast, the worst case scenario described in chapter 3 should be compared with the real situation. If the cross-section profile exceeds 2 m depth an 18 m width, there is no need for risk mitigation measures as the factors dilution, time after introduction and effect size include sufficient safety for any application situation. Only if the water course is only 2 m deep at a width of less than 18 m, dilution by the flow regimen and percentage and timing of bank coverage have to be considered to exclude a risk for aquatic communities.

5. References

Böhn (2009a): Summary of results – Elastocoast Eluaten. Testing for potential estrogen and anti-estrogen activity using the YES-assay [ERa] (Yeast Estrogen Screening). Non-GLP study performed by BASF SE, Ludwigshafen, Germany; sponsored by ELASTOGRAN GmbH, Lemfoerde, Germany.

Böhn (2009b): Summary of results – Elastocoast Eluaten. Testing for potential androgen and anti-abdrogen activity using the YAS-assay [AR] (Yeast Androgen Screening). Non-GLP study performed by BASF SE, Ludwigshafen, Germany; sponsored by ELASTOGRAN GmbH, Lemfoerde, Germany.

DIN EN ISO 10712: Water quality – *Pseudomonas putida* growth inhibition test (*Pseudomonas* cell multiplication inhibition test). February 1996.

Hund-Rinke (2009): umu-Test (ISO 13829, DIN 38412-3). Effect of water samples derived from an application simulation of stones treated with Elastocoast. Non-GLP study performed by Fraunhofer IME-AE, Schmallenberg, Germany; sponsored by ELASTOGRAN GmbH, Lemfoerde, Germany.

DIN 38415-3: Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung - Suborganismische Testverfahren (Gruppe T) - Teil 3: Bestimmung des erbgutverändernden Potentials von Wasser mit dem umu-Test (T 3). December 1996.

ISO 13829: Water quality – Determination of the genotoxicity of water and waste water using the umu-test. March 15, 2000.

OECD (2006): Guideline for Testing of Chemicals – Fish Embryo Toxicity (FET) Test. Draft proposal for a new guideline. 1st version, May 30, 2006.

OECD 201: OECD guideline for testing of chemicals - Freshwater Alga and Cyanobacteria, Growth Inhibition Test. March 23, 2006.

OECD 202: Guideline for Testing of Chemicals - Daphnia sp., Acute Immobilization Test. April 13, 2004.

OECD 203: OECD guideline for testing of chemicals – Fish, Acute Toxicity Test. July 17, 1992.

OECD 211: OECD guideline for testing of chemicals – Daphnia magna Reproduction Test. September 21, 1998

OECD 219: OECD guideline for testing of chemicals – Sediment-Water Chironomid Toxicity Test using Spiked Water. April 13, 2004.

Simon (2009a): Estimation of emissions to the aqueous environment. Semi-technical testing for estimation of leaching from coated stones treated with Elastocoast. Non-GLP study performed by Fraunhofer IME-AE, Schmallenberg, Germany; sponsored by ELASTOGRAN GmbH, Lemfoerde, Germany.

Simon (2009b): *Pseudomonas putida*, Growth Inhibition Test. Effects on cell multiplication of *Pseudomonas putida* by water samples derived from an application simulation of stones treated with Elastocoast. GLP study ELA-002/3-07 performed by Fraunhofer IME-AE, Schmallenberg, Germany; sponsored by ELASTOGRAN GmbH, Lemfoerde, Germany.

Simon (2009c): *Daphnia magna*, acute immobilisation test (OECD 202), static exposure. Effect of water samples derived from an application simulation of stones treated with Elastocoast on the immobilisation of *Daphnia magna.* GLP study ELA-002/4-20 performed by Fraunhofer IME-AE, Schmallenberg, Germany; sponsored by ELASTOGRAN GmbH, Lemfoerde, Germany.

Simon (2009d): *Daphnia magna*, reproduction test (OECD 211), semi-static exposure. Effect of water samples derived from an application simulation of stones treated with Elastocoast on the reproduction of *Daphnia magna.* GLP study ELA-002/4-21 performed by Fraunhofer IME-AE, Schmallenberg, Germany; sponsored by ELASTOGRAN GmbH, Lemfoerde, Germany.

Simon (2009e): Sediment – water chironomid toxicity test using spiked water. Effect of water samples derived from an application simulation of stones treated with Elastocoast on the development of *Chironomus riparius*. GLP study ELA-002/4-29 performed by Fraunhofer IME-AE, Schmallenberg, Germany; sponsored by ELASTOGRAN GmbH, Lemfoerde, Germany.

Teigeler (2009): Zebrafish (*Danio rerio*), fish embryo toxicity test. Effect of water samples derived from an application simulation of stones treated with Elastocoast on the embryonic stages of *Danio rerio*. GLP study ELA-002/4-14 performed by Fraunhofer IME-AE, Schmallenberg, Germany; sponsored by ELASTOGRAN GmbH, Lemfoerde, Germany.

Wenzel (2009): Alga, growth inhibition test. Effect of water samples derived from an application simulation of stones treated with Elastocoast on the growth of *Desmodesmus subspicatus*, static conditions. GLP study ELA-002/4-30 performed by Fraunhofer IME-AE, Schmallenberg, Germany; sponsored by ELASTOGRAN GmbH, Lemfoerde, Germany.