

18 December 2023

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Dear Tanya,

**ADDENDUM TO CAWTHRON REPORT 3895: 'PRELIMINARY ASSESSMENT OF THE IMPACTS OF THE GREEN ISLAND LANDFILL LEACHATE ON THE RECEIVING ENVIRONMENT USING PASSIVE SAMPLERS AND TOXICITY TESTING'**

Please find herewith the results for the blue mussel embryo-larval development test conducted on extracts.

**Embryo-larval development assay**

The blue mussel (*Mytilus galloprovincialis*) 48-hour embryo-larval development (survival) acute test is a widely used and well-validated test (ASTM 2021). Briefly, freshly fertilised eggs are exposed to dilutions of the test solution under static conditions (no renewal of the test solution) for a fixed period of 48 hours. At the end of the test, larvae are fixed in a solution of formalin and D-larvae are numbered. The number of abnormal D-larvae in the test solution gives an indication of embryo toxicity in early life stage development. A test solution is considered toxic when a statistically significant, dose-dependent increasing percentage of abnormalities occurs. The test is conducted during the mussel's early life stages, which are highly sensitive. The test parameters and sensitivity control are reported in Table 1.

Table 1. Exposure parameters for the embryo-larval development assay

<b>Bivalve – embryo-larval development</b>	
Test start–end dates	29 November 2023–1 December 2023
Standard	ASTM E724-21 (2021)
Cawthron SOP	ETX 4
Test species	<i>Mytilus galloprovincialis</i>
Source	Duncan Bay
Density (no. per container)	~ 400
Test containers	6-well plate (10 mL/well)
Exposure time (hr)	48
Sample pre-treatment	None
Replicates	5
Light	None
Temperature (°C)	16.3 ± 0.8
Dissolved oxygen (at beginning) (mg/L)	8.7 (108%)
pH	8.4
Dilution water	Reconstituted seawater
Aeration	None
Salinity (beginning; PSU)	33.4
End point	Survival
Sensitivity (EC <sub>50</sub> with 95% CI)	0.230 (0.224–0.236) mg Zn <sup>2+</sup> /L
Control quality for sensitivity (mean with 2SD)	0.183 (0.118–0.247) mg Zn <sup>2+</sup> /L ( <i>n</i> = 10)
Test acceptability (in controls)	> 60% development
Test compliance to procedure	Yes
Notes	Collection date: 28 November 2023 Spawning method: thermal stimulation

## Results and discussion

Mussel embryo survival rates (as larvae D-yield) for the tested extracts are presented in Figure 1 (raw data are reported in Appendix 1, Table A.1). The assay did not show any significant difference between the reconstituted seawater control (Control) and the extracts resuspension solvent (DMSO [dimethyl sulfoxide] control). Nor was any difference detected between the DMSO control and the extracts for the tested sites. Only the difference between the Control (without DMSO) and the sites was significant ( $P < 0.05$ ). A previous trial showed the same trend (results not shown as the standard deviations were too wide to be used). As no difference was detected between field blanks and tested sites, this could indicate a low toxicity, if any, of extracts towards the tested organism.

Water used for the test with the mussel embryos was saltier (33.4 PSU) than media used with the other tested organisms (20 PSU and 26 PSU for the marine bacterium *Aliivibrio fischeri* and the green microalga *Dunaliella tertiolecta*, respectively). This may have buffered

the impact on the embryo-larval development of the blue mussel, by reducing the bioavailability of the different compounds (Heugens et al. 2021).<sup>1</sup>

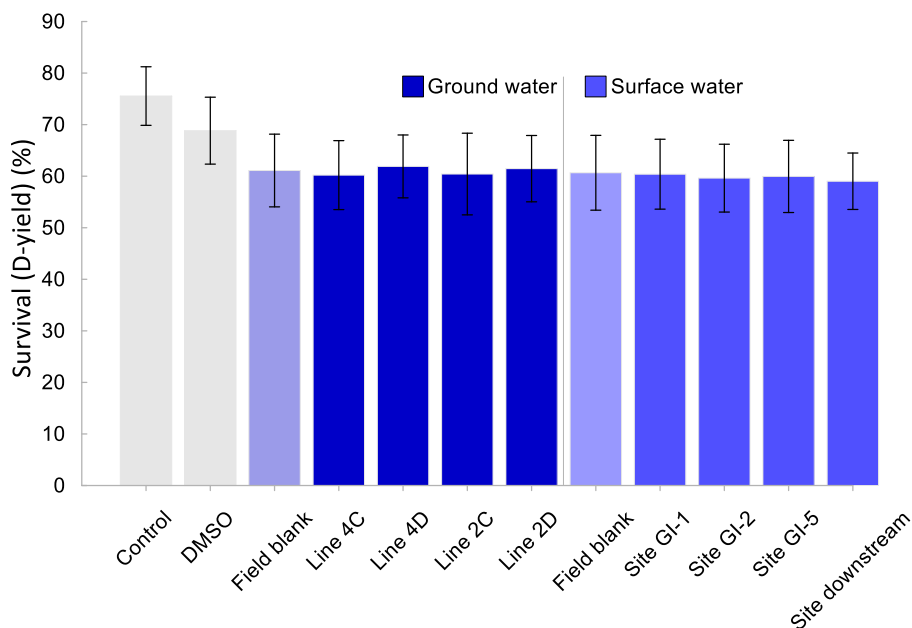


Figure 1. Blue mussel larvae survival (% D-yield) (average  $\pm$  standard deviation).

Yours sincerely

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<sup>1</sup> Heugens E, Hendriks A, Dekker T, Straalen N, Admiraal W. 2001. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. *Critical Reviews in Toxicology*. 31:247–284. <https://doi.org/10.1080/20014091111695>

## Appendix 1

Table A.1 Blue mussel embryo survival (% D-yield larvae) after a 48-hour exposure to extracts from test sites

<b>Seawater control</b>	<b>GI.1 DMSO control</b>	<b>GI.2 Field blank GW</b>	<b>GI.5 Line 4C</b>	<b>GI.6 Line 4D</b>	<b>GI.3 Line 2C</b>	<b>GI.4 Line 2D</b>	<b>GI.7 Field blank S</b>	<b>GI.8 Site GI-1</b>	<b>GI.9 Site GI-2</b>	<b>GI.10 Site GI-5</b>	<b>GI.11 Site downstream</b>
71.2	64.2	53.5	55.5	57.8	60.8	61.1	65.3	61.0	56.5	60.5	53.4
69.0	60.9	54.3	52.5	53.7	51.9	56.8	57.2	50.5	50.0	49.1	54.8
77.2	70.6	62.2	59.8	65.3	61.2	53.6	57.9	58.5	61.9	61.5	64.8
78.7	77.6	69.4	69.4	69.0	72.8	68.2	70.6	69.2	67.1	68.6	64.9
79.6	70.8	66.1	63.8	63.8	55.3	67.6	52.3	62.7	62.6	60.0	57.2
67.5											
71.2											
69.4											
81.7											
73.5											