



Certificate of Analysis

Whole Effluent Toxicity Testing

WSP Group Africa (Pty) Ltd. Earth and Environment Laboratory

Submitted to:

Leandra Jonker

Scientific Aquatic Services

P. O. Box 751779

Garden View

2047





Type of Document (Version) Confidential

Project no. 1164 - SAS

Our Ref. No. COA 2024/200

Date: 28-10-2024

Enclosed please find the test Certificate of Analysis number COA 2024/200. The results relate only to the sample(s) tested. WSP Group Africa (Pty) Ltd. Earth and Environment Laboratory does not accept responsibility for any matters arising from further use of the results contained within this COA. Tests marked "Not SANAS Accredited" in this COA are not included in the SANAS Schedule of Accreditation for this laboratory.

The Test(s) conducted in this COA have fulfilled the requirements of the method specific pass/fail criteria. No specifications of conformity for interpretation of the test results have been made or implied in this report.

No part of this COA may be quoted in isolation from the rest of the text, without the written permission of WSP Group Africa (Pty) Ltd. Earth and Environment Laboratory. Opinions and interpretations expressed within this document are outside the scope of SANAS accreditation.

This COA supersedes any results reported telephonically or otherwise.

Please contact the laboratory if further information is required.

We look forward to being of assistance to you.

Yours faithfully,

Mahadi Motsumi

(Quality Coordinator)

Quality Control

	Report:	Technical Signatory:	Designation:
Prepared by:	Claire Volschenk	LAB-GUI-ENV-001 LAB-GUI-ENV-002 LAB-GUI-ENV-004	Bench Supervisor
Signature:			
Checked by:	Bridget Shaddock	LAB-GUI-ENV-001 LAB-GUI-ENV-002 LAB-GUI-ENV-003 LAB-GUI-ENV-004 LAB-GUI-ENV-006 LAB-GUI-ENV-007 LAB-GUI-ENV-009 LAB-GUI-ENV-010 LAB-GUI-ENV-011 LAB-GUI-ENV-012 LAB-GUI-ENV-013	Laboratory Manager
Signature:			
Authorised by:	Mahadi Motsumi	LAB-GUI-ENV-001 LAB-GUI-ENV-002 LAB-GUI-ENV-003 LAB-GUI-ENV-004 LAB-GUI-ENV-006 LAB-GUI-ENV-009 LAB-GUI-ENV-010 LAB-GUI-ENV-011 LAB-GUI-ENV-012 LAB-GUI-ENV-013	Quality Coordinator
Signature:			
Remarks:	Revision 0		
Date:	28-10-2024		

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1. Certificate of Analysis

1.1 Customer Details

Requested By:	Leandra Jonker
Company Name:	Scientific Aquatic Services
Address:	P. O. Box 751779
	Garden View
	2047
Telephone Number:	+27 11 616 7893
E-mail Address:	leandra@sasenvgroup.co.za

1.2 Laboratory Details

Company Name:	WSP Group Africa (Pty) Ltd. Earth and Environment Laboratory
Division:	Toxicity Division
Physical Address:	25 Main Avenue
	Florida
	1709
Telephone Number:	+27 11 254 4800
Registration Number:	1999/008928/07

2. Sample Information

2.1 Sample Receipt

Sampling Technique:	Grab
Name of Sampler(s):	Unknown
Description of Sample Container(s):	Plastic bottle
Date and Time of Sample Receipt at the Laboratory:	19.07.2024 Time 15:45
Comments:	The Damfix product was mixed according to the manufacturers specifications (x1) and additionally to twice (x2) and three (x3) times the recommended dosages

2.2 Samples Received

Sample Reference Name(s):	Collection Date and Time:	Sample Reference Number(s):
DAMFIX		
Damfix x1	04.10.2024 Time 14:00	24/1697
Damfix x2	04.10.2024 Time 14:00	24/1698
Damfix x3	04.10.2024 Time 14:00	24/1699
Damfix x1 deposit	04.10.2024 Time 14:00	24/1700
Damfix x3 deposit	04.10.2024 Time 14:00	24/1701

2.3 Introduction to Tests Requested

License Number:	Not applicable
License Toxicity Testing Requirements:	Not applicable
Plant Name and/or Location:	Not available
Name of Receiving Water Body(s) Up and Downstream of Discharge:	Not available

2.4 Requested Analysis

Analysis Performed:	Sample Reference Numbers:
15- and 30-minute <i>Vibrio fischeri</i> Bioluminescent Screening Test	24/1697 – 24/1699
72-hour <i>Selenastrum capricornutum</i> Growth Inhibition Screening Test	24/1697 – 24/1699
24- and 48-hour <i>Daphnia pulex</i> Acute Toxicity Screening Test	24/1697 – 24/1699
96-hour <i>Poecilia reticulata</i> Acute Toxicity Screening Test	24/1697 – 24/1699
6-day <i>Heterocypris incongruens</i> Mortality and Growth Inhibition Test	24/1700 – 24/1701 (without sediment) 24/1700 – 24/1701 (with sediment)

3. Methodology

Test Conditions

All toxicity tests were conducted in environmentally controlled rooms using standard techniques.

Quality assurance

The WSP Group Africa (Pty) Ltd. Earth and Environment Laboratory's Policy and Quality Manual, intended to support and maintain all aspects of the Quality System, is based on the application of ISO/IEC 17025. The following Quality Assurance information will be made available on request: in-house reference toxicant test data and control charts, Proficiency Testing Scheme (PTS) test data, additional lot and batch numbers and raw toxicity test data.

Toxicity units

The toxicity unit (TUa) for each test performed is calculated as 100% (full strength effluent expressed as a percentage) divided by the effective concentration or LC₅₀ expressed as percentage sample dilution (e.g., *Daphnia pulex* and *Poecilia reticulata* Acute Toxicity Tests) and EC₅₀ (e.g., *Vibrio fischeri* Bioluminescent Test and *Selenastrum capricornutum* Growth Inhibition Test) (Tonkes & Baltus, 1997). If there is insufficient toxicity in a sample to allow for the determination of an EC₅₀/LC₅₀ value, then an acute toxicity unit of <1 will be assigned to the sample.

Table 1: Toxicity Units (Tonkes and Baltus, 1997)

Toxicity Unit	Conclusion
<1	Limited to Not Acutely Toxic
1 - 2	Negligibly Acutely Toxic
2 - 10	Mildly Acutely Toxic
10 - 100	Acutely Toxic
> 100	Highly Acutely Toxic

Physical and Chemical Properties

Parameter	LAB-GUI-ENV Number	Analysis	Date and time analysed in Room 4
pH	011	Voltammetry	08.10.2024 Time 10:04
Electrical Conductivity	010	Ion Exchange	
Dissolved Oxygen	Not SANAS Accredited	Luminescence	
Residual Total Chlorine		Colorimetric	

LAB-GUI-ENV-002

<i>Vibrio fischeri</i> Bioluminescent Test, EN ISO 11348-3 (2007)	
Test endpoint:	<u>Screening</u> : % growth inhibition/stimulation relative to control <u>Definitive test</u> : % growth inhibition/stimulation relative to control and EC ₅₀ and EC ₂₀ values
Test acceptability:	Correction factor = 0.6 to 1.8 No replicates differ from mean by > 3%
Light quality:	Ambient laboratory illumination
Photoperiod:	Not relevant
Aeration during test:	None
Test temperature:	16°C ± 2°C
Test chamber type:	Polystyrene luminometer cuvette
Test sample volume per container:	500 µL
Number of replicate containers per sample:	2
Volume of organisms per test chamber:	500 µL
Test concentrations:	<u>Screening</u> : 100% sample and a control <u>Definitive</u> : Minimum of five effluent concentrations and a control
Exposure period:	15- and 30-minutes
Deviation from reference method:	None
Test organism species name:	Lyophilized <i>Vibrio fischeri</i> luminescent bacteria (NRRL B-11177)
Synonym:	<i>Aliivibrio fischeri</i>
Reagent batch number:	VF 240318
Statistical methods used:	Microsoft Excel® and Regression analysis
Date of performance of the test in Room 2:	09.10.2024

LAB-GUI-ENV-003

<i>Selenastrum capricornutum</i> Growth Inhibition Test, OECD Guideline 201 (2011)	
Test endpoint:	<u>Screening</u> : % growth inhibition/stimulation relative to control <u>Definitive test</u> : % growth inhibition/stimulation relative to control and EC ₅₀ and EC ₂₀ values
Test acceptability:	Algal biomass in the control increased by at least a factor of 67 during exposure period
Light quality:	Cool white light: 3 500 to 4 100 K lateral illumination
LUX:	≤4 440 Lux
Photoperiod:	Continuous illumination during test period in an incubator
Aeration during test:	None
Test temperature:	23°C ± 2°C (preferably 24°C)
Test chamber type:	10 cm path length long cells (cuvettes)
Test sample volume per container:	25 mL
Number of replicates per sample	2
Number of organisms per test chamber:	1 x 10 ⁴ algae/mL
Test concentrations:	<u>Screening</u> : 100% sample and a control <u>Definitive</u> : Minimum of five effluent concentrations and a control
Exposure period:	72-hours
Deviation from reference method:	None
Test organism species name:	<i>Selenastrum capricornutum</i>
Synonym:	<i>Raphidocelis subcapitata</i> , <i>Pseudokirchneriella subcapitata</i>
Test organism source:	Printz algae beads (CCAP 278/4 Cambridge, UK)
OD measurement:	Jenway 6300 Spectrophotometer
Algal beads batch number:	SC240924
Statistical methods used:	Microsoft Excel® and Regression analysis
Date of performance of the test in Room 2:	14.10.2024 – 17.10.2024

LAB-GUI-ENV-004

<i>Daphnia pulex</i> Acute Toxicity Test, US EPA (2002)	
Test endpoint:	Screenings: % Mortality Definitive: % Mortality, LC ₁₀ and LC ₅₀ values
Test acceptability:	10% or less mortality in the controls
Light quality:	Ambient laboratory illumination
Photoperiod:	16 hours light, 8 hours darkness
Aeration during test:	None
Test temperature:	21°C ± 2°C
Test chamber type:	50 mL disposable polystyrene cups
Test sample volume per container:	25 mL
Number of replicate containers per sample:	4
Number of organisms per test chamber:	5 test organisms per sample container 20 test organisms per sample concentration and control
Test concentrations:	Screening: 100% sample concentration and a control Definitive: Minimum of five sample concentrations and a control
Exposure period:	24- and 48-hours
Deviation from reference method:	None
Test organism species name:	<i>Daphnia pulex</i>
Test organism source:	In-house sub-cultures
Test organism age:	Less than 24 hours old
Statistical methods used:	Probit software\TSK for definitive exposures
Date of performance of the test in Room 4:	08.10.2024 – 10.10.2024

LAB-GUI-ENV-001

<i>Poecilia reticulata</i> Acute Toxicity Test, US EPA (1996)	
Test endpoint:	Screenings: % Mortality Definitive: % Mortality, LC ₁₀ and LC ₅₀ values
Test acceptability:	10% or less mortality in the controls
Light quality:	Ambient laboratory illumination
Photoperiod:	16 hours light, 8 hours darkness
Aeration during test:	None
Test temperature:	23°C ± 2°C
Test chamber type:	250 mL disposable polystyrene cups
Test sample volume per container:	200 mL
Number of replicate containers per sample	2
Number of organisms per test chamber:	5 test organisms per control chamber Minimum of 4 test organisms per test chamber (i.e. 10 test organisms per control, minimum of 8 test organisms per sample/concentration)
Test concentrations:	Screening: 100% sample concentration and a control Definitive: Minimum of five sample concentrations and a control
Exposure period:	96-hours
Deviation from reference method:	None
Test organism species name:	<i>Poecilia reticulata</i>
Test organism source:	Obtained from Internal stock.
Test organism age:	7 to 21 days old.
Statistical methods used:	Probit software\TSK for definitive exposures
Date of performance of the test in Room 4:	11.10.2024 – 15.10.2024

LAB-GUI-ENV-007

<i>Heterocypris incongruens</i> Mortality and Growth Inhibition Test, EN ISO 14371 (2012)	
Test endpoint:	<u>Screening:</u> % mortality and growth inhibition/stimulation relative to control, <u>Reference:</u> LC ₅₀ /LC ₁₀ values and EC ₅₀ /EC ₁₀ values
Test acceptability:	Mortality in control <20% Growth length in controls at the end of the test should have increased by a factor x 1.5 in comparison to T ₀ measurement.
Light quality:	3 000 to 4 000 lux for hatching of cysts
Photoperiod:	No Illumination during test exposure
Aeration during test:	None
Test temperature:	25°C ± 2°C.
Test chamber type:	Polystyrene multiwell test plates
Test sample volume per well:	1 000 µL sediment
Number of replicate wells per sample	<u>Environmental samples:</u> minimum 5 replicates per sample <u>Reference:</u> minimum 1 replicate per concentration
Volume of organisms per test well:	10
Test concentrations:	<u>Screening:</u> 100% sample concentration and a control <u>Reference:</u> Minimum of five sample concentrations and a control
Exposure period:	6 days
Deviation from reference method:	None
Test organism species name:	<i>Heterocypris incongruens</i> cysts
Cysts batch number:	HI211022
Measurement:	Calibrated micro-meter cover slip, IRMECO IM1910-D compound microscope, USB.2.0 Camera Viewer, Image J©
Statistical methods used:	Regression analyses
Date of performance of the test in Sediment Toxicity Area:	11.10.2024 – 17.10.2024

4. Results

Table 2: 24/1697, 24/1698 and 24/1699 Water Toxicity Results: DAMFIX

Physical and Chemical Data	LAB-GUI-ENV Number	Sample Reference Number(s) and Description		
		24/1697 Damfix x1	24/1698 Damfix x2	24/1699 Damfix x3
pH	011	7.21	7.87	7.67
Electrical Conductivity (µS/cm)	010	285	289	296
Dissolved Oxygen concentration (mg/L)	Not SANAS Accredited	6.76	7.09	6.92
Residual Total Chlorine (present ✓/not present ×)*		×	×	×
Temperature (°C)		23	23	23
Toxicity Test Results				
15-minute <i>Vibrio fischeri</i> Bioluminescent Screening Test (average % inhibition (-) or stimulation (+))	002	-0.54	+4.3	+42
30-minute <i>Vibrio fischeri</i> Bioluminescent Screening Test (average % inhibition (-) or stimulation (+))		+9.1	+12	+59
30-minute <i>Vibrio fischeri</i> Bioluminescent Test, Toxicity Unit (TUa)		<1	<1	<1
72-hour <i>Selenastrum capricornutum</i> Growth Inhibition Screening Test (% growth inhibition (-) or growth stimulation (+))	003	+21	-19	-1.0
72-hour <i>Selenastrum capricornutum</i> Growth Inhibition Test, Toxicity Unit (TUa)		<1	<1	<1
24-hours <i>Daphnia pulex</i> Acute Toxicity Screening Test (% mortality)	004	0	0	10
48-hour <i>Daphnia pulex</i> Acute Toxicity Screening Test (% mortality)		10	30	45
48-hour <i>Daphnia pulex</i> Acute Toxicity Test, Toxicity Unit (TUa)		<1	<1	<1
96-hour <i>Poecilia reticulata</i> Acute Toxicity Screening Test (% mortality)	001	0	10	20
96-hour <i>Poecilia reticulata</i> Acute Toxicity Test, Toxicity Unit (TUa)		<1	<1	<1

Table 3: 24/1700 *Heterocypris incongruens* Sediment Toxicity Results: DAMFIX

Toxicity Test Results	LAB-GUI-ENV Number	Sample Reference Number(s) and Description
		24/1700 Damfix x1 deposit
6-day <i>Heterocypris incongruens</i> mortality test (average % mortality)	007	0
6-day <i>Heterocypris incongruens</i> mortality test toxicity unit (TUa)		<1
6-day <i>Heterocypris incongruens</i> growth inhibition test (average % inhibition (-) or stimulation (+))		+119
6-day <i>Heterocypris incongruens</i> growth inhibition test toxicity unit (TUa)		<1

UR Insufficient toxicity data available from the screening results to determine TUa with certainty.

Table 4: 24/1701 *Heterocypris incongruens* Sediment Toxicity Results: DAMFIX

Toxicity Test Results	LAB-GUI-ENV Number	Sample Reference Number(s) and Description
		24/1701 Damfix x3 deposit
6-day <i>Heterocypris incongruens</i> mortality test (average % mortality)	007	0
6-day <i>Heterocypris incongruens</i> mortality test toxicity unit (TUa)		<1
6-day <i>Heterocypris incongruens</i> growth inhibition test (average % inhibition (-) or stimulation (+))		+115
6-day <i>Heterocypris incongruens</i> growth inhibition test toxicity unit (TUa)		<1

UR Insufficient toxicity data available from the screening results to determine TUa with certainty.

5. Additional Requirements or Comments:

Note: Opinions and interpretations expressed within this document are outside the scope of SANAS accreditation.

*** Due to the mechanism of the DPD colorimetric method used for the determination of the presence of Residual Total Chlorine, there is a potential for a false positive chlorine result to occur. This may occur due to the presence of any of the additional Group VII Halogen elements (fluorine, bromine, iodine, and astatine).**

Any queries, pertaining to the results within this COA, should be lodged with the Quality Coordinator, Mahadi Motsumi, within 5 working days from the date of customer receipt of this COA. Samples will be discarded 1 week after the date of customer receipt of this COA and will not be kept on the laboratory premises for an extended period. Should any queries arise, that relate directly to specific samples within this COA, re-sampling will have to take place if queries have been brought to the laboratory's attention more than 5 working days after the date of customer receipt of this COA.

6. References

EUROPEAN STANDARD, 2007. "Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) – Part 3 for the method using freeze-dried bacteria". EN ISO 11348-3. European Committee for Standardization, Brussels.

DEUTSCHES INSTITUT FUER NORMUNG, 1991. Testverfahren mit Wassreorganismen. DIN 38 412 Standard, Teil 341, (Gruppe L).

OSTRACODTOXKIT F™.2001. Chronic "direct contact" toxicity test for freshwater sediments. Standard Operational Procedure. Dienze, Belgium: Creasel.

ORGANIZATION FOR ECONOMIC COOPORATION AND DEVELOPMENT, (OECD). 2011. Guideline for testing chemicals: Alga, Growth Inhibition Tests. Document no 201, Organization for Economic Cooperation and Development, Paris, France.

SOUTH AFRICAN NATIONAL STANDARD (SANS), ISO/IEC 17025.2017. General requirements for the competence of testing and calibration laboratories.3rd Edition. South African Bureau of Standards, Pretoria.

TONKES M. and BALTUS C.A.M. 1997. Praktijkonderzoek aan complexe efflueten met de Totaal Effluent Milieubezwaarlijkheid (TEM) – methodiek. RIZA – rapportnummer 97.033. RIZA, Lelystad, The Netherlands.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (US EPA), 1996. Ecological effects test guidelines. Fish acute toxicity test, Freshwater and marine. OPPTS 850.1075. Certificate of analysis number EPA-712-C-96-118.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (US EPA). 2002. Method for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA-821-R-02-012, 5th Edition. Office of Research and Development, Washington DC 20460.



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