

## TOXICITY TEST REPORT

**For:**  
GroundTruth

9 Quarry Street, Hilton, KwaZulu-Natal  
[patsy@groundtruth.co.za](mailto:patsy@groundtruth.co.za)

**Survey:**  
2023-10-02

**Report reference:**  
GRT-GTP-Q-23\_TOX

**Revision:**  
0

**Project:**  
GT1231 UPL

**Samples:**  
**Water:** SW04, SW10, SW21, SW23, PCD (Pre), PCD (Post)

Tests performed by: Praise Manyenga (Senior Analyst); Lethabo Mothupi (Senior Analyst)  
Classification (DEEEP) performed by: Lizet Swart (Quality Manager)

Report approved by:

Results approved by:



Praise Manyenga  
Praise Manyenga (Oct 23, 2023 12:58 GMT+2)

Lethabo Mothupi  
Lethabo Mothupi (Oct 23, 2023 14:32 GMT+2)

Lizet Swart  
Quality Manager

Praise Manyenga  
Technical Signatory

Lethabo Mothupi  
Technical Signatory

Report issue date  
23 October 2023

## Table of contents

1. ANALYSES REQUESTED AND SAMPLE INFORMATION .....	3
2. METHODOLOGY .....	3
Sampling and sample handling .....	3
Test Conditions .....	3
Quality Assurance .....	3
Assessments .....	4
3. HAZARD CLASSIFICATION METHODOLOGY .....	7
4. RESULTS AND HAZARD CLASSIFICATION DATA.....	8
5. COMMENTS .....	11
6. REFERENCES.....	11
END OF REPORT.....	11

## List of Tables

<b>Table 1:</b> Analyses requested and description for the different samples, including sampling and delivery dates. ....	3
<b>Table 2:</b> Toxicity Units (Tonkes and Baltus, 1997).....	7
<b>Table 3:</b> Hazard classification system for effluent/waste <u>samples</u> .....	7
<b>Table 4:</b> Weight score allocation for each test type (Persoone <i>et al.</i> (2003)) .....	7
<b>Table 5:</b> Hazard class per dilution level scale .....	8
<b>Table 6:</b> Hazard classification of water <u>samples</u> .....	8
<b>Table 7:</b> Hazard classification of water <u>sample dilutions (as per Table 5 methodology)</u> .....	8
<b>Table 8:</b> Site hazard classification of water <u>samples</u> .....	10

## 1. ANALYSES REQUESTED AND SAMPLE INFORMATION

Enclosed please find Test Certificate of analysis number GRT-GTP-Q-23\_TOX. The results relate only to the samples tested. BioToxLab does not accept responsibility for any matters arising from the further use of the results. Tests marked "Not SANAS accredited" (NA or OS) in this Certificate of Analyses are not included in the SANAS Schedule of Accreditation for this Laboratory.

No part of this Certificate of Analyses may be quoted in isolation of the rest of the text without the written permission of BioToxLab. Opinions and Interpretations expressed herein are outside the scope of SANAS accreditation.

Please contact the Laboratory if further information is required.

**Table 1:** Analyses requested and description for the different samples, including sampling and delivery dates.

Sample name	Sampling date	Sample type (water, sediment, product etc)	Sampled by	Delivery date	Delivered by	Additional comments (sample description or deviations)	Oxygenation required for sample, please tick (lab use): ✓   ✗   N/A	Tests requested - Marked with X								
								Screening	Definitive	Water					Sediment	
										<i>Daphnia magna</i>	<i>Poecilia reticulata</i>	<i>Aliivibrio fischeri</i>	<i>Selenastrum capricornutum</i>	<i>Spiridula polyhiza</i>	Phyto seeds	Ostracod
SW04	2023-10-02	Surface Water	GroundTruth	2023-10-09	Courier	Delivered >3 days after sampling Slightly discoloured	✗		X	X	X	X	X			
SW10	2023-10-02	Surface Water	GroundTruth	2023-10-09	Courier	Delivered >3 days after sampling Slightly discoloured with particles	✗		X	X	X	X	X			
SW21	2023-10-02	Surface Water	GroundTruth	2023-10-09	Courier	Delivered >3 days after sampling Slightly discoloured with particles	✗		X	X	X	X	X			
SW23	2023-10-02	Surface Water	GroundTruth	2023-10-09	Courier	Delivered >3 days after sampling Slightly discoloured with particles	✗		X	X	X	X	X			
PCD (Pre)	2023-10-02	Surface Water	GroundTruth	2023-10-09	Courier	Delivered >3 days after sampling Discoloured	✗		X	X	X	X	X			
PCD (Post)	2023-10-02	Surface Water	GroundTruth	2023-10-09	Courier	Delivered >3 days after sampling	✗		X	X	X	X	X			

**Key:**  
 Screening = 100% (undiluted) sample tested only  
 Definitive = Series of sample dilutions tested to enhance classification accuracy and to determine safe dilution

## 2. METHODOLOGY

### Sampling and sample handling

Samples were analysed as received from the Client. The water samples received from GroundTruth were exposed as definitives on 4 trophic levels (*Aliivibrio fischeri*, *Selenastrum capricornutum*, *Daphnia magna* and *Poecilia reticulata*).

### Test Conditions

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

### Quality Assurance

The BioToxLab Aquatic Toxicology 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 can be made available on request (1) inhouse reference toxicant test data and control charts (2) Proficiency Testing Scheme (PTS) test data (3) lot and batch numbers (4) raw toxicity test data.

## Assessments

Given the limitations of substance-specific assessments, and the risk of allowing ecological toxicity hazards to go unchecked/undetected, water resource managers and scientists have for some time called for methodologies that will allow more complete assessments of ecological toxicity hazards to be used in addition to the substance-specific approach. The National Water Act (Act no. 36 of 1998), providing for water in sufficient quantity and in sufficient quality for basic human needs and for maintenance of aquatic ecosystem function, implemented an approach known as the Direct Estimation of Ecological Effect Potential (DEEEP) protocol as a means of circumventing the shortcomings of direct toxicant monitoring. This protocol consists of a battery of tests to directly assess lethal (acute) and sub-lethal (chronic) toxicity, using test organisms from a range of trophic levels. These toxicity tests can demonstrate whether contaminants are bioavailable, it can evaluate the aggregate toxic effects of all contaminants in the medium and it can evaluate the toxicity of substances whose biological effects may not have been well characterized.

Lethal or sub-lethal toxicity testing (as applied for this assessment) is applied by exposing biota to water sources in order to determine the potential risk of such waters to the biota/biological integrity of the receiving water bodies and the environment. A risk category is determined based on the percentage of mortalities (lethal) or inhibition (sub-lethal) of the exposed biota. It is important to note that the hazard classification is based on the standardised battery of selected test biota and therefore represents the risk/hazard towards similar biota in the receiving aquatic environment. The toxicity hazard is therefore in terms of the aquatic biotic integrity and does in no way represent toxicology towards humans or other mammals.

Physical and chemical properties as required to be performed by the standard toxicity methods are also presented in this report as supplementary data to the toxicity testing data.

Standard, internationally accepted methods and materials were applied in order to conduct lethal and sub-lethal toxicity testing.

### **Aliivibrio fischeri sub-lethal bioluminescent test (A)**

BioToxLab method number:	QM7.2/TMH-01
Standard method:	SANS 11348-3:2013
Deviation from the method:	None
Test endpoint:	EC <sub>20</sub> /EC <sub>50</sub>
Exposure period:	15- and 30-minutes
Test chamber type:	Polystyrene cuvettes for luminometer
Test sample volume:	500 µL
Number of replicates per sample:	2
Test temperature (14-16°C):	14.6°C – 15.4°C
Test organism species name and source:	Lyophilized <i>Aliivibrio fischeri</i> luminescent bacteria (NRRL B-11177)
Luminescent measurement:	Luminoscan TL, Hygiene Monitoring System
<i>Aliivibrio fischeri</i> batch number(s):	VF 221107 / 2025-09
Reagent batch number(s):	RD 230723 / 2025-09
Sample diluent batch number(s):	SD 221003 / 2024-10
Statistical methods used:	Microsoft Excel® and Regression analysis
Date(s) of performance of the test(s):	2023.10.16 2023.10.17 2023.10.18
Uncertainty of measurement:	Available on request
Validity criteria (CF 0.6-1.8):	0.9/0.8/1.4/0.7

**Selenastrum capricornutum sub-lethal growth inhibition test (A)**

Synonym:	<i>Raphidocelis subcapitata</i> ; <i>Pseudokirchneriella subcapitata</i>			
BioToxLab method number:	QM7.2/TMH-02			
Standard method:	SANS 8692:2015			
Deviation from the method:	None			
Test endpoint:	EC <sub>20</sub> /EC <sub>50</sub>			
Exposure period:	72-hours			
Test chamber type:	10cm path length long cells			
Test sample volume:	25 mL			
Number of replicates per sample:	3			
Test temperature (21-25°C):	22.2°C – 24.2°C			
Test organism species name and source:	<i>Selenastrum capricornutum</i> , Printz algae beads (CCAP 278/4 Cambridge, UK)			
Optical density measurement:	Jenway 6300 Spectrophotometer			
Algal beads batch number(s):	SC 090523			
Matrix dissolving batch number(s):	MD 070122			
Nutrient batch number(s):	A: SC050123	B: SC080722	C: SC080722	D: SC080722
Statistical methods used:	Microsoft Excel® spreadsheet formulated by supplier (MicroBioTests Inc., Belgium) – RegTox and Regression analysis			
Date(s) of performance of the test(s):	2023.10.17			
Uncertainty of measurement:	Available on request			
Validity (from Regtox sheet: cell density factor ≥67):	Yes			

**Daphnia magna lethality toxicity test (A)**

BioToxLab method number:	QM7.2/TMH-03			
Standard method:	SANS 6341: 2015			
Deviation from the method:	None			
Test endpoint:	LC <sub>10</sub> /LC <sub>50</sub>			
Exposure period:	24- and 48-hours			
Test chamber type:	Polycarbonate test plates (6 rinsing wells and 24 testing wells)			
Test sample volume:	25 mL			
Number of replicates per sample:	4			
Number of test organisms per chamber:	5			
Test temperature (20-22°C):	21.1°C			
Test organism species name, age & source:	<i>Daphnia magna</i> – ehippia obtained from MicroBiotests, <24h old			
Feeding frequency during testing:	None			
Ehippia batch number(s):	DM 220323			
ISO media batch number(s):	ISO 120423			
Statistical methods used:	Microsoft Excel®			
Date(s) of performance of the test(s):	2023.10.16			
Uncertainty of measurement:	Available on request			
Validity criteria (control mortality ≤10%):	0%			

**Poecilia reticulata lethality toxicity test (A)**

BioToxLab method number:	QM7.2/TMH-04
Standard method:	SANS 7346-1: 2013
Deviation from the method:	None
Test endpoint:	LC <sub>10</sub> /LC <sub>50</sub>
Exposure period:	96-hours
Test chamber type:	250 mL disposable polystyrene cups
Test sample volume:	200 mL
Number of replicates per sample:	2
Number of test organisms per chamber:	6
Test temperature (22-24°C):	22.6°C – 23.6°C
Test organism species name, age & source:	<i>Poecilia reticulata</i> – 7-21 days old. Obtained from external stock
Feeding frequency during testing:	None
ISO media batch number(s):	ISO 120423
Statistical methods used:	Microsoft Excel®
Date(s) of performance of the test(s):	2023.10.12
Uncertainty of measurement:	Available on request
Validity criteria (control mortality ≤ 10%):	8.33%

**Physical and chemical properties**

Parameter	BioToxLab Method number	Test temperature (25°C ± 3°C)	Instrument	Batch numbers	Date of tests
pH (NA)	QM7.2/TMC-05	26.0°C	HQ440d	pH1.67: C02990 pH4: A2262 pH7: A2293A pH10: A2290	2023.10.10
EC (NA)	QM7.2/TMC-06	26.0°C	HQ440d	1413µS/m: A2279A	2023.10.10
Dissolved oxygen (NA)	QM7.2/TMC-07	26.0°C	HQ440d	N/A	2023.10.10

### 3. HAZARD CLASSIFICATION METHODOLOGY

The toxicity unit (TU) for each test performed is calculated as 100% (full strength effluent expressed as percentage) divided by the effective concentration or LC<sub>50</sub> expressed as percentage sample dilution (e.g. *Daphnia magna* and *Poecilia reticulata* lethal toxicity tests) and EC<sub>50</sub> (e.g. *Aliivibrio fischeri* bioluminescent test and *Selenastrum capricornutum* growth inhibition tests) (Tonkes & Baltus, 1997) (Table 2). If there is insufficient toxicity in a sample to allow for the determination of an EC<sub>50</sub>/LC<sub>50</sub> value, then a toxicity unit of <1 will be assigned to the sample.

**Table 2:** Toxicity Units (Tonkes and Baltus, 1997)

Toxicity Unit	Conclusion/Description
<1	Limited to no toxicity
1 – 2	Negligibly toxic
2 – 10	Mildly toxic
10 – 100	Acutely toxic
> 100	Highly toxic

A risk/hazard category is determined by using a hazard classification system developed by Persoone *et al.* (2003) whereby one can classify sites using the toxicity data of the non-diluted samples. The percentage effect (PE) of toxicity (mortalities, growth inhibition, luminescence inhibition) is used to rank the sample into one of five classes (Table 3 – effluent/waste samples) based on the highest toxic response obtained in at least one of the tests applied.

**Table 3:** Hazard classification system for effluent/waste samples

Class	Symbol	Hazard rating	PE	Percentage effect
I	☺	No lethal/sub-lethal hazard	≤10/20%	None of the tests show a toxic effect (i.e. an effect value that is significantly higher than that noted in the controls)
II	☹	Slight lethal/sub-lethal hazard	10/20%≤PE<50%	The effect percentage observed in at least one toxicity test is significantly higher than that in the control, but is below 50% (< 1 TU)
III	☠	Lethal/sub-lethal hazard	50%≤PE<100%	The L(E)C <sub>50</sub> is reached or exceeded in at least one test, but in the 10-fold dilution of the sample, the effect is less than 50% (= 1-10 TU)
IV	☠☠	High lethal/sub-lethal hazard	PE 100% in at least one test	The L(E)C <sub>50</sub> is reached in the 10-fold dilution for at least one test but not in the 100-fold dilution (=10-100 TU)
V	☠☠☠	Very high lethal/sub-lethal hazard	PE 100% in all tests	The L(E)C <sub>50</sub> is reached in the 100-fold dilution for at least one test (≥ 100 TU)

Each sample is furthermore weighted (Table 4) according to its relative toxicity level (out of 100%). Higher values indicate that more of the individual tests indicated toxicity within a specific class.

**Table 4:** Weight score allocation for each test type (Persoone *et al.* (2003))

Score	Category
0	No significant toxicity effect
1	Significant toxicity effect < PE50
2	Toxicity effect >PE50 but <PE100
3	The PE100 is reached

Class weight score calculated as follows:

Class weight score = (∑ all test scores)/n where n is the number of tests performed

Class weight score % = (class score) / (maximum class weight score) x 100

EP (Percentage effect) = an effect measured either as mortality or inhibition (depending on the type of test). A >10% effect is regarded as slight lethal toxicity for *Daphnia* and *Poecilia*, while a >20% effect is regarded as slight sub-lethal toxicity for *Selenastrum* and *Aliivibrio*. A 50% effect is regarded as a lethal/sub-lethal toxicity for all the tests (*Daphnia*, *Poecilia*, *Selenastrum* and *Aliivibrio*).

The toxicity hazard for each dilution level used to assess the hazard and perform calculations and classifications are presented in Table 5 to asses/review data trends and are done according to the following scale:

**Table 5:** Hazard class per dilution level scale

Scale	Description
0-≤10% ( <i>Daphnia</i> , <i>Poecilia</i> ) 0-≤20% ( <i>Aliivibrio</i> , <i>Selenastrum</i> )	Not toxic
10-<50 ( <i>Daphnia</i> , <i>Poecilia</i> ) 20-<50 ( <i>Aliivibrio</i> , <i>Selenastrum</i> )	Slightly toxic
50-<100 ( <i>Daphnia</i> , <i>Poecilia</i> , <i>Aliivibrio</i> , <i>Selenastrum</i> )	Toxic
≥100 ( <i>Daphnia</i> , <i>Poecilia</i> , <i>Aliivibrio</i> , <i>Selenastrum</i> )	Highly toxic

#### 4. RESULTS AND HAZARD CLASSIFICATION DATA

**Table 6:** Hazard classification of water samples

Site/ sample	Bacteria (A)			Microalgae (A)			Crustacea (A)			Vertebrates (A)			Weight %
	<i>Aliivibrio fischeri</i>			<i>Selenastrum capricornutum</i>			<i>Daphnia magna</i>			<i>Poecilia reticulata</i>			
	% effect	TU	Test score	% effect	TU	Test score	% effect	TU	Test score	% effect	TU	Test score	
SW04	-3.70	<1	0	-0.73	<1	0	0	<1	0	0	<1	0	0
SW10	+9.67	<1	0	-46.06	<1	1	0	<1	0	-8.33	<1	0	25
SW21	+1.37	<1	0	-27.05	<1	1	0	<1	0	0	<1	0	25
SW23	+96.93	<1	0	-4.61	<1	0	0	<1	0	0	<1	0	0
PCD (Pre)	-27.84	<1	1	-19.13	<1	1*	0	<1	0	-8.33	<1	0	50
PCD (Post)	-5.81	<1	0	+3.68	<1	0	0	<1	0	-8.33	<1	0	0

\*although no significant growth inhibition effect was noted on the 100% concentration (although close to the ≤20% level), slight inhibitions were noted on other dilutions of the sample

**Table 7:** Hazard classification of water sample dilutions (as per Table 5 methodology)

Sample name	Dilution level	Toxicity hazard	Sample name	Toxicity hazard	Sample name	Toxicity hazard	Sample name	Toxicity hazard
A. fischeri SW04	100%	Not toxic	S. capricornutum SW04	Not toxic	D. magna SW04	Not toxic	P. reticulata SW04	Not toxic
	50%	Not toxic		Not toxic		Not toxic		Not toxic
	25%	Not toxic		Not toxic		Not toxic		Not toxic
	12.5%	Not toxic		-		-		-
	10%	-		Not toxic		Not toxic		Not toxic
	6.25%	Not toxic		-		-		-
	3.125%	Not toxic		-		-		-
	1.56%	Not toxic		-		-		-
	1%	-		Not toxic		Not toxic		Not toxic
	0.78%	Not toxic		-		-		-
	0.39%	Not toxic		-		-		-
	0.195%	Not toxic		-		-		-
0.1%	-	Not toxic	Not toxic	Not toxic	Not toxic	Not toxic		
A. fischeri SW10	100%	Not toxic	S. capricornutum SW10	Slightly toxic	D. magna SW10	Not toxic	P. reticulata SW10	Not toxic
	50%	Not toxic		Slightly toxic		Not toxic		Not toxic
	25%	Not toxic		Slightly toxic		Not toxic		Not toxic
	12.5%	Not toxic		-		-		-
	10%	-		Not toxic		Not toxic		Not toxic
	6.25%	Not toxic		-		-		-
	3.125%	Not toxic		-		-		-
	1.56%	Not toxic		-		-		-
	1%	-		Not toxic		Not toxic		Not toxic
	0.78%	Not toxic		-		-		-
	0.39%	Not toxic		-		-		-
	0.195%	Not toxic		-		-		-
0.1%	-	Not toxic	Not toxic	Not toxic	Not toxic			



**Table 7: Hazard classification of water sample dilutions (as per Table 5 methodology)(continued)**

A.fischeri SW21	100%	Not toxic	S. capricornutum SW21	Slightly toxic	D. magna SW21	Not toxic	P. reticulata SW21	Not toxic
	50%	Not toxic		Slightly toxic		Not toxic		
	25%	Not toxic		Slightly toxic		Not toxic		
	12.5%	Not toxic		-		-		
	10%	-		Not toxic		Not toxic		
	6.25%	Not toxic		-		-		
	3.125%	Not toxic		-		-		
	1.56%	Not toxic		-		-		
	1%	-		Not toxic		Not toxic		
	0.78%	Not toxic		-		-		
	0.39%	Not toxic		-		-		
	0.195%	Not toxic		-		-		
	0.1%	-		Not toxic		Not toxic		
A.fischeri SW23	100%	Not toxic	S. capricornutum SW23	Not toxic	D. magna SW23	Not toxic	P. reticulata SW23	Not toxic
	50%	Not toxic		Not toxic		Not toxic		
	25%	Not toxic		Not toxic		Not toxic		
	12.5%	Not toxic		-		-		
	10%	-		Not toxic		Not toxic		
	6.25%	Not toxic		-		-		
	3.125%	Not toxic		-		-		
	1.56%	Not toxic		-		-		
	1%	-		Not toxic		Not toxic		
	0.78%	Not toxic		-		-		
	0.39%	Not toxic		-		-		
	0.195%	Not toxic		-		-		
	0.1%	-		Not toxic		Not toxic		
A.fischeri PCD (Pre)	100%	Slightly toxic	S. capricornutum PCD (Pre)	Not toxic	D. magna PCD (Pre)	Not toxic	P. reticulata PCD (Pre)	Not toxic
	50%	Slightly toxic		Slightly toxic		Not toxic		
	25%	Slightly toxic		Slightly toxic		Not toxic		
	12.5%	Slightly toxic		-		-		
	10%	-		Not toxic		Not toxic		
	6.25%	Slightly toxic		-		-		
	3.125%	Slightly toxic		-		-		
	1.56%	Slightly toxic		-		-		
	1%	-		Not toxic		Not toxic		
	0.78%	Slightly toxic		-		-		
	0.39%	Slightly toxic		-		-		
	0.195%	Not toxic		-		-		
	0.1%	-		Not toxic		Not toxic		
A.fischeri PCD (Post)	100%	Not toxic	S. capricornutum PCD (Post)	Not toxic	D. magna PCD (Post)	Not toxic	P. reticulata PCD (Post)	Not toxic
	50%	Not toxic		Not toxic		Not toxic		
	25%	Not toxic		Not toxic		Not toxic		
	12.5%	Not toxic		-		-		
	10%	-		Not toxic		Not toxic		
	6.25%	Not toxic		-		-		
	3.125%	Not toxic		-		-		
	1.56%	Not toxic		-		-		
	1%	-		Not toxic		Not toxic		
	0.78%	Not toxic		-		-		
	0.39%	Not toxic		-		-		
	0.195%	Not toxic		-		-		
	0.1%	-		Not toxic		Not toxic		

**Table 8: Site hazard classification of water samples**

Results		SW04	SW10	SW21	SW23	PCD (Pre)	PCD (Post)
Water quality	Test date yy/mm/dd	2023.10.10	2023.10.10	2023.10.10	2023.10.10	2023.10.10	2023.10.10
	pH @ 25°C (NA)	7.6	8.3	8.3	7.3	7.8	6.3
	EC (Electrical conductivity) (mS/m) @ 25°C (NA)	70.3	94.5	93.6	70.6	33.3	37.3
	Dissolved oxygen (mg/l) (NA)	7.3	7.7	7.1	5.1	7.4	7.8
A. fischeri (bacteria) (A)	Test started on yy/mm/dd	2023.10.16	2023.10.17	2023.10.17	2023.10.17	2023.10.17	2023.10.18
	%30min inhibition (-) / stimulation (+) (%)	-4	10	1	97	-28	-6
	EC/LC20 (30 mins)	n.r.	n.r.	n.r.	n.r.	0.2	n.r.
	EC/LC50 (30 mins)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	Toxicity unit (TU) / Description	<1	<1	<1	<1	<1	<1
S. capricornutum (micro-algae) (A)	Test started on yy/mm/dd	2023.10.17	2023.10.17	2023.10.17	2023.10.17	2023.10.17	2023.10.17
	%72hour inhibition (-) / stimulation (+) (%)	-1	-46	-27	-5	-19(F)	4
	EC/LC20 (72hours)	n.r.	17	15	n.r.	12	n.r.
	EC/LC50 (72hours)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	Toxicity unit (TU) / Description	<1	<1	<1	<1	<1	<1
D. magna (waterflea) (A)	Test started on yy/mm/dd	2023.10.16	2023.10.16	2023.10.16	2023.10.16	2023.10.16	2023.10.16
	%48hour mortality rate (-%)	0	0	0	0	0	0
	EC/LC10 (48hours)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	EC/LC50 (48hours)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Toxicity unit (TU) / Description	<1	<1	<1	<1	<1	<1	
P. reticulata (guppy) (A)	Test started on yy/mm/dd	2023.10.12	2023.10.12	2023.10.12	2023.10.12	2023.10.12	2023.10.12
	%96hour mortality rate (-%)	0	-8	0	0	-8	-8
	EC/LC10 (96hours)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	EC/LC50 (96hours)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Toxicity unit (TU) / Description	<1	<1	<1	<1	<1	<1	
<b>Estimated safe dilution factor (%) [for definitive testing only]</b>		<b>None required</b>	<b>17</b>	<b>15</b>	<b>None required</b>	<b>&lt;1</b>	<b>None required</b>
<b>Overall classification - Hazard class***</b>		Class I - No lethal/sub-lethal hazard	Class II - Slight sub-lethal hazard	Class II - Slight sub-lethal hazard	Class I - No lethal/sub-lethal hazard	Class II - Slight sub-lethal hazard	Class I - No lethal/sub-lethal hazard
Weight (%)		0	25	25	0	50	0

**Key:**  
 % = for definitive testing, only the 100% concentration (undiluted) sample mortality/inhibition/stimulation is reflected by this summary table. The dilution series results are considered for EC/LC values and Toxicity unit determinations  
 n.r. = not relevant, i.e. the 100% concentration caused less than 10/20/50% (effective concentration) mortalities or inhibition  
 (F) = Inhibition/Mortality rate with "(F)" indicates that the sample was filtered, this is often essential with turbid or coloured samples to perform the *Selenastrum* test. Filtration could potentially lower the toxicity for the specific test, but *Daphnia* and *Poecilia* test samples are never filtered and hence toxicity will still be detected if affected by filtration  
 \*\*\* = The overall hazard classification takes into account the full battery of tests and is not based on a single test result. Note that the overall hazard classification is expressed as both lethal (*Daphnia* & *Poecilia*) and sub-lethal (*Aliivibrio* & *Selenastrum*) levels of toxicity  
 Weight (%) = relative toxicity levels (out of 100%), higher values indicate that more of the individual tests indicated toxicity within a specific class  
 Site/sample name shaded in orange = definitive test

Site	Hazard classification		Percentage Effect
SW04	I	☺	No lethal/sub-lethal hazard
SW23			
PCD (post)			
SW10	II	☹	Slight sub-lethal hazard
SW21			
PCD (pre)	The effect percentage observed in at least one toxicity test is significantly higher than that in the control, but is below 50% (< 1 TU)		

Water samples SW04, SW23 and PCD (post) showed no lethal or sub-lethal environmental toxicity hazard (Class I).

Water samples SW10, SW21 and PC (pre) were allocated a Hazard Class II (slight sub-lethal environmental toxicity hazard) based on the 46% and 27% (for samples SW10 and SW21 respectively) micro-algae (*S. capricornutum*) growth inhibition effects noted on the 100% concentration of the samples, and the 29.65% – 42.72% growth inhibitions noted from the 50% to the 25% dilutions of sample PCD (pre) (with an inhibition of 19.13% on the 100% samples which is very close to the ≤20% acceptable limit) together with the 28% bacterial (*A. fischeri*) light emission inhibition effect noted for sample PCD (Pre). Minimum acceptable effect levels of 17% (SW10) and 15% (SW21) were established. Although the bacterial (*A. fischeri*) light emission inhibition effect noted for sample PCD (pre) was slight, this effect could only be diluted out to acceptable levels as from the 0.195% dilution of the sample.

## 5. COMMENTS

Six water samples were delivered to the BioToxLab office on 2023.10.09. The pH levels of the water samples ranged between 6.30 and 8.30 which are within the acceptable range (pH 6-9) in which pH can be excluded as a driving factor for toxicity (USEPA, 1996). The conductivities (ECs) of the water samples ranged between 33.3 mS/m and 94.5 mS/m. A dissolved oxygen (DO) concentration above 4 mg/L is required for aquatic organisms (USEPA, 1996) to survive. The DO levels for the 6 water samples ranged from 5.08 mg/L to 7.77 mg/L.

Any queries regarding the results can be lodged with Lizet Swart within 14 days from the date of receiving this report after which the samples will be discarded. It is not advised to use these samples for any retesting other than range confirmation of chemical parameters – re-sampling must be done in the case of any queries relating to the results associated with the samples.

## 6. REFERENCES

- DEPARTMENT OF WATER AFFAIRS AND FORESTRY, 2003. The Management of Complex Industrial Waste Water Discharges. Introducing the Direct Estimation of Ecological Effect Potential (DEEEP) approach, a discussion document. Institute of Water Quality Studies, Pretoria.
- PERSOONE G, MARSALEK B, BLINOVA I, TÖRÖKNE A, ZARINA T, MANUSADZIANAS L, NALECZ-JAWECKI G, TOFAN L, STEPANOVA L, TOTHOVA L, KOLAR B (2003). A practical and user-friendly toxicity classification system with Microbiotests for natural waters and wastewaters (personal communication).
- SOUTH AFRICAN NATIONAL STANDARD, (SANS), ISO/IEC 17025:2017. General requirements for the competence of testing and calibration laboratories. 3<sup>rd</sup> Edition. South African Bureau of Standards, Pretoria.
- SOUTH AFRICAN NATIONAL STANDARD, SANS 6341:2015. “Water quality – Determination of the inhibition of the mobility of *Daphnia magna* Straus (*Cladocera*, *Crustacea*) – Acute toxicity test.
- SOUTH AFRICAN NATIONAL STANDARD, SANS 8692: 2015. “Water quality – Fresh water algal growth inhibition test with unicellular green algae
- SOUTH AFRICAN NATIONAL STANDARD, SANS 11348-3: 2013. “Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test). Part 3: Method using freeze-dried bacteria
- SOUTH AFRICAN NATIONAL STANDARD, SANS 7346-1:2013. “Water quality – Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae) Part 1: Static method – also applicable to *Poecilia reticulata* (Teleostei, Poeciliidae)
- TONKES M. and BALTUS C.A.M. 1997. Praktijkonderzoek aan complexe effluënten met de Totaal Effluent Milieubezwaarlijkheid (TEM) – methodiek. RIZA – rapportnummer 97.033. RIZA, Lelystad, The Netherlands.
- UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA), 1996. Ecological effects test guidelines. Fish acute toxicity test, Freshwater and marine. OPPTS 850.1075.

**END OF REPORT**









# GRT-GTP-Q-23\_TOX(final)

Final Audit Report

2023-10-23

Created:	2023-10-23
By:	Lizet Moore (lizet@biotoxsa.co.za)
Status:	Signed
Transaction ID:	CBJCHBCAABAAIEz0ATIUX88b_Xohsf9i8454dwzMhHY2

## "GRT-GTP-Q-23\_TOX(final)" History

-  Document created by Lizet Moore (lizet@biotoxsa.co.za)  
2023-10-23 - 10:52:58 AM GMT - IP address: 197.184.169.69
-  Document emailed to Praise Manyenga (praise@biotoxsa.co.za) for signature  
2023-10-23 - 10:53:02 AM GMT
-  Document emailed to Lethabo Mothupi (lethabo@biotoxsa.co.za) for signature  
2023-10-23 - 10:53:02 AM GMT
-  Email viewed by Praise Manyenga (praise@biotoxsa.co.za)  
2023-10-23 - 10:58:30 AM GMT - IP address: 197.184.169.69
-  Document e-signed by Praise Manyenga (praise@biotoxsa.co.za)  
Signature Date: 2023-10-23 - 10:58:43 AM GMT - Time Source: server- IP address: 197.184.169.69
-  Email viewed by Lethabo Mothupi (lethabo@biotoxsa.co.za)  
2023-10-23 - 12:32:02 PM GMT - IP address: 165.73.227.131
-  Document e-signed by Lethabo Mothupi (lethabo@biotoxsa.co.za)  
Signature Date: 2023-10-23 - 12:32:21 PM GMT - Time Source: server- IP address: 165.73.227.131
-  Agreement completed.  
2023-10-23 - 12:32:21 PM GMT