Acute Toxicity Test Results

Sample PLAY IT COOL,
collected January 03, 2023

Final Report

January 20, 2023

Submitted to: **Target Technologies**
Burnaby, BC

8664 Commerce Court, Burnaby, BC V5A 4N7

SAMPLE INFORMATION

Sample ID	Dates			Receipt temperature
	Collected	Received	Rainbow trout test initiation	
PLAY IT COOL	03-Jan-23 at N/A	04-Jan-23 at 1345h	10-Jan-23 at 1418h	10.0°C

N/A = Not Available

TESTS

- Rainbow trout 96-h LC50 test

RESULTS

Toxicity test results

Test Concentration (g/L)	Survival (%)
Control	100
12.5	100
25	100
50	100
100	100
200	100

Sample ID	LC50 (g/L)
PLAY IT COOL	> 200 *

LC = Lethal Concentration, * Highest concentration comprised of 2.4 kg of the product in 12 L of dilution water which is equivalent to 200 g/L.

The rainbow trout toxicity test exhibited 100% survival in the 200 g/L sample concentration (highest concentration tested) after 96-h exposure period.

Fish survival (100%) in all the sample concentrations tested exceeded any definitive calculations of a median lethal concentration (LC50) value for this sample and, therefore, the LC50 could only be estimated as greater than the highest concentration tested (LC50 >200 g/L).

QA/QC

QA/QC summary	Rainbow trout
Reference toxicant LC50 (95% CL)	1.8 (1.6 – 2.2) g/L KCl[1]
Reference toxicant historical mean (2 SD range)	1.3 (0.7 – 2.6) g/L KCl
Reference toxicant CV	35%
Organism health history	Acceptable
Protocol deviations	None
Water quality range deviations	None
Control performance	Acceptable
Test performance	Valid

[1] Test Date: January 06, 2023, LC = Lethal Concentration, CL = Confidence Limits, SD = Standard Deviation, CV = Coefficient of Variation

The test treatments were set up by adding the corresponding sample amounts into each test vessel and topped up to the test volume of 12L with dechlorinated water. For example, 2400 g of the sample was added to prepare the highest test concentration, which corresponded to 200 g/L sample concentration (see Table 1).

Report By:
Ditty Kakkassery, R.P. Bio.
Laboratory Biologist

Reviewed By:
Edmund Canaria, R.P. Bio.
Senior Analyst

APPENDIX A – Summary of test conditions

Table 1. Summary of test conditions: 96-h rainbow trout (*Oncorhynchus mykiss*) LC50 test.

Test species	*Oncorhynchus mykiss*
Organism source	Hatchery
Organism age	Juvenile
Test type	Static
Test duration	96 hours
Test vessel	20-L glass aquarium
Test volume	12 L
Test solution depth	≥15 cm
Test concentrations	Five concentrations 200 g/l, 100 g/l, 50 g/l, 25 g/l and 12.5 g/l plus laboratory control.
Test replicates	1 per treatment
Number of organisms	10 per replicate
Control/dilution water	Dechlorinated Metro Vancouver municipal tapwater
Test solution renewal	None
Test temperature	15 ± 1°C
Feeding	None
Light intensity	100 to 500 lux
Photoperiod	16 hours light / 8 hours dark
Aeration	6.5 ± 1 mL/min/L
Test measurements	Temperature, dissolved oxygen and pH measured daily; conductivity measured at test initiation and termination; survival checked daily
Test protocol	Environment Canada (1990), EPS 1/RM/9, with 1996 & 2007 amendments
Statistical software	CETIS Version 2.1.4
Test endpoints	Survival (96-hour LC50)
Test acceptability criterion for controls	Survival ≥90%
Reference toxicant	Potassium Chloride (KCl)

APPENDIX B – Toxicity test data

Rainbow Trout Summary Sheet

Client: Target Technologies

Start Date/Time: Jan. 10, 2023 /1418h

Work Order No.: 230030

Test Species: *Oncorhynchus mykiss*

Sample Information:

Sample ID: PLAY IT COOL
Sample Date: Jan. 3, 2023
Date Received: Jan. 4, 2023
Sample Volume: 2 BAGS (15 lbs)
Other: -

Test Validity Criteria:
≥ 90% Control Survival
WQ Ranges:
T (°C) = 15 ± 1; DO (mg/L) = 7.0 to 10.3; pH = 5.5 to 8.5

Dilution Water:

Type: Dechlorinated Municipal Tap Water
Hardness (mg/L $CaCO_3$): 20
Alkalinity (mg/L $CaCO_3$): 21

Test Organism Information:

Batch No.: 120622B
Source: DUNCAN
No. Fish/Volume (L): 10/12
Loading Density (g/L): 0.29
Mean Length ± SD (mm): 35 ± 3 Range: 30 - 41
Mean Weight ± SD (g): 0.34 ± 0.09 Range: 0.21 - 0.52

KCl Reference Toxicant Results:

Reference Toxicant ID: RBTKS7
KCl Lot #: 213248
Date Initiated: Jan. 6, 2023
96-h LC50 (95% CL) [g/L KCl]: 1.8 (1.6 - 2.2)

Reference Toxicant Mean and Historical Range [g/L KCl]: 1.3 (0.7 - 2.6)
Reference Toxicant CV (%): 35%

Test Results: The 96h LC50 is estimated to be >200g/L (w/v)* ~~>100% (v/v)~~
* Highest concentration Tested

Reviewed by: [signature]

Date reviewed: Jan - 19, 2023

96-Hour Rainbow Trout Toxicity Test Data Sheet

Client/Project#: Target Technologies
Sample I.D. PLAY IT COOL
W.O. # 230030
RBT Batch #: 120622B
Date Collected/Time: Jan. 03, 2023 / N/A
Date Setup/Time: Jan. 10, 2023 / 1418 h
CER #: 2
Sample Setup By: T.C.

Thermometer: CER #2
D.O. meter/probe: S / S
Cond./Salinity meter/probe: S / S
pH meter/probe: S / S

Number Fish/Volume: 10/12
7-d % Mortality: 0.08
Total Pre-aeration Time (mins): -
Aeration rate adjusted to 6.5 ± 1 mL/min/L? (Y/N): Y

Undiluted Sample WQ			
Parameters	Initial WQ	Adjustment	30 min WQ
Temp °C			
D.O. (mg/L)			
pH			
Cond. (µS/cm)			
Salinity (ppt)			

Concentration g/L (~~% v/v~~ T.C.)	# Survivors							Temperature (°C)					Dissolved Oxygen (mg/L)					pH ①					Conductivity (µS/cm)	
	1	2	4	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	96
Ctrl				10	10	10	10	15.0	14.5	15.0	15.0	15.0	9.8	9.8	9.8	9.7	9.7	7.1	7.1	7.1	7.1	7.2	51	57
12.5				10	10	10	10	15.0	14.5	15.0	15.0	15.0	9.8	9.8	9.8	9.8	9.7	8.2	7.6	7.3	7.3	7.4	66	146
25				10	10	10	10	15.0	14.5	15.0	15.0	15.0	9.8	9.9	9.8	9.8	9.6	8.3	7.7	7.4	7.3	7.5	72	180
50				10	10	10	10	15.0	14.5	15.0	15.0	15.0	9.8	9.8	9.8	9.7	9.8	8.5	7.9	7.5	7.4	7.5	75	201
100				10	10	10	10	15.0	14.5	15.0	15.0	15.0	9.9	9.9	9.9	9.8	9.8	8.7	8.0	7.6	7.4	7.4	79	261
200				10	10	10	10	15.0	14.5	15.0	15.0	15.0	9.9	9.8	9.9	9.8	9.7	9.3	8.1	7.7	7.5	7.6	103	295
Initials				T.C.	T.C.	T.C.	~~T.C.~~	T.C.	T.C.	T.C.	T.C.	~~T.C.~~	T.C	T.C.	T.C.	T.C.	~~T.C.~~	T.C.	T.C.	T.C.	T.C.	~~T.C.~~	T.C.	T.C.

Sample Description/Comments: 2.4 kg of chemical added to 12L of dechlorinated tap water to make highest concentration 200g/L

Fish Description at 96 h: All fish appear normal

Number of Stressed Fish at 96 h: 0

Other Observations:

Test solution depth in each vessel ≥15 cm? (Y/N) Y

Reviewed by: [signature]

① Confirmed with pH probes #2 & #5

Date Reviewed: Jan. 19, 2023

APPENDIX C – Chain-of-custody form

NAUTILUS ENVIRONMENTAL

TESTING LOCATION (Please Circle)

Burnaby
8664 Commerce Court
Burnaby, British Columbia, Canada
V5A 4N7
Phone 604.420.8773

Calgary
#4, 6125 12 Street SE
Calgary, Alberta, Canada
T2H 2K1
Phone 403.253.7121

Chain of Custody

Date_____ Page 1 of 1

Report to:		Invoice To:	
Company	TARGET TECHNOLOGIES	Company	SAME
Address	8535 EASTLAKE DR.	Address	
City/Prov/PC	BURNABY BC	City/Prov/PC	
Contact	NADIA MINATO	Contact	
Phone	604.421.3620	Phone	
Email	nadia.minato@ttionline.com	Email	
		PO No.	

Sample Collection By: **Sample Type:** Grab (D) OR Composite ○

	SAMPLE ID	DATE (DD/MM/YY) (F)	TIME	MATRIX	# OF CONTAINERS AND VOLUME (e.g. 1 x 20 L)	COMMENTS	ANALYSES REQUIRED: 96h RBT LC50	WO # 2300305	Receipt Temperature (°C)
1	PLAY IT COOL	03.01.~~22~~ 23 R			2 BAGS =	LC 50 TEST	X		10°C
2					15 LBS				
3									
4									
5									
6									
7									
8									
9									
10									

SPECIAL INSTRUCTIONS/COMMENTS (CLIENT)

SAMPLE RECEIPT DETAILS (LABORATORY)

1. Total No. of Containers	2 bags	4. Ice Present in Cooler?	Y / (N)
2. Courier	~~Purolator~~ Client	5. Seal Present?	Y / (N)
3. Good Condition?	(Y) / N	6. Initials Present on Seal?	Y / (N)

SAMPLE DESCRIPTION AND COMMENTS (LABORATORY)

(F) Client confirmed the year of collection was 2023 - R

RELINQUISHED BY (CLIENT)

NADIA MINATO N. Minato
(Printed Name) (Signature)

TARGET TECHNOLOGIES 03.01.22
(Company) (Date DD/MM/YY and Time)

RECEIVED BY (LABORATORY)

Nair Yamamoto NY
(Printed Name) (Signature)

Nautilus Jan 4, 2023; 1345h
(Company) (Date DD/MM/YY and Time)

Additional costs may be required for sample disposal or storage. Payment net 30 unless otherwise contracted.

END OF REPORT