FILE: USGREENTECH/1705041.RTL

DATE: 17 May 2017

TO: Ross Vocke
US Greentech
5076 Wooster Road
Cincinnati, OH
45226

REPORT ON: **RAINBOW TROUT BIOASSAY RESULT**

SAMPLE DESCRIPTION:

IRC Sample ID No.:	1705041
Sample Name:	**Powerfill 3.0**
Sample collected by:	N/A
Date collected:	04 May 2017
Date, time received:	09 May 2017; 1155 hrs.
Collection Method:	Grab
Amount, Container:	1 x 20 L plastic container
Physical description:	Medium sized green puck-shaped pellets
Date, time tested:	09 May 2017; 1500 hrs.

RAINBOW TROUT 96 HR RESULTS:
The 96 hour (static) LC_{50} was greater than 20% (w/v sample).
40% trout mortality in 20%* concentration.

*Highest concentration tested

The LC_{50} is defined as the median lethal concentration or the concentration at which there is 50% fish mortality. Results are calculated following the methods described in "Guidance Document on Statistical Methods for Environmental Toxicity Tests" EPS 1/RM/46 – March 2005 (with June 2007 amendments), using the software CETIS, ver 1.8.7E (2014).

The method used for this test was as per the IRC laboratory "Standard Operating Procedure for Rainbow Trout Holding and Testing" RTver5. This procedure was modified from "Biological Test Method: Acute Lethality Test Using Rainbow Trout" EPS 1/RM/9, July 1990 (with May 1996 and May 2007 amendments) and the "Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout" EPS 1/RM/13, Second Edition – December 2000 (with May 2007 and February 2016 amendments). Testing was carried out using under gravel filtration system. Test volume was 12 litres with 10 fish in each test vessel. Aeration was by forced air through airstones at a rate of approximately 6.5 ± 1 ml/L/min. The sample was not pH adjusted or filtered prior to testing.

The initial dissolved oxygen was 10.0 mg/L at 15.5°C, the conductivity was 52 µS/cm and the initial pH was 6.9. After pre-aerating the sample for 120 minutes, the dissolved oxygen level was 10.1 mg/L. Although the dissolved oxygen level was greater than 100% saturation the maximum aeration time had been reached and so the test was initiated at this time. The test set-up technician was CW.

IRC Integrated Resource Consultants Inc.

Richard Chea
Laboratory Supervisor
b503
enclosure

RAW DATA:

TEST CONCENTRATION:		HOURS:				
		0	24	48	72	96
20%	Percent Survival	100%	100%	100%	100%	60%
	Dissolved Oxygen (mg/L)	10.1	8.4	7.0	5.0	3.7
	Temperature (°C)	15.5	15.5	15.5	15.5	15.5
	pH	7.0	6.9	6.9	6.9	6.9
	Conductivity (µS/cm)	66				226
	Symptoms	1	2	2	2	2
	Loading Density (g/L)	0.25	0.25	0.25	0.25	0.15
10%	Percent Survival	100%	100%	100%	100%	100%
	Dissolved Oxygen (mg/L)	10.0	8.8	7.9	6.2	5.1
	Temperature (°C)	15.5	15.5	15.5	15.5	15.5
	pH	7.0	6.9	6.9	6.9	6.9
	Conductivity (µS/cm)	56				187
	Symptoms	1	2	2	2	2
	Loading Density (g/L)	0.25	0.25	0.25	0.25	0.25
5%	Percent Survival	100%	100%	100%	100%	90%
	Dissolved Oxygen (mg/L)	10.0	9.0	8.5	6.9	5.7
	Temperature (°C)	15.5	15.5	15.5	15.5	15.5
	pH	6.9	6.8	6.8	6.9	6.8
	Conductivity (µS/cm)	52				143
	Symptoms	1	1,2	1,2	1,2	2
	Loading Density (g/L)	0.25	0.25	0.25	0.25	0.22
2.5%	Percent Survival	100%	100%	100%	100%	100%
	Dissolved Oxygen (mg/L)	10.1	8.6	7.8	7.2	6.5
	Temperature (°C)	15.5	15.5	15.5	15.5	15.5
	pH	6.9	6.7	6.7	6.8	6.8
	Conductivity (µS/cm)	49				103
	Symptoms	1	1,2	1,2	1,2	1,2
	Loading Density (g/L)	0.25	0.25	0.25	0.25	0.25
1.25%	Percent Survival	100%	100%	100%	100%	100%
	Dissolved Oxygen (mg/L)	10.0	7.9	6.7	6.3	5.6
	Temperature (°C)	15.5	15.5	15.5	15.5	15.5
	pH	6.9	6.6	6.7	6.8	6.9
	Conductivity (µS/cm)	47				81
	Symptoms	1	1	1	1,2	1,2
	Loading Density (g/L)	0.25	0.25	0.25	0.25	0.25
CONTROL	Percent Survival	100%	100%	100%	100%	100%
	Dissolved Oxygen (mg/L)	9.9	8.7	8.2	7.5	6.8
	Temperature (°C)	15.5	15.5	15.5	15.5	15.5
	pH	6.8	6.6	6.8	6.9	6.9
	Conductivity (µS/cm)	45				48
	Symptoms	1	1	1	1	1
	Loading Density (g/L)	0.25	0.25	0.25	0.25	0.25
	Technician	CW	CW	CW	CW	WB

KEY TO SYMPTOMS:
1 = no apparent effect
2 = fish showing signs of stress
3 = loss of equilibrium

TEST FISH STOCK INFORMATION:

Date received:	24 April 2017
Source:	AquaFarms JV
Species:	*Oncorhynchus mykiss* (Rainbow Trout)

Fork Length:	Mean:	32.1 mm ± 1.8 mm
	Range:	30.0 mm – 36.0 mm

Wet weight:	Mean:	0.30 g ± 0.04 g
	Range:	0.23 g – 0.38 g

Condition Factor (100xWt/length^{3}cm):	0.89

Acclimation History	
Acclimation temperature:	13.5-15.5 °C
Treatments:	None
Water:	Dechlorinated tap water
Feeding:	BioVita Starter #1 Crum
Mortality:	1.74%

RAINBOW TROUT REFERENCE TOXICANT DATA

Stock Arrival Date (y/m/d)	Test Date (y/m/d)	Toxicant	LC50 (mg/L)	95% Confidence Interval
16.04.03	16.04.18	Phenol	11.22	10.42 to 12.08
16.04.10	16.04.25	"	10.02	9.56 to 10.51
16.04.25	16.04.28	"	14.10	12.06 to 16.42
16.04.25	16.05.09	"	8.94	7.96 to 10.04
16.05.24	16.06.06	"	12.60	10.61 to 14.85
16.06.19	16.07.05	"	9.35	7.89 to 11.29
16.07.17	16.08.02	"	9.06	4.89 to 12.08
16.07.29	16.08.16	"	10.73	8.82 to 12.83
16.08.26	16.09.09	"	9.36	8.58 to 10.21
16.09.11	16.09.28	"	10.24	8.44 to 12.03
16.09.23	16.10.07	"	12.62	10.43 to 15.03
16.10.14	16.10.21	"	17.62	14.57 to 21.84
16.10.14	16.10.27	"	10.24	8.44 to 12.03
16.10.31	16.11.11	"	11.96	9.87 to 14.28
16.11.04	16.11.25	"	9.80	8.00 to 12.00
16.11.21	16.12.05	"	12.62	10.47 to 15.03
16.12.05	16.12.19	"	10.18	9.44 to 10.98
16.12.21	17.01.05	"	10.20	9.45 to 11.02
16.12.02	17.01.16	"	8.30	6.73 to 9.87
17.01.24	17.02.17	"	8.94	7.96 to 10.04
17.02.10	17.02.24	"	15.81	14.41 to 17.35
17.02.10	17.03.02	"	14.70	12.00 to 18.00
17.03.02	17.03.16	"	13.58	11.37 to 16.05
17.03.02B	17.03.27	"	8.94	7.96 to 10.04
17.03.11	17.03.27	"	9.75	8.68 to 10.95
17.03.29	17.04.17	"	12.50	11.02 to 14.17
17.04.17	17.05.01	"	9.35	7.89 to 11.29
17.04.24	17.05.08	"	9.36	8.58 to 10.21
LAB GEOMETRIC MEAN ± 2 standard deviations:				10.877 mg/L ± 4.647
Warning Limits:				6.230 mg/L to 15.524 mg/L

CONTROL/DILUTION WATER QUALITY:

Hardness:	18 mg/L
Total Residual Chlorine:	04 μg/L

IRC

Integrated Resource Consultants Inc.

SAMPLE TRANSMITTAL AND CHAIN-OF-CUSTODY FORM

Client Name:	U3greentech
Client Contact Name:	Adam Coleman
Shipping Date:	5-4-2017
Purchase Order:	
Sample Collected by:	

Ship to:

IRC
160-14480 River Road
Richmond BC
V6V 1L4

Attn:

Analysis Requested

Sample Identification	Collection Date/Time	Sample Collection Method (G=grab, C=composite)	Sample Container(s)¹	Sample Condition Upon Receipt²	96 hour LC50 Trout	96 hour LC50 Trout (RM/50-pH stabilized)	96 hour LT50 Trout	48 hour LC50 Daphnia	48 hour LT50 Daphnia	15 Min. Microtox	Ceriodaphnia (sub-lethal)	TSS	IRC ID
PowerFill 3.0	5-4-17		Bucket	17.0	X								1705041

Comments

Released by:				Received by:		
Name:		Date:		Name:	CW	Date: 05 May 17 Time: 1155
Affiliation:		Time:		Affiliation:	IRC	Courier: FedEx
Name:		Date:		Name:		Date: Time:
Affiliation:		Time:		Affiliation:		Courier:

Distribution: original accompanies sample and is returned with results.
¹indicate # of containers, and type of container: P=plastic; CG=clear glass jar; BG= brown glass jar; O=other (indicate type in comments section).
²to be filled in upon arrival, include temperature, container condition, and/or other observations.

Feb 2004

STCOC ver 5.2

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