

July 31, 2020

Mr. Christopher Bittner Standards Coordinator Utah Dept. of Environmental Quality 195 N 1950 W Salt Lake City, UT 84116 Dr. Gary Belovsky Environ. Res. Center & Dept. Biol Sci. University of Notre Dame Notre Dame, IN 46556

Subject: Results of Analytical Data for Experiment #17

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the analytical data for the acute brine shrimp experiment with copper initiated on May 28, 2020. Total copper samples were collected in old test solutions.

Characterization of Recon Water

Sample	рН	Hard.	Alk.	Spec. Cond.	TRC	NH ₃ -N	Salinity
No.		(mg/L) ^a	(mg/L) ^a	(μS/cm)	(mg/L) ^b	(mg/L)	(ppt)
RW#13937	8.0	NM	NM	134,600	NM	NM	120

^aAs CaCO3

Results of Copper Analysis

Total Cop	Percent of				
Nominal Value					
0	U <100				
94	144	153			
188	180	96			
375	336	90			
750	774	103			
1,500	1,440	96			

U= below method detection limit (100 μg/L)

Measured copper values in the four highest treatments were similar to nominal values. The measured value in the lowest treatment was about 50% above nominal, but still provided for a clean monotonically increasing scale. Average measured copper concentrations were then used to recalculate the test endpoint on a measured basis. Both nominal and measured median lethal concentrations are presented in the following table for comparison.

^bTotal residual chlorine

Test Endpoints

Survival 96-hour LC ₅₀	Value (μg/L Copper)				
Nominal	1,336 (C.L. 1,082 – 1,650)				
Measured	1,298 (C.L 1,075 – 1,568)				

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions or concerns.

Rami B. Naddy, Ph.D.

naddyrb.tre@gmail.com

Manager / Environmental Toxicologist

Sincerely,

Amanda Bidlack Project Specialist / QA Officer

bidlackac.tre@gmail.com

17001-474-072

Attachment

cc: David Pillard, TRE

CETIS Analytical Report Brine shrimp

Report Date:

29 Jul-20 16:39 (p 1 of 2)

TRE Environmental Strategies

Test Code:

474-072 | 05-2923-3275

-Fathead Minn	ow 9 6-h Acute	Survival Test
Analysis ID:	09-8235-2129	Endpo

29 Jul-20 16:39

96h Survival Rate	CETIS Version:	CETISv1.8.7
Trimmed Spearman-Kärber	Official Results:	Yes

Official Results: Yes

Batch ID: 09-2953-2358 Test Type: Survival (96h) Start Date: 21 May-20 14:40 Protocol:

Lab Tech rGSL

Ending Date: 25 May-20 14:15

Analyst: Diluent:

Duration: 96h

EPA/821/R-02-012 (2002) Species: Artemia franciscana Source: In-House Culture

Brine: Age:

Crystal Sea 48h

Sample ID: 20-5963-3281

Code:

Analysis:

7AC38281

research

Endpoint: 96h Survival Rate

Client:

Sample Date: 14 May-20 11:00

Analyzed:

Material:

Copper sulfate

Notre Dame

Receive Date: 21 May-20 11:00 Sample Age: 7d 4h

Source: Station: Project: Special Studies

Trimmed Spearman-Kärber Estimates

Threshold	d Option	Threshold	Trim	Mu	Sigma		LC50	95% LCL	95% UCL			
Control Th	reshold	0	40.00%	3.113	0.04102		1298	1075	1568			
96h Survi	val Rate Summar	у			Calc	ulated Varia	ite(A/B)					
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В	

Jon Gui Vi	vai Kate Sulfilliary		Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	В
100	Dilution Water	2	1	1	1	0	0	0.0%	0.0%	20	20
144		2	1	1	1	0	0	0.0%	0.0%	20	20
180		2	1	1	1	0	0	0.0%	0.0%	20	20
336		2	1	1	1	0	0	0.0%	0.0%	20	20
774		2	1	1	1	0	0	0.0%	0.0%	20	20
1440		2	0.4	0.3	0.5	0.1	0.1414	35.4%	60.0%	8	20

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2
100	Dilution Water	1	1
144		1	1
180		1	1
336		1	1
774		1	1
1440		0.5	0.3

96h Survival Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	
100	Dilution Water	10/10	10/10	
144		10/10	10/10	
180		10/10	10/10	
336		10/10	10/10	
774		10/10	10/10	
1440		5/10	3/10	

(1) DAP 7/30/20 E

Brine Shring

Report Date: Test Code:

29 Jul-20 16:39 (p 2 of 2) 474-072 | 05-2923-3275

Fathead Minnow-96-h Acute Survival Test

TRE Environmental Strategies

Analysis ID: Analyzed:

09-8235-2129 29 Jul-20 16:39

Endpoint: 96h Survival Rate Analysis:

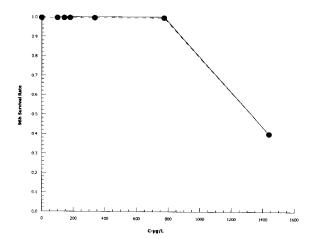
Trimmed Spearman-Kärber

CETIS Version:

CETISv1.8.7

Official Results: Yes

Graphics



Open 7/30/20 E

TRE Environmental Strategies, LLC 100 Racquette Drive, Unit A, Fort Collins, Colorado, 80524 T 970.416.0916 F 970.490.2963



June 11, 2020

Mr. Christopher Bittner Standards Coordinator Utah Dept. of Environmental Quality 195 N 1950 W Salt Lake City, UT 84116 Dr. Gary Belovsky Environ. Res. Center & Dept. Biol Sci. University of Notre Dame Notre Dame, IN 46556

Subject: Results of Acute Brine Shrimp Experiment #17

Mr. Bittner/ Dr. Belovsky:

Below is a summary of the acute brine shrimp experiment initiated on May 21, 2020. The purpose of this experiment was to investigate an appropriate range for a short term chronic *Artemia franciscana* test with copper as the toxicant.

Along with a control, five different nominal copper concentrations (prepared with CuCl₂) were tested:

94, 188, 375, 750, and 1,500 μg/L

The results of these studies will help determine the experimental design of the definitive short-term chronic toxicity tests. The test volume was consistent at 50 ml.

Species: Artemia franciscana

Test type:

Test duration: 4 days

- Test type: static-renewal (solutions and food renewed daily)
- Algae: Dunaliella viridis
- Food concentration: 72.5 µg/L Chla and 0.3 ml YTC¹
- Temperature: 20°CTest volume(s): 50 ml
- Replicates: 2

• Organisms/Rep: 10

• Test media: 120 ppt rGSL media (per Notre Dame recipe)

Pretest conditions: <24-h old *A. franciscana* were hatched out in ~29 ppt artificial seawater (Crystal Sea Marine Mix) and ~200 organisms were placed in 120 ppt rGSL water and fed *Dunaliella viridis* at a density of 72.5 μg/L Chl*a* and 0.3 ml YTC. Solutions were gently aerated.

¹ yeast-trout chow-cerophyl mixture used as a typical food for water fleas in whole effluent toxicity testing (USEPA 2002)

Characterization of Recon Water

Sample No.	рН	Hard. (mg/L) ^a	Alk. (mg/L) ^a	Spec. Cond. (μS/cm)	TRC (mg/L) ^b	NH ₃ -N (mg/L)	Salinity (ppt)
RW#13937	8.0	NM	NM	134,600	NM	NM	120

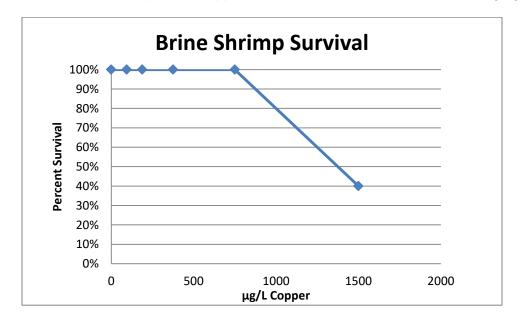
^aAs CaCO3

Test activities:

- Biological observations (primarily survival) taken daily.
- Chemistries taken daily (i.e., pH, dissolved oxygen, and temperature).
- Conductivity was measured at test termination or when there was 0% survival in that treatment.
- Copper was added to 120 rGSL media containing food and allowed to equilibrate for 3 hours prior to use in the toxicity tests.

Results:

The survival of the brine shrimp in the copper treatments is illustrated in the following figure:



^bTotal residual chlorine

Test Endpoints

Test Concentration (µg/L Copper)	Percent Survival of Artemia franciscana					
(nominal)	24 hours	48 hours	72 hours	96 hours		
0 (rGSL)	100	100	100	100		
94	100	100	100	100		
188	100	100	100	100		
375	100	100	100	100		
750	100	100	100	100		
1,500	95	50	45	40		
Control Performance		Accept	able			

Data Analysis and Test Endpoints

Biological Endpoint	Statistical Endpoint	Value (μg/L Copper) (nominal)		
Survival	96-hour LC ₅₀	1,336 (C.L. 1,082 -1,650)		

Summary and findings:

- Organism survival was ≥ 90% for the controls.
- Acute copper toxicity was clearly demonstrated at the highest test concentration.
 - Test concentrations were selected based on the round 1 acute toxicity tests with copper.
- Samples were collected for copper analysis.
- Copper concentrations for a short term chronic test will be similar to those used here.

We greatly appreciate the opportunity to complete this study for you. Please do not hesitate to call if you have any questions or concerns.

Sincerely,

Amanda Bidlack

Project Specialist / QA Officer

bidlackac.tre@gmail.com

ander Billed

17001-474-072

Attachment

cc: David Pillard, TRE

Rami B. Naddy, Ph.D.

Manager / Environmental Toxicologist
naddyrb.tre@gmail.com

Page 1 of 7 QA Form No. 051 Revision 5 Effective 02/14

TOXICITY DATA PACKAGE COVER SHEET

QX: NAP 6/8/20

Test Type:	Chronic		Project Number:	<u></u>	17001-474-0	72
Test Substance:	Copper (Cut	CI2)	Species:	Artemia franci	scana	
Dilution Water:	rGSL		Organism Lot	or Batch Numbe	er: _	051920
Concurrent Control Water:	NA		Age: 48HR	(48 hr)	Supplier: _	TRE
Date and Time Test Began:	5/21/20	@ 1440	Date and Time	Test Ended:	5/25/20	@ 1415
Protocol Number:			Investigator(s)	CP/ABIG	#AFIES	
Background Information			pH control?:	Yes	No	
Type of Test:	Static-Renev	wal (48 h)	If yes, give %		NA	
Test Temperature:	20 ± 1 °C		Env. Chmbr/Bath #: _25	<u>i_</u>	Test	Chmbrs: 147-ml cups
Photoperiod:	16 h light : 8	h dark	Light intensity:		50-100 ft-c.	
Test Solution Vol.:	5	0 ml	Replicates per	Treatment:	2	
Length of Test:	96 hr		Organisms pe	r Replicate:	10	
Type of Food and Quantity pe	r Chamber:	72.5 ug/L Chla/	0.3 ml YT Feeding Frequ	uency:	Initiation an	d Renwals
Test Substance Characteriza	ation Parame	eters and Frequ	uency:			
Hardness: <u>Test Initiation</u>	Alkalinity:	Test Initiation	NH ₃ : <u>Test Initiation</u>	TRC: Test Ini	tiation	
pH: <u>Daily</u>	Conductivity	: Daily				
Test Concentrations (Volume:	Volume):	rGSL, 94, 188,	375, 750, and 1,500 μg/L	as Cu		
Agency Summary Sheet(s)?:		None	-			
Reference Toxicant Data:	Test Dates:		to		IC ₂₅ :	
Hist. 95% Control Limits:		to	Method for Determining	Ref. Tox. Value	: Linear Inter	rpolation
Special Procedures and Cor						
Organisms hatched 2 days pri	or to initiation	and held in rGs	SL with 72.5 ug/L Chla/ 0.	3 ml YTC		
				-		
	- · · · · · · · · · · · · · · · · · · ·			<u> </u>		
Appropriate correction factors	have been a	pplied to all tem	peratures recorded in this	data package		
Study Director Initials:		Date: 5/20	ze			

Page 2 of 7 QA Form No. 014 Revision 1 Effective 02/14

TEST SUBSTANCE USAGE LOG

Project	Number:

17001-474-072	
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QA: NAP 6/8/20

	Sample 1	Sample 2	Sample 3	Sample 4
Test Substance Number	ENSR # 19122			
	From:	From:	From:	From:
Test Substance Collection	@	@	@	@
Date and Time	To:	То:	To:	To:
	@	@	@	@
Sample Type (Grab or Comp)				
Date Test Substance Received				
Dilution Water Number RW# or TRE#, circle one	13937			
Concurrent Control Water RW#				
	5/21/20			
Date(s) Used	5/25/20			
	05/23/20			

Preparation of Test Solutions

Test	Test	Dilution	Total	Test	Dilution	Total	Test	Dilution	Total
Substance	Substance	Water	Volume	Substance	Water	Volume	Substance	Water	Volume
Conc.	Volume	Volume	(ml)	Volume	Volume	(ml)	Volume	Volume	(ml)
(µg/L)	(ml)	(ml)		(mi)	(ml)		(ml)	(ml)	
0	0	125	125						
94	8	117	125						
188	16	109	125						
375	31	94	125						
750	63	63	125						
1500	125	0	125						
	242	508	750						
Initials / Date	AS 5/2	120 mi	cael BS						
Initials / Date	R 51	13/2 U	۱٠						
Initials / Date	AD T								
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									
Initials / Date									

OAF5123120E

Artemia franciscana CHRONIC BIOLOGICAL DATA

QA: NOP 6/4/20

Project N	lumber:	17001 ₋	<u>-474-072</u>							
										2 m/vm
								ing Orgar		0
μg/L	Test Replicate	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Remarks
0	Α	10	10	10	(°	10	\angle			(b)
	В	10	10	10	(b)	10				
	С									
	D						\angle			
94	Α	10	Õ	10	(0)	10				[62
	В	10	10	10	(O)	10	\angle			
	С									
	D						_			
188	Α	D	(0	10	oj	10	/			180
	В	lo	10	10	(0	10				•
	С				\backslash					
	D		/							
375	Α	10	10	10	()	10	$\overline{}$			loo
	В	10	10	10	(0	10	\backslash			·
	С						\backslash			
	D									
750	Α	10	10	10	(0	10	//			100
	В	10	10	10	10	10				•
	С									
	D									
1500	Α	10	10	σ	6	5				ilo
	В	10	9	4	3	3				
	С									
	D									
	А									
	В									·
	С									
	D									
	Date:	5/21/20	5/22/20	5/23/20	5124120	5125120				
	Time:	1440	1615	1140	1115	1415				
	Initials:	CP/Dn	CP	AF/	5	AF				

CHRONIC CHEMICAL DATA (INITIAL)

QA: NAP 6/9/20

Project Number:	17001-474-072			
Test Species	s: Artemia franciscana			

μg/L	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Meter #	Remarks
Conc.: 0									All Conc.	
pH	8.0		7.8						FM27	
D.O. (mg/L)	5.4		6.3						17	
Temp. (°C)	ro		20						IRI	
Cond. (µS/cm)	134,600		133,200						15	
Hard. (mg/L)				\angle				\angle		
Alk. (mg/L)				\angle		/		\angle		
TRC (mg/L)				_		\angle		\angle		
NH ₃ (mg/L)				_						
Conc.: 94				_						
рН	8.0		7.8	_		\angle		/		
D.O. (mg/L)	5.7		W-4	_						
Temp. (°C)	20		20	\angle		\angle				
Cond. (µS/cm)	134,400		133,500			\angle				
Hard. (mg/L)				\angle		\angle				
Alk. (mg/L)				\angle						
TRC (mg/L)										
NH ₃ (mg/L)										
Conc.: 188										
pH	7.9		7.8							
D.O. (mg/L)	5.5		0.4							
Temp. (°C)	20		20							
Cond. (µS/cm)	134,600		133,900							
Conc.: 375										
рН	7.9		7.8							
D.O. (mg/L)	5.6		io-i							
Temp. (°C)	20		20							
Cond. (µS/cm)	134,400		134,400							
Date:	5/21/20		5/23/20							
Time:	1430		1125							
Initials:	co		AF							

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

*Dilution/control water and effluent were brought to 25C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25C.

CHRONIC CHEMICAL DATA (INITIAL)

QA: DMD 6/8/20

Project Number:	17001-474-072			
Test Species:	Artemia franciscana			

μg/L	Day	Day	Day	Day	Day	Day	Day	Day	Meter #	Remarks
	0	1	2	Day 3	4	5	6	7		rtomanto
Conc.: 750									All Conc.	
pН	7.9		7.8					/		
D.O. (mg/L)	5.2		i0.0							
Temp. (°C)	20		20							
Cond. (µS/cm)	134,500		134,000							
Conc.:				/						
pH		/		/	\setminus	\setminus				
D.O. (mg/L)		/		/		\setminus	\backslash			
Temp. (°C)				/			/			
Cond. (µS/cm)				/						
Conc.:				/						
рН										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)				/						
Conc.:										
рН										
D.O. (mg/L)										
Temp. (°C)										
Cond. (µS/cm)				/						
Conc.: 1500										
pH	7.9		7.9							
D.O. (mg/L)	5.1		6.0							
Temp. (°C)	20		20							
Cond. (µS/cm)	134,700		134,000							
Hard. (mg/L)										
Alk. (mg/L)										
TRC (mg/L) NH ₃ (mg/L)										
NH ₃ (mg/L)										
Date:	5/21/20		5123120							
Time:	1430		5 <u> 13 10</u> 1125							
Initials:	ce		AF							

Note: Hardness, alkalinity, TRC, and NH3 data appearing on this page have been transcribed from the wet chemistry log QA Form No. 084.

^{*}Dilution/control water and effluent were brought to 25C prior to making the dilution series. The temperature of resulting effluent dilution is assumed to also be 25C.

Page 6 of 7 QA Form No. 059 Revision 3 Effective 02/14

CHRONIC CHEMICAL DATA (FINAL)

Qx: psp 6/8/20

Project Number: 17001-474-072
Test Species: Artemia franciscana

μg/L		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Meter #	Remarks
Conc.:	0	•			159,100				$\overline{}$	All Conc.	* conductivity 15
рН		7.9	8.0	7.8	7.7					FM27	
D.O. (mg/L)		5.5	5.5	4,6	959					17	
Temp (°C)		21	224	20	20				/	L-13	
Conc.:	94				100,300				/		* conductivity
рН		7.9	7.9	7.9	7.7						
D.O. (mg/L)		5.4	5.8	4.3	4.0						
Temp (°C)		21	ZÌ	<u></u>	20				/		
Conc.:	188				159,500						* conductivity
рН		7.9	7.9	7.9	7.7				\angle		
D.O. (mg/L)		5.3	5.5	4.4	0.0	\angle					
Temp (°C)		21	224	10	20						
Conc.:	375				160:100	\angle					* conductivity
pН		7.9	7.9	7.9	7.7						
D.O. (mg/L)		5.2	5.6	4,5	6.2	/					
Temp (°C)		21	21	w	20						
Conc.:	750				100,400						* conductivity
pН		7.9	7.9	7.9	1.7						
D.O. (mg/L)		5.2	4.9	4.3	0.2						
Temp (°C)		224	224	w	20						
Conc.:	1500				160:200			\angle			* conductivity
рН		7.8	7.8	7.9	7.7	/	/				
D.O. (mg/L)		5.1	49	4.3	6-1	/	/				
Temp (°C)		224	224	W	20						
Conc.:											
рН						/					
D.O. (mg/L)						/					
Temp (°C)										<u> </u>	
	Date:				5/25/20						
	Time:	1620		1130	1425						
	Initials:	CP	AF	ES	AF						

^{*}checked both reps

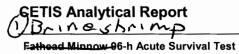
Page 7 of 7 QA Form No. 055 Revision 3 Effective 02/14

DAILY TOXICITY TEST LOG

CA: NAP 6/8/20

Project Number:	17001-474-072	
Test Species:	Artemia franciscana	

General		Feeding	Initials/Date
Comments		72.5 ug/l Chla	
	Random Chart: Min/Max Thermometer # M-15	0.3 ml YTC	
Test Day 0	Test Solution Mixed at: 100	Fed @ 1100	
	Test Organisms Added at: 1440		CP
			CP 5/21/20
Test Day 1	Real Time: 20 °C Min-Max Range: 20 - 22 °C		
		NONE	CP
			5/22/20
Test Day 2	Real Time: 22 °C Min-Max Range: 21 -22 °C	Fed @ 0340	AF/
			1 ' 1
			5/23/20
Test Day 3	Real Time: 21 °C Min-Max Range: 21-22 °C		
		nne	5/24/20
Test Day 4	Real Time: 20 °C Min-Max Range: 20 - 22 °C		AF
		NONE	
			5/25/20



Report Date:

26 May-20 14:02 (p 1 of 2)

474-072 | 05-2923-3275 **Test Code:**

TRE Environmental Strategies

I-activate total	Da-00-II Acate Sulv	IVai 165t				THE Environmental outlegion
Analysis ID: Analyzed:	10-2289-6469 26 May-20 14:02	Endpoint: Analysis:	96h Survival Rate Trimmed Spearman-Kärber	CETIS Vers Official Res		CETISv1.8.7 Yes
Batch ID:	09-2953-2358	Test Type:	Survival (96h)	Analyst:	Lab T	Tech
Start Date:	21 May-20 14:40	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	rGSL	
Ending Date:	25 May-20 14:15	Species:	Artemia franciscana	Brine:	Cryst	al Sea
Duration:	96h	Source:	In-House Culture	Age:	48h	
Sample ID:	20-5963-3281	Code:	7AC38281	Client:	Notre	Dame
Sample Date:	21 May-20 11:00	Material:	Copper sulfate	Project:	Spec	ial Studies
Receive Date:	21 May-20 11:00	Source:	research			
Sample Age:	4h	Station:				

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	40.00%	3.126	0.0458	1336	1082	1650

96h Survi	ival Rate Summary				Cal	culated Varia	te(A/B)				
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
0	Dilution Water	2	1	1	1	0	0	0.0%	0.0%	20	20
94		2	1	1	1	0	0	0.0%	0.0%	20	20
188		2	1	1	1	0	0	0.0%	0.0%	20	20
375		2	1	1	1	0	0	0.0%	0.0%	20	20
750		2	1	1	1	0	0	0.0%	0.0%	20	20
1500		2	0.4	0.3	0.5	0.1	0.1414	35.4%	60.0%	8	20

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	1	1
94		1	1
188		1	1
375		1	1
750		1	1
1500		0.5	0.3

96h Survival Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	10/10	10/10
94		10/10	10/10
188		10/10	10/10
375		10/10	10/10
750		10/10	10/10
1500		5/10	3/10

ODAP 6/8/20 E

Analyst: 43 QA: 040 QA

CETIS™ v1.8.7.16

CETIS Analytical Report Fathead Minnew 96-h Acute Survival Test Report Date:

26 May-20 14:02 (p 2 of 2)

Test Code:

474-072 | 05-2923-3275

TRE Environmental Strategies

Analysis ID: Analyzed:

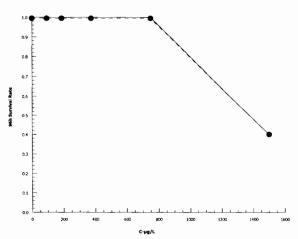
10-2289-6469 26 May-20 14:02

Analysis:

Endpoint: 96h Survival Rate Trimmed Spearman-Kärber **CETIS Version:** Official Results: Yes

CETISv1.8.7

Graphics



DAP 6/8/20 E

000-470-187-3

Analyst: Analyst: QA: Analyst: