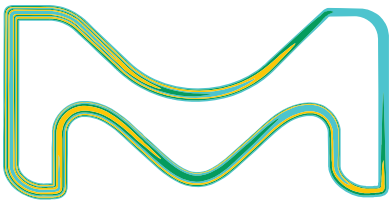
PROFICIENCY TESTING

Evaluation Report

Scheduled Study	**DMRQA 40**
Study Type	WPCHEM_MICRO
Open Date	2020-03-20
Close Date	2020-09-18
Report Generated	2020-10-07
Laboratory	Coastal Bioanalysts, Inc Pete DeLisle 6400 Enterprise Court Gloucester VA 23061 US
Account Number	49480494
US EPA Lab Code	VA01116



Supelco®
Analytical Products

Provider of the proficiency test

Sigma-Aldrich RTC, Inc.
2931 Soldier Springs Road
Laramie, WY 82070 USA
ptservice@milliporesigma.com

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Authorized release of the report

Alexus Horton
(PT coordinator)

Sign:

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Sigma-Aldrich RTC, Inc. is accredited by ANAB to provide PT programs for the scope of accreditation under ANAB Certificate # AP-1469.

All batch numbers of proficiency testing samples, including microbiological materials, are manufactured and tested in accordance with ISO/IEC 17043 requirements. For further information on proficiency testing samples, please check the PT product code information on each product detail page located on our website.

Accreditors

Evaluations of this study will be sent to the accreditor(s) listed below. If any of the information listed below is not correct, please contact Sigma-Aldrich RTC immediately.

Accrediting Agency

Commonwealth of Virginia DGS-DCLS

Agency lab code: 00067

Lab Certification
600 North 5th St.
Richmond VA 23219-3691 US

Accrediting Agency

Kentucky DEP

Agency lab code: VA01116

Laboratory Certification
300 Sower Blvd. 3rd floor
Frankfort KY 40601 US

Accrediting Agency

Maryland Department of the Environment

Agency lab code: VA01116

Ron Wicks
MDE - Water Supply Program
1800 Washington Blvd., Ste 450
Water Supply Program
Baltimore MD 21230-1708 US

Summary Results for DMRQA 40

WET019-1EA Ceriodaphnia Acute MHSF 25ºC

LRAC5791

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

Analyte	Reported Value	Assigned Value	Acceptance Window	z-score*
EPA 2002.0 - Ceriodaphnia dubia, 48-hr Acute, renewal, MHSF 25°C (2002) 10214809				
Test Code 19 / EPA Method 2002				
Ceriodaphnia Acute MHSF 25° - LC50[1,2] 764 Analyst: Arianna Krueger Analysis Date: 2020-04-01	37.8 %	43.0 % *Evaluation Criteria – 5** *Parameters*: deviations:2*	5.89 - 80.1 %	**-0.3** Acceptable
Group Analysis Summary	Acceptable: 1/1		**Score: 100% - Acceptable**	

* Evaluation parameters used for the statistical analysis: explanation at the end of report; a yellow highlighted results is acceptable but to be checked.

** Unable to calculate a study mean due to <4 data points being received, therefore an effective evaluation could not be performed.

[1] TNI Compliant, covered by Sigma-Aldrich RTC's ANAB Proficiency Testing Provider accreditation, Cert. AP-1469

[2] ISO/IEC 17043 Accredited, covered by Sigma-Aldrich RTC's ANAB Proficiency Testing Provider accreditation, Cert AP-1469

Summary Results for DMRQA 40

WET021-1EA Ceriodaphnia Chronic MHSF

LRAC5793

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

Analyte	Reported Value	Assigned Value	Acceptance Window	z-score*
EPA 1002.0 - Ceriodaphnia dubia, 7-day Chronic, daily renewal, MHSF 25°C (2002) 10215006				
Test Code 21 / EPA Method 1002				
Ceriodaphnia Chronic MHSF - Survival NOEC[1,2] 766 Analyst: Arianna Krueger Analysis Date: 2020-04-02	12.5 %	12.5 % *Evaluation Criteria – 8** *Parameters*: ± 1 dilution*	6.25 - 25.0 %	Acceptable
Ceriodaphnia Chronic MHSF - Reproduction IC25[1,2] 767 Analyst: Arianna Krueger Analysis Date: 2020-04-02	10.2 %	10.8 % *Evaluation Criteria – 5** *Parameters*: deviations:2*	3.62 - 17.9 %	**-0.2** Acceptable
Ceriodaphnia Chronic MHSF - Reproduction NOEC[1,2] 768 Analyst: Arianna Krueger Analysis Date: 2020-04-02	6.25 %	6.25 % *Evaluation Criteria – 8** *Parameters*: ± 1 dilution*	<6.25 - 12.5 %	Acceptable
Group Analysis Summary	Acceptable: 3/3		**Score: 100% - Acceptable**	

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Summary Results for DMRQA 40

WET032-1EA Daphnia Magna Acute MHSF 25ºC

LRAC5795

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

Analyte	Reported Value	Assigned Value	Acceptance Window	z-score*
EPA 2021.0 - Daphnia magna, 48-hr Acute, nonrenewal, MHSF 25°C (2002) 10215415				
Test Code 32 / EPA Method 2021				
Daphnia Magna Acute MHSF 25° - LC50[1,2] 788 Analyst: CV Analysis Date: 2020-04-07	13.9 % *voluntary*	14.6 % *Evaluation Criteria – 5** *Parameters*: deviations:2*	1.46 - 32.2 %	**-0.1** Acceptable
Group Analysis Summary	Acceptable: 1/1		**Score: 100% - Acceptable**	

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Summary Results for DMRQA 40

WET013-1EA Fathead Minnow Acute MHSF 25ºC

LRAC5789

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

Analyte	Reported Value	Assigned Value	Acceptance Window	z-score*
EPA 2000.0 - Fathead minnow, 48-hr Acute, nonrenewal, MHSF 25°C (2002) 10213602				
Test Code 13 / EPA Method 2000				
Fathead Minnow Acute MHSF 25° - LC50[1,2] 754 Analyst: LT Analysis Date: 2020-05-26	35.4 %	27.4 % *Evaluation Criteria – 5** *Parameters*: deviations:2*	5.05 - 49.8 %	**0.7** Acceptable
Group Analysis Summary	Acceptable: 1/1		**Score: 100% - Acceptable**	

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Summary Results for DMRQA 40
WET015-1EA Fathead Minnow, 7Day, MHSF
LRAC5790

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

Analyte	Reported Value	Assigned Value	Acceptance Window	z-score*
EPA 1000.0 - Fathead minnow, 7-day Chronic, daily renewal, MHSF 25°C (2002) 10214207				
Test Code 15 / EPA Method 1000				
Fathead Minnow Chronic MHSF - Survival NOEC[1,2] 756 Analyst: Arianna Krueger Analysis Date: 2020-05-26	12.5 %	25.0 % *Evaluation Criteria – 8** *Parameters*: ± 1 dilution*	12.5 - 50.0 %	Acceptable
Fathead Minnow Chronic MHSF - Growth IC25 (ON)[1,2] 808 Analyst: Arianna Krueger Analysis Date: 2020-05-26	12.5 %	26.2 % *Evaluation Criteria – 5** *Parameters*: deviations:2*	9.64 - 42.7 %	**-1.7** Acceptable
Fathead Minnow Chronic MHSF - Growth NOEC (ON)[1,2] 810 Analyst: Arianna Krueger Analysis Date: 2020-05-26	6.25 %	25.0 % *Evaluation Criteria – 8** *Parameters*: ± 1 dilution*	12.5 - 50.0 %	Not Acceptable
Group Analysis Summary	Acceptable: 2/3		**Score: 67% - Acceptable**	

Note: See attached corrective action report

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Summary Results for DMRQA 40

WET042-1EA Mysid Acute 40 Fathoms Seawater 25ºC

LRAC5797

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

Analyte	Reported Value	Assigned Value	Acceptance Window	z-score*
EPA 2007.0 - Mysid, 48-hr Acute, nonrenewal, 40-fath SW, 25°C (2002) 10216009				
Test Code 42 / EPA Method 2007				
Mysid Acute 40 F 25° - LC50[1,2] 798 Analyst: Arianna Krueger Analysis Date: 2020-05-29	20.4 % 	21.2 % *Evaluation Criteria – 5* *Parameters*: deviations:2*	3.67 - 38.7 %	**-0.1** Acceptable
Group Analysis Summary	Acceptable: 1/1		**Score: 100% - Acceptable**	

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Summary Results for DMRQA 40

WET043-1EA Mysid Chronic 40 Fathoms Seawater

LRAC5798

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

Analyte	Reported Value	Assigned Value	Acceptance Window	z-score*
EPA 1007.0 - Mysid, 7-day Chronic, daily renewal, 40-fathoms SW 26°C (2002) 10254009				
Test Code 43 / EPA Method 1007				
Mysid Chronic 40 F Survival NOEC[1,2] 799 Analyst: Arianna Krueger Analysis Date: 2020-05-28	100 %	100 % *Evaluation Criteria – 8** *Parameters*: ± 1 dilution*	50.0 - >100 %	Acceptable
Mysid Chronic 40 F Growth IC25 (ON)[1,2] 816 Analyst: Arianna Krueger Analysis Date: 2020-05-28	>100 %	95.0 % *Evaluation Criteria – 7** *Parameters*: a:1, b:0, c:0.3333, d:0*	31.7 - >100 %	Acceptable
Mysid Chronic 40 F Growth NOEC (ON)[2] 818 Analyst: Arianna Krueger Analysis Date: 2020-05-28	50 %	50.0 % *Evaluation Criteria – 8** *Parameters*: ± 1 dilution*	25.0 - 100 %	Acceptable
Group Analysis Summary	Acceptable: 3/3		**Score: 100% - Acceptable**	

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Summary Results for DMRQA 40

WET046-1EA Sheepshead Minnow Acute 40 Fathoms Seawater 25ºC

LRAC5799

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

Analyte	Reported Value	Assigned Value	Acceptance Window	z-score*
EPA 2004.0 - Sheepshead Minnow, 48-hr Acute, nonrenewal, 40-fathoms SW 25°C (2002) 10216623				
Test Code 46 / EPA Method 2004				
Sheepshead Minnow Acute 40 F 25° - LC50[1,2] 804 Analyst: Arianna Krueger Analysis Date: 2020-05-27	34.2 % 	25.0 % *Evaluation Criteria – 7** *Parameters*: a:1, b:0, c:0.3333, d:0*	8.34 - 41.7 % 	**1.1** Acceptable
Group Analysis Summary	Acceptable: 1/1		**Score: 100% - Acceptable**	

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Summary Results for DMRQA 40

WET047-1EA Sheepshead Minnow Chronic 40 Fathoms Seawater

LRAC5800

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

Analyte	Reported Value	Assigned Value	Acceptance Window	z-score*
EPA 1004.0 - Sheepshead Minnow, 7-day Chronic, daily renewal, 40-fathoms SW 25°C (2002) 10216805				
Test Code 47 / EPA Method 1004				
Sheepshead Minnow Chronic 40 F - Survival NOEC[2] 805 Analyst: CV Analysis Date: 2020-04-02	12.5 %	25.0 % *Evaluation Criteria – 8** *Parameters*: ± 1 dilution*	12.5 - 50.0 %	Acceptable
Sheepshead Minnow Chronic 40 F - Growth IC25 (ON)[2] 820 Analyst: CV Analysis Date: 2020-04-02	17.2 %	30.0 % *Evaluation Criteria – 7** *Parameters*: a:1, b:0, c:0.3333, d:0*	10.0 - 50.0 %	**-1.3** Acceptable
Sheepshead Minnow Chronic 40 F - Growth NOEC (ON)[2] 822 Analyst: CV Analysis Date: 2020-04-02	12.5 %	25.0 % *Evaluation Criteria – 8** *Parameters*: ± 1 dilution*	12.5 - 50.0 %	Acceptable
Group Analysis Summary	Acceptable: 3/3			**Score: 100% - Acceptable**

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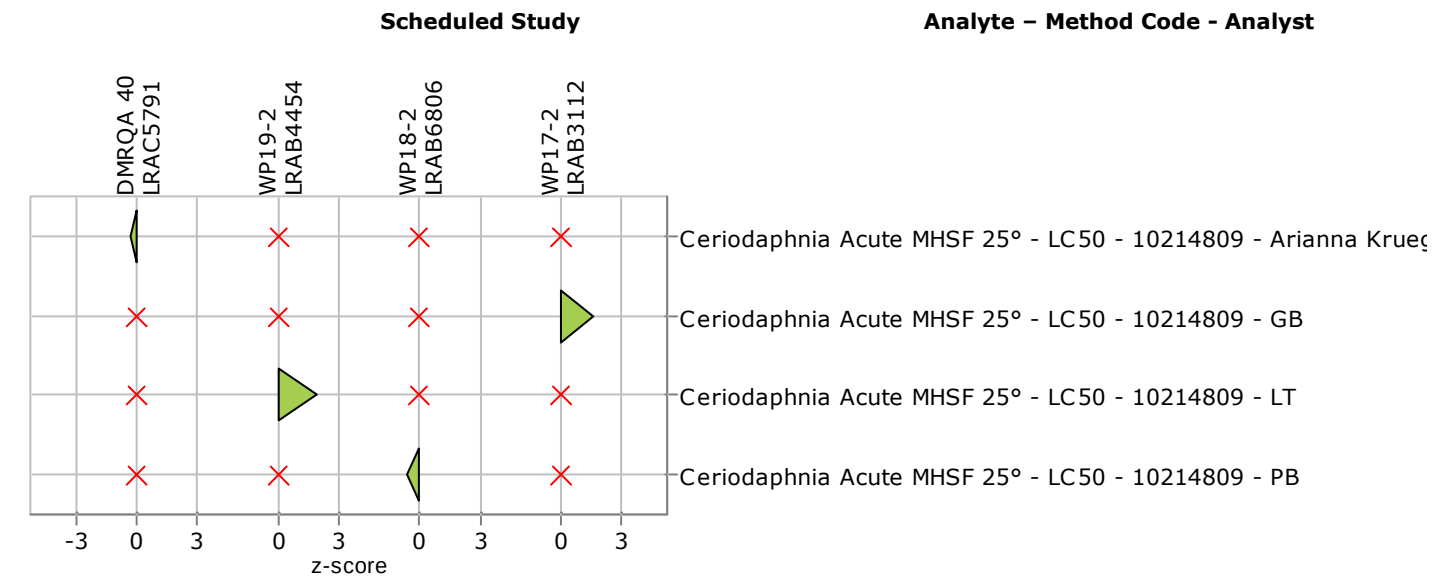
Graphical z-score Overview for DMRQA 40
WET019-1EA Ceriodaphnia Acute MHSF 25ºC

z-score Overview* for DMRQA 40 and the Previous three Scheduled Studies of this Study Type

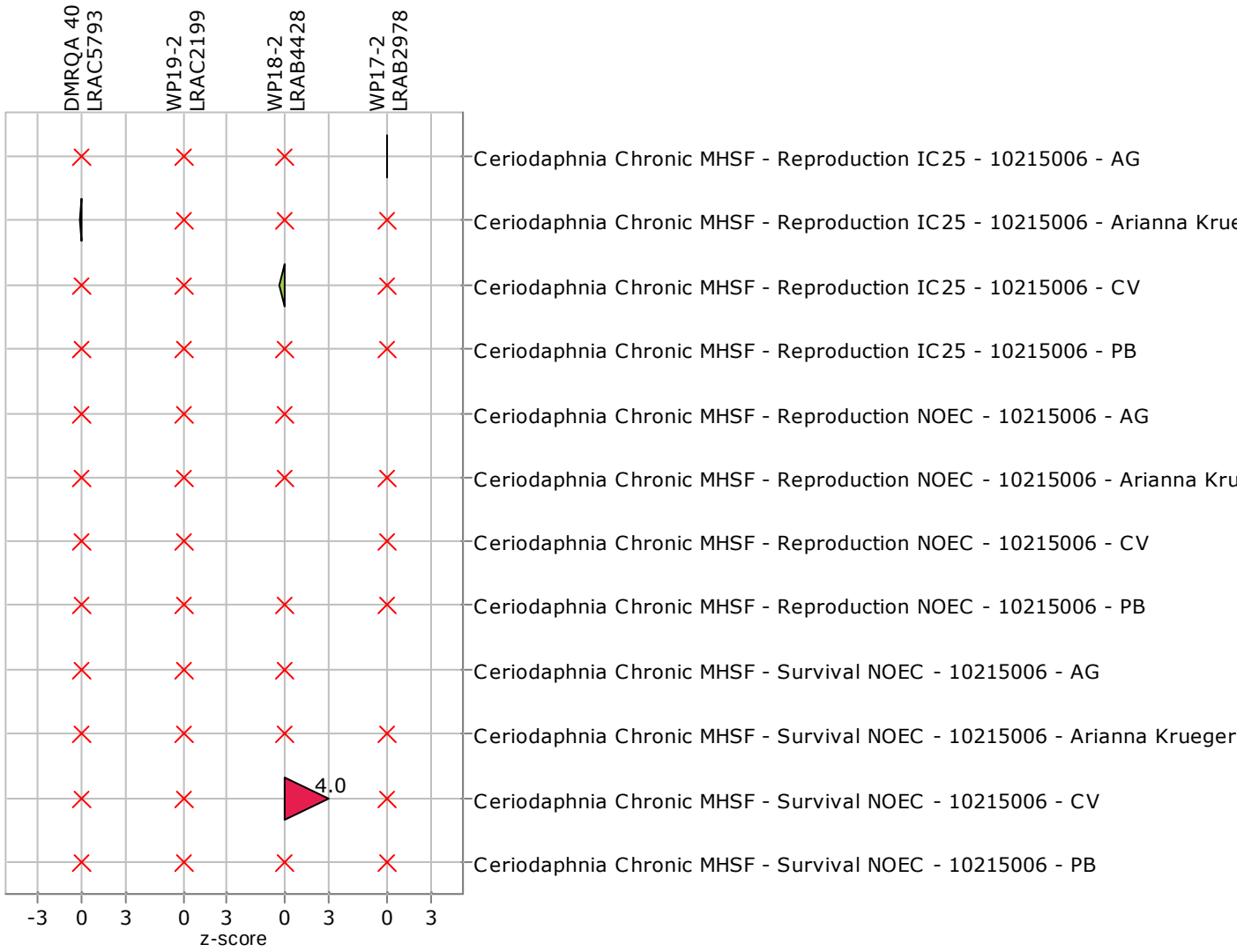


* Evaluation parameters used for the statistical analysis; explanation at the end of report

Graphical z-score Overview for DMRQA 40
WET021-1EA Ceriodaphnia Chronic MHSF

z-score Overview* for DMRQA 40 and the Previous three Scheduled Studies of this Study Type

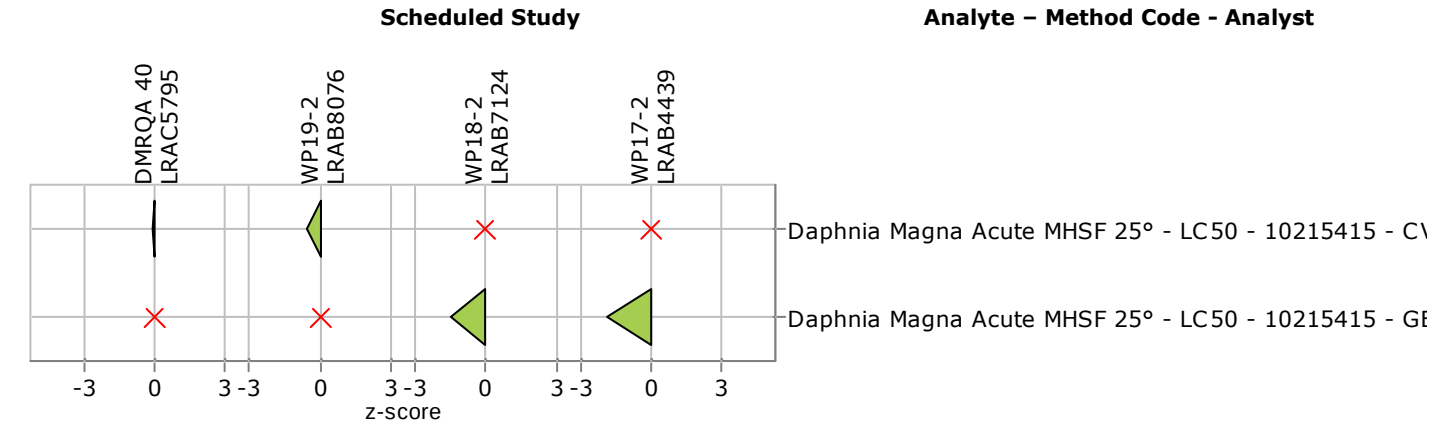
Scheduled Study **Analyte – Method Code - Analyst**



* Evaluation parameters used for the statistical analysis; explanation at the end of report

Graphical z-score Overview for DMRQA 40
WET032-1EA Daphnia Magna Acute MHSF 25ºC

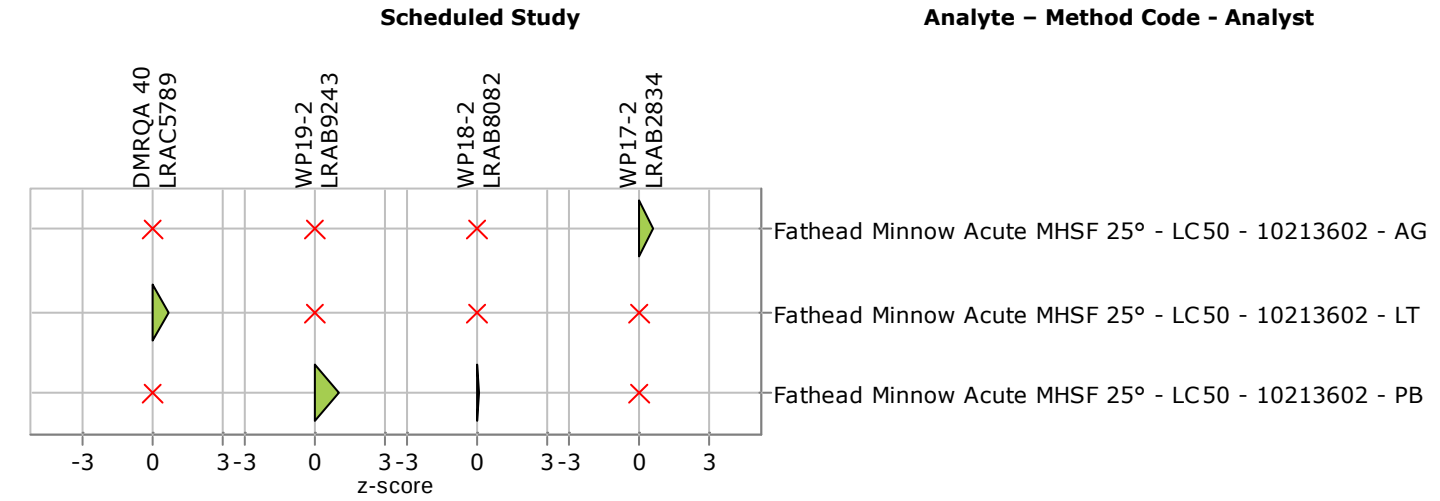
z-score Overview* for DMRQA 40 and the Previous three Scheduled Studies of this Study Type



* Evaluation parameters used for the statistical analysis; explanation at the end of report

Graphical z-score Overview for DMRQA 40
WET013-1EA Fathead Minnow Acute MHSF 25ºC

z-score Overview* for DMRQA 40 and the Previous three Scheduled Studies of this Study Type

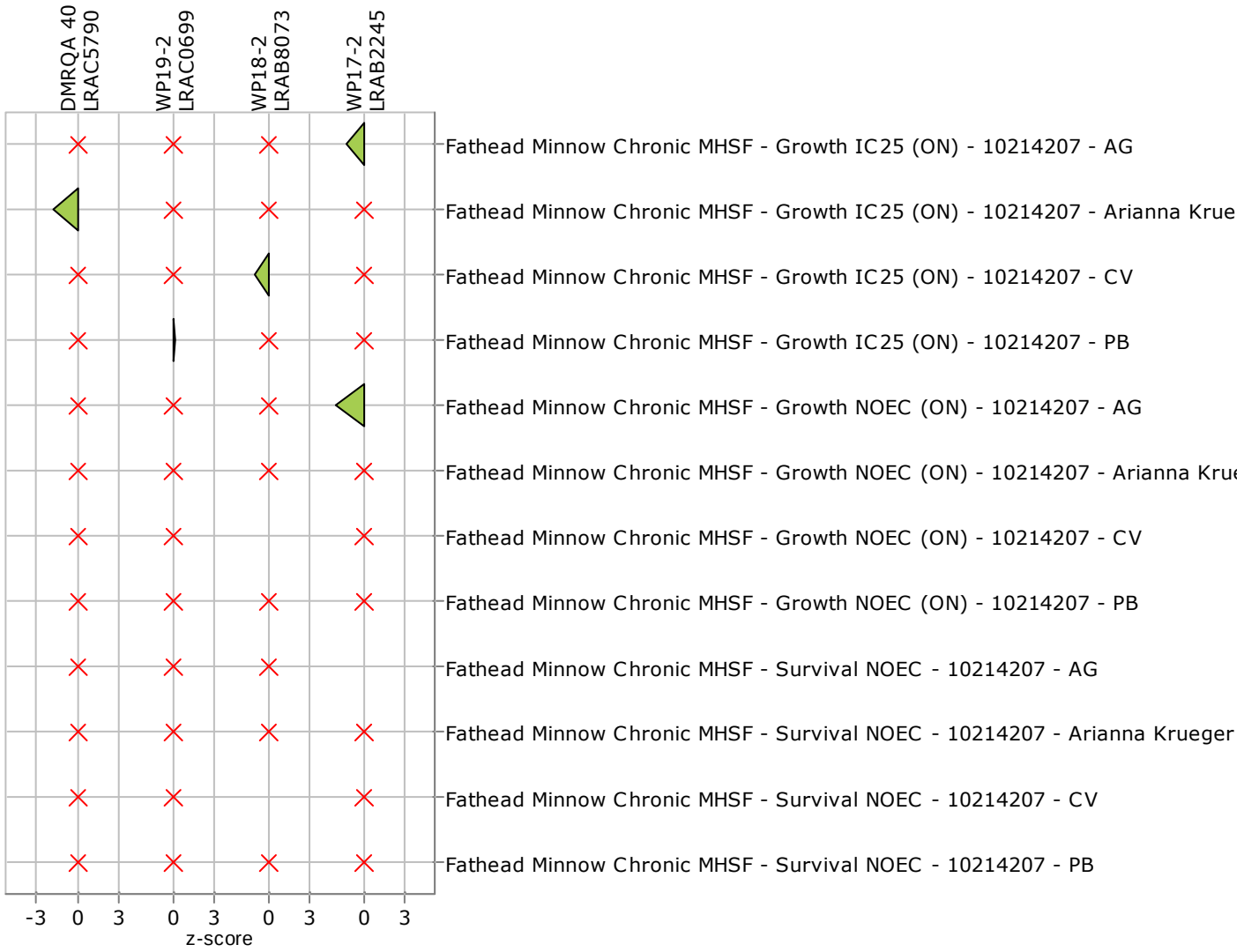


* Evaluation parameters used for the statistical analysis; explanation at the end of report

Graphical z-score Overview for DMRQA 40
WET015-1EA Fathead Minnow, 7Day, MHSF

z-score Overview* for DMRQA 40 and the Previous three Scheduled Studies of this Study Type

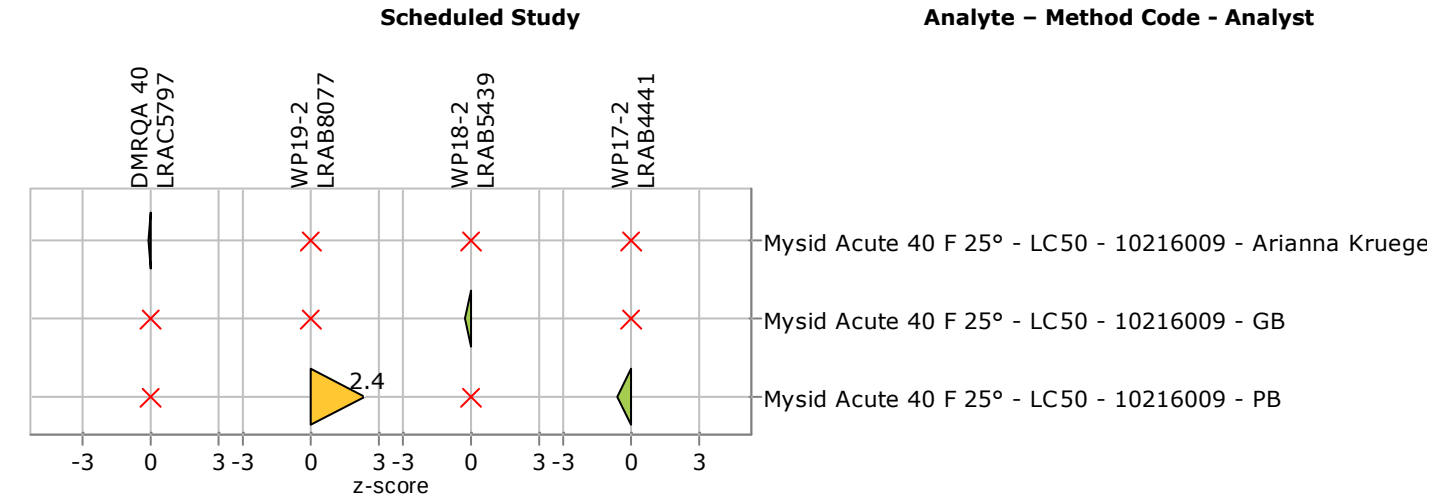
Scheduled Study **Analyte – Method Code - Analyst**



* Evaluation parameters used for the statistical analysis; explanation at the end of report

Graphical z-score Overview for DMRQA 40
WET042-1EA Mysid Acute 40 Fathoms Seawater 25ºC

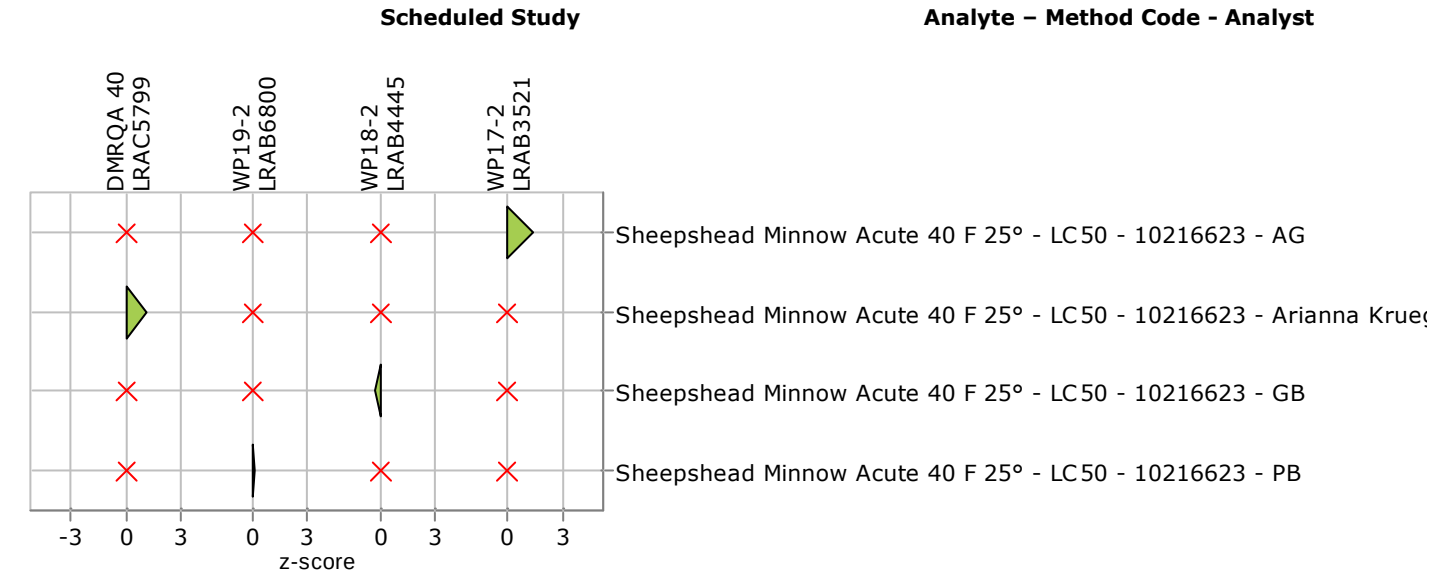
z-score Overview* for DMRQA 40 and the Previous three Scheduled Studies of this Study Type



* Evaluation parameters used for the statistical analysis; explanation at the end of report

Graphical z-score Overview for DMRQA 40
WET046-1EA Sheepshead Minnow Acute 40 Fathoms Seawater 25ºC

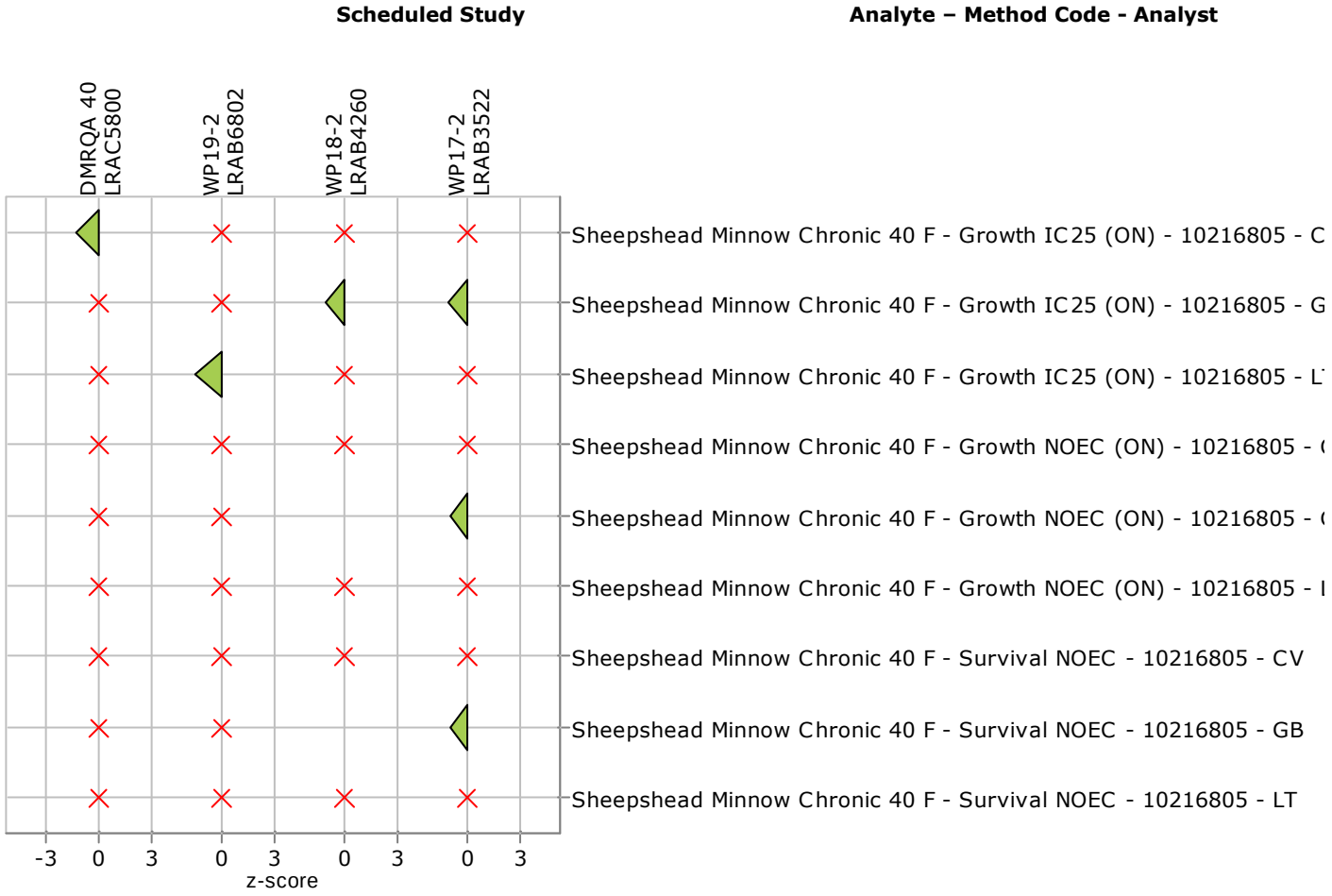
z-score Overview* for DMRQA 40 and the Previous three Scheduled Studies of this Study Type



* Evaluation parameters used for the statistical analysis; explanation at the end of report

Graphical z-score Overview for DMRQA 40
WET047-1EA Sheepshead Minnow Chronic 40 Fathoms Seawater

z-score Overview* for DMRQA 40 and the Previous three Scheduled Studies of this Study Type



* Evaluation parameters used for the statistical analysis; explanation at the end of report

Contents

1 Aim of the Proficiency Test

This interlaboratory study is a proficiency test for the assessment of laboratory performance. It was conducted in the framework of external quality assurance and the report provides an external appraisal of the participant laboratories' competence in the particular testing field.

2 Sample Information

WET019-1EA Ceriodaphnia Acute MHSF 25ºC LRAC5791

Analyte	Unit	Gravimetric Value	PTRL	Study Mean*	Study Std. Dev.*
Ceriodaphnia Acute MHSF 25° - LC50 764	%	43.9	---	43.0	18.5

WET021-1EA Ceriodaphnia Chronic MHSF LRAC5793

Analyte	Unit	Gravimetric Value	PTRL	Study Mean*	Study Std. Dev.*
Ceriodaphnia Chronic MHSF - Survival NOEC 766	%	12.5	---	12.5	0
Ceriodaphnia Chronic MHSF - Reproduction IC25 767	%	10.8	---	10.8	3.57
Ceriodaphnia Chronic MHSF - Reproduction NOEC 768	%	6.25	---	6.25	0

WET032-1EA Daphnia Magna Acute MHSF 25ºC LRAC5795

Analyte	Unit	Gravimetric Value	PTRL	Study Mean*	Study Std. Dev.*
Daphnia Magna Acute MHSF 25° - LC50 788	%	12.5	---	14.6	8.77

WET013-1EA Fathead Minnow Acute MHSF 25ºC LRAC5789

Analyte	Unit	Gravimetric Value	PTRL	Study Mean*	Study Std. Dev.*
Fathead Minnow Acute MHSF 25° - LC50 754	%	27.4	---	27.4	11.2

* If there are not enough data available to provide Study mean and Study Std. Dev, this is indicated by "---".

WET015-1EA Fathead Minnow, 7Day, MHSF
LRAC5790

Analyte	Unit	Gravimetric Value	PTRL	Study Mean*	Study Std. Dev.*
Fathead Minnow Chronic MHSF - Survival NOEC 756	%	25.0	---	23.1	10.5
Fathead Minnow Chronic MHSF - Growth IC25 (ON) 808	%	27.5	---	26.2	8.26
Fathead Minnow Chronic MHSF - Growth NOEC (ON) 810	%	25.0	---	25.0	0

WET042-1EA Mysid Acute 40 Fathoms Seawater 25ºC
LRAC5797

Analyte	Unit	Gravimetric Value	PTRL	Study Mean*	Study Std. Dev.*
Mysid Acute 40 F 25° - LC50 798	%	25.0	---	21.2	8.75

WET043-1EA Mysid Chronic 40 Fathoms Seawater
LRAC5798

Analyte	Unit	Gravimetric Value	PTRL	Study Mean*	Study Std. Dev.*
Mysid Chronic 40 F Survival NOEC 799	%	100	---	83.3	34.4
Mysid Chronic 40 F Growth IC25 (ON) 816	%	95.0	---	---	---
Mysid Chronic 40 F Growth NOEC (ON) 818	%	100	---	70.0	36.8

WET046-1EA Sheepshead Minnow Acute 40 Fathoms Seawater 25ºC
LRAC5799

Analyte	Unit	Gravimetric Value	PTRL	Study Mean*	Study Std. Dev.*
Sheepshead Minnow Acute 40 F 25° - LC50 804	%	25.0	---	18.3	1.33

* If there are not enough data available to provide Study mean and Study Std. Dev, this is indicated by "---".

WET047-1EA Sheepshead Minnow Chronic 40 Fathoms Seawater LRAC5800

Analyte	Unit	Gravimetric Value	PTRL	Study Mean*	Study Std. Dev.*
Sheepshead Minnow Chronic 40 F - Survival NOEC 805	%	25.0	---	---	---
Sheepshead Minnow Chronic 40 F - Growth IC25 (ON) 820	%	30.0	---	---	---
Sheepshead Minnow Chronic 40 F - Growth NOEC (ON) 822	%	25.0	---	---	---

* If there are not enough data available to provide Study mean and Study Std. Dev, this is indicated by "---".

3 Data Availability

WET019-1EA Ceriodaphnia Acute MHSF 25ºC LRAC5791

Analyte	Number of participating laboratories		Number of data points	
	in total	with quantitative data points only*	in total	quantitative only*
Ceriodaphnia Acute MHSF 25° - LC50 764	15	15	15	15

WET021-1EA Ceriodaphnia Chronic MHSF LRAC5793

Analyte	Number of participating laboratories		Number of data points	
	in total	with quantitative data points only*	in total	quantitative only*
Ceriodaphnia Chronic MHSF - Survival NOEC 766	17	17	17	17
Ceriodaphnia Chronic MHSF - Reproduction IC25 767	17	17	17	17
Ceriodaphnia Chronic MHSF - Reproduction NOEC 768	17	17	17	17

WET032-1EA Daphnia Magna Acute MHSF 25ºC LRAC5795

Analyte	Number of participating laboratories		Number of data points	
	in total	with quantitative data points only*	in total	quantitative only*
Daphnia Magna Acute MHSF 25° - LC50 788	8	8	8	8

WET013-1EA Fathead Minnow Acute MHSF 25ºC LRAC5789

Analyte	Number of participating laboratories		Number of data points	
	in total	with quantitative data points only*	in total	quantitative only*
Fathead Minnow Acute MHSF 25° - LC50 754	16	16	16	16

* Only quantitative values are taken into account in the calculation of study mean and study std.dev. (i.e. without missing results, without less-than results, without larger-than results).

WET015-1EA Fathead Minnow, 7Day, MHSF
LRAC5790

Analyte	Number of participating laboratories		Number of data points	
	in total	with quantitative data points only*	in total	quantitative only*
Fathead Minnow Chronic MHSF - Survival NOEC 756	18	18	18	18
Fathead Minnow Chronic MHSF - Growth IC25 (ON) 808	17	17	17	17
Fathead Minnow Chronic MHSF - Growth NOEC (ON) 810	17	17	17	17

WET042-1EA Mysid Acute 40 Fathoms Seawater 25ºC
LRAC5797

Analyte	Number of participating laboratories		Number of data points	
	in total	with quantitative data points only*	in total	quantitative only*
Mysid Acute 40 F 25° - LC50 798	8	8	8	8

WET043-1EA Mysid Chronic 40 Fathoms Seawater
LRAC5798

Analyte	Number of participating laboratories		Number of data points	
	in total	with quantitative data points only*	in total	quantitative only*
Mysid Chronic 40 F Survival NOEC 799	6	6	6	6
Mysid Chronic 40 F Growth IC25 (ON) 816	5	3	5	3
Mysid Chronic 40 F Growth NOEC (ON) 818	5	5	5	5

* Only quantitative values are taken into account in the calculation of study mean and study std.dev. (i.e. without missing results, without less-than results, without larger-than results).

WET046-1EA Sheepshead Minnow Acute 40 Fathoms Seawater 25ºC LRAC5799

Analyte	Number of participating laboratories		Number of data points	
	in total	with quantitative data points only*	in total	quantitative only*
Sheepshead Minnow Acute 40 F 25° - LC50 804	4	4	4	4

WET047-1EA Sheepshead Minnow Chronic 40 Fathoms Seawater LRAC5800

Analyte	Number of participating laboratories		Number of data points	
	in total	with quantitative data points only*	in total	quantitative only*
Sheepshead Minnow Chronic 40 F - Survival NOEC 805	3	3	3	3
Sheepshead Minnow Chronic 40 F - Growth IC25 (ON) 820	3	3	3	3
Sheepshead Minnow Chronic 40 F - Growth NOEC (ON) 822	3	3	3	3

* Only quantitative values are taken into account in the calculation of study mean and study std.dev. (i.e. without missing results, without less-than results, without larger-than results).

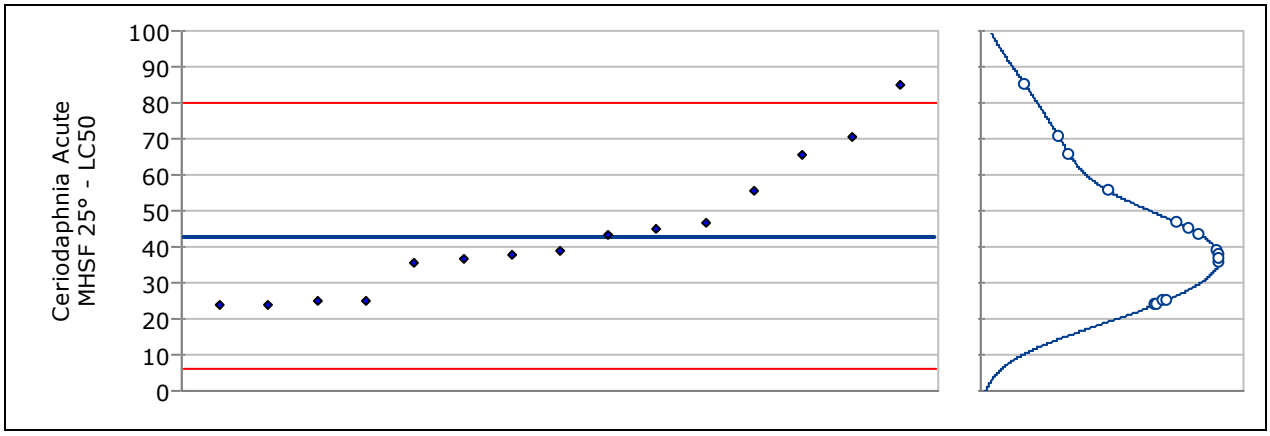
4 Results

4.1 WET019-1EA Ceriodaphnia Acute MHSF 25ºC / LRAC5791

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

4.1.1 Ceriodaphnia Acute MHSF 25° - LC50

No. of participating laboratories (in total / with quant. data points only)	15 / 15
No. of data points (in total / quantitative)	15 / 15
Assigned value	43.0 %
Proficiency std. dev.	18.5 %
Acceptance window	5.89 - 80.1 %

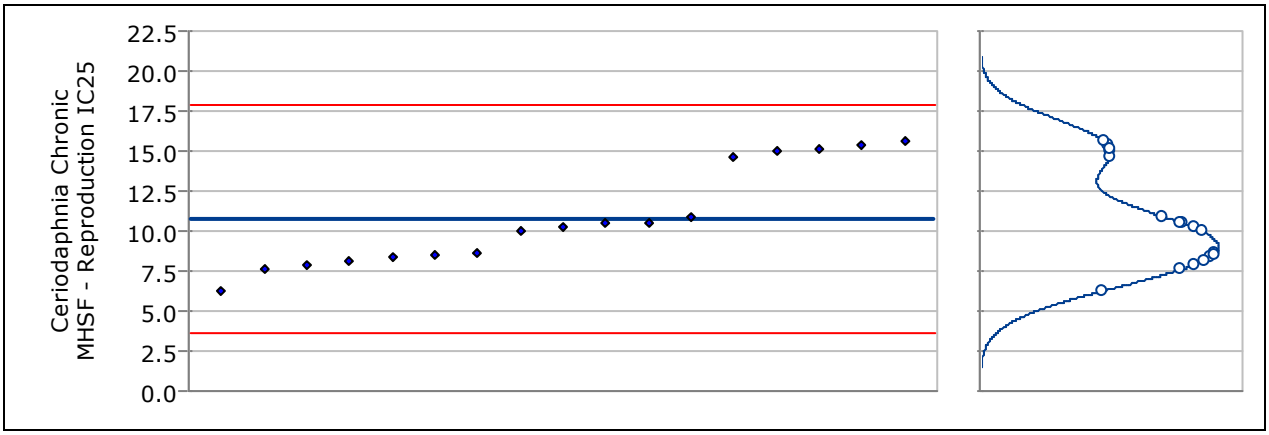


4.2 WET021-1EA Ceriodaphnia Chronic MHSF / LRAC5793

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

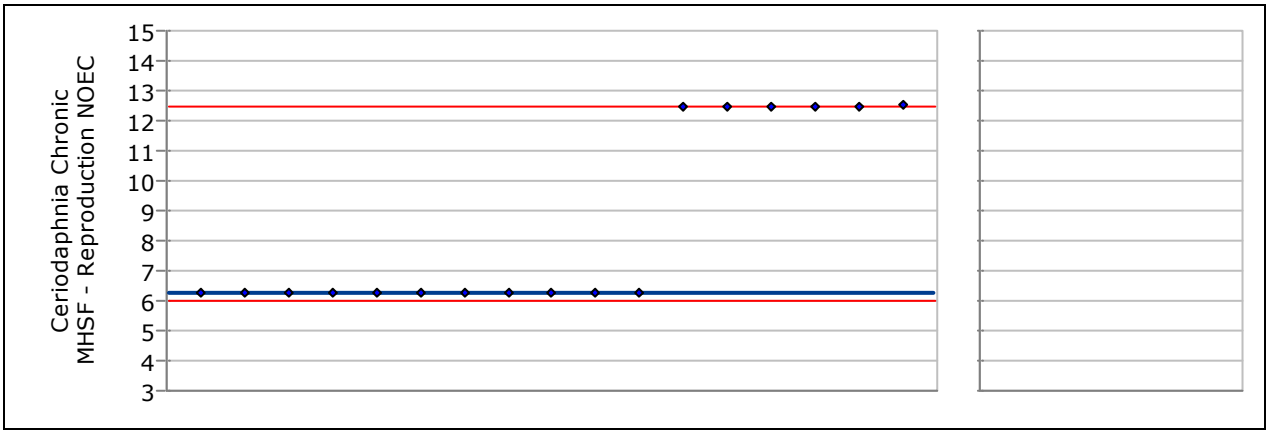
4.2.1 Ceriodaphnia Chronic MHSF - Reproduction IC25

No. of participating laboratories (in total / with quant. data points only)	17 / 17
No. of data points (in total / quantitative)	17 / 17
Assigned value	10.8 %
Proficiency std. dev.	3.57 %
Acceptance window	3.62 - 17.9 %



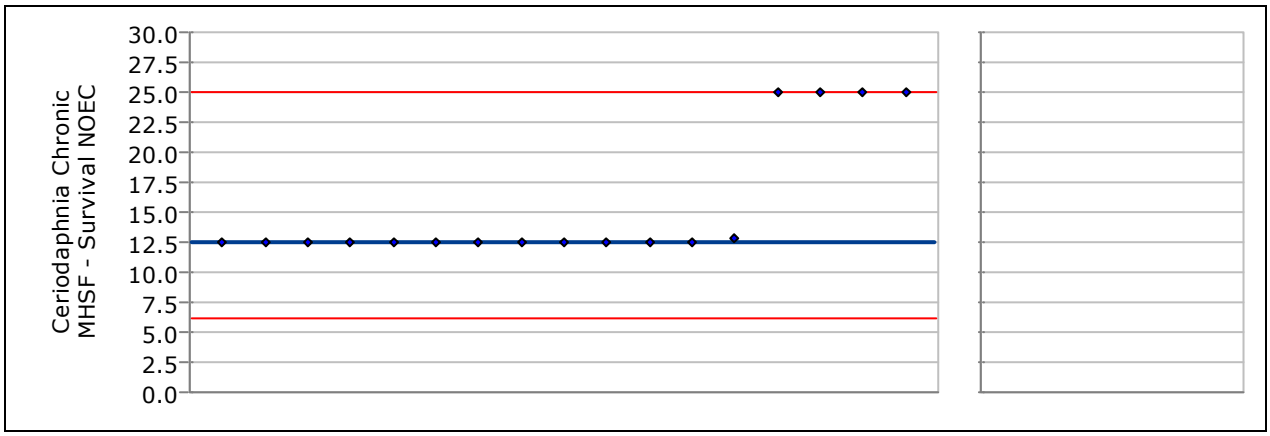
4.2.2 Ceriodaphnia Chronic MHSF - Reproduction NOEC

No. of participating laboratories (in total / with quant. data points only)	17 / 17
No. of data points (in total / quantitative)	17 / 17
Assigned value	6.25 %
Proficiency std. dev.	--- %
Acceptance window	6.00 - 12.5 %



4.2.3 Ceriodaphnia Chronic MHSF - Survival NOEC

No. of participating laboratories (in total / with quant. data points only)	17 / 17
No. of data points (in total / quantitative)	17 / 17
Assigned value	12.5 %
Proficiency std. dev.	--- %
Acceptance window	6.25 - 25.0 %

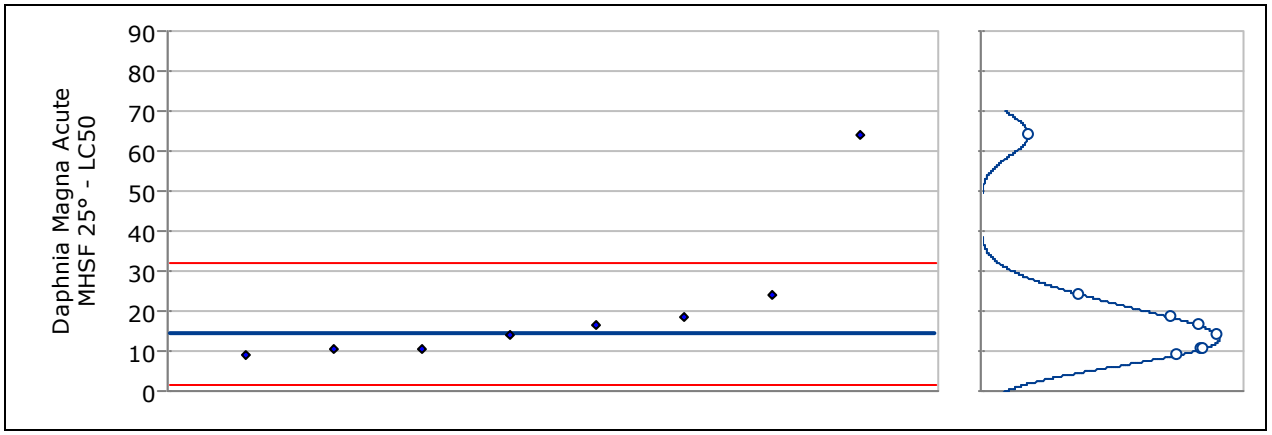


4.3 WET032-1EA Daphnia Magna Acute MHSF 25ºC / LRAC5795

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

4.3.1 Daphnia Magna Acute MHSF 25° - LC50

No. of participating laboratories (in total / with quant. data points only)	8 / 8
No. of data points (in total / quantitative)	8 / 8
Assigned value	14.6 %
Proficiency std. dev.	8.77 %
Acceptance window	1.46 - 32.2 %

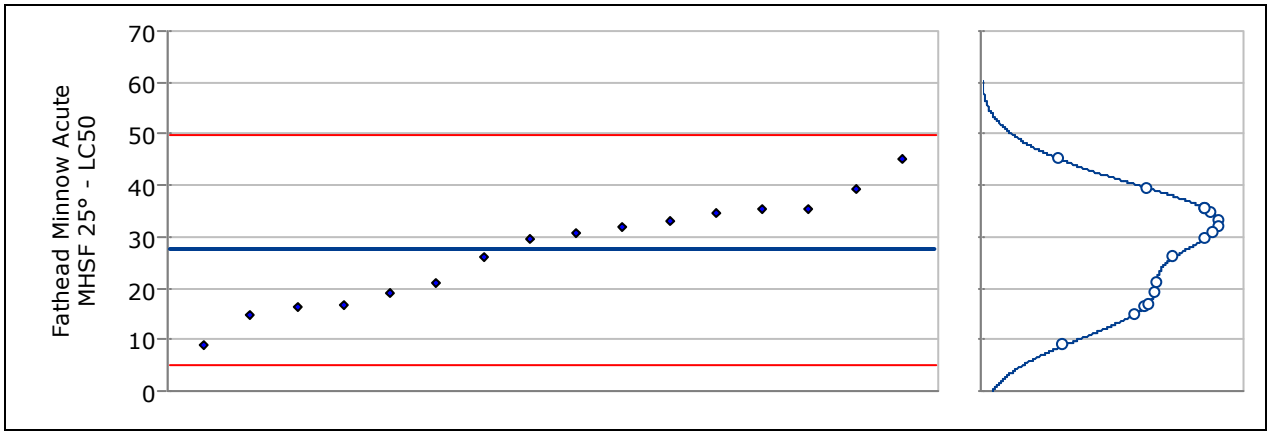


4.4 WET013-1EA Fathead Minnow Acute MHSF 25ºC / LRAC5789

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

4.4.1 Fathead Minnow Acute MHSF 25° - LC50

No. of participating laboratories (in total / with quant. data points only)	16 / 16
No. of data points (in total / quantitative)	16 / 16
Assigned value	27.4 %
Proficiency std. dev.	11.2 %
Acceptance window	5.05 - 49.8 %

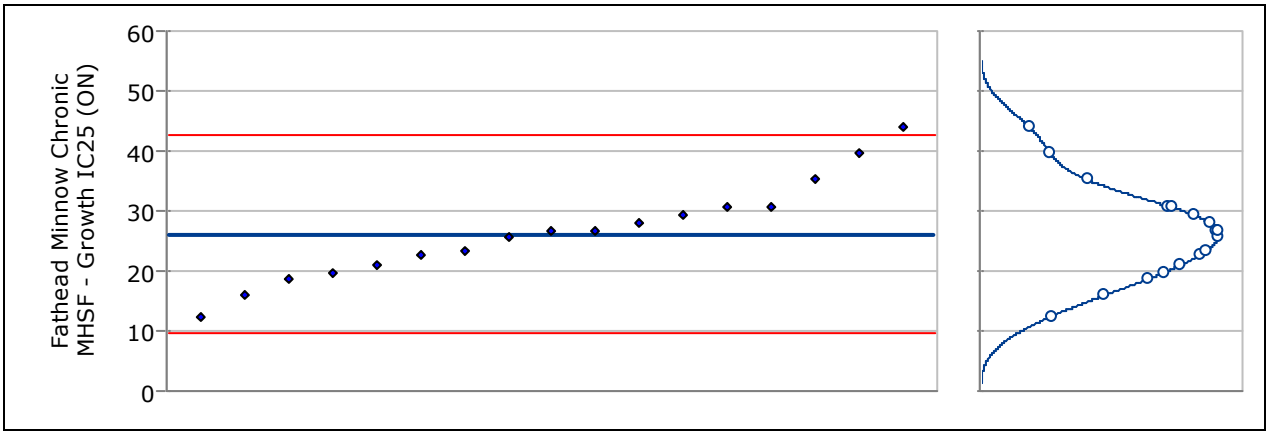


4.5 WET015-1EA Fathead Minnow, 7Day, MHSF / LRAC5790

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

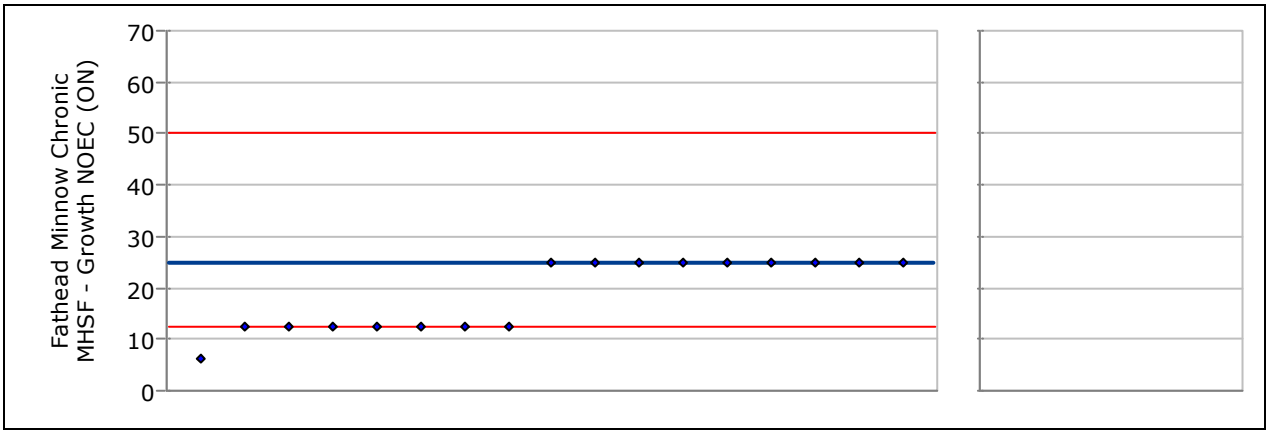
4.5.1 Fathead Minnow Chronic MHSF - Growth IC25 (ON)

No. of participating laboratories (in total / with quant. data points only)	17 / 17
No. of data points (in total / quantitative)	17 / 17
Assigned value	26.2 %
Proficiency std. dev.	8.26 %
Acceptance window	9.64 - 42.7 %



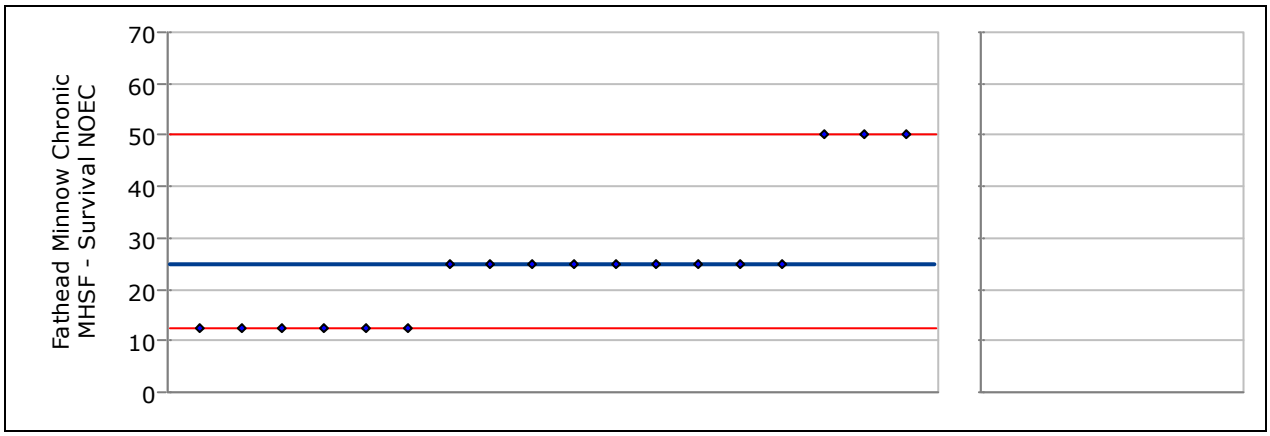
4.5.2 Fathead Minnow Chronic MHSF - Growth NOEC (ON)

No. of participating laboratories (in total / with quant. data points only)	17 / 17
No. of data points (in total / quantitative)	17 / 17
Assigned value	25.0 %
Proficiency std. dev.	--- %
Acceptance window	12.5 - 50.0 %



4.5.3 Fathead Minnow Chronic MHSF - Survival NOEC

No. of participating laboratories (in total / with quant. data points only)	18 / 18
No. of data points (in total / quantitative)	18 / 18
Assigned value	25.0 %
Proficiency std. dev.	--- %
Acceptance window	12.5 - 50.0 %

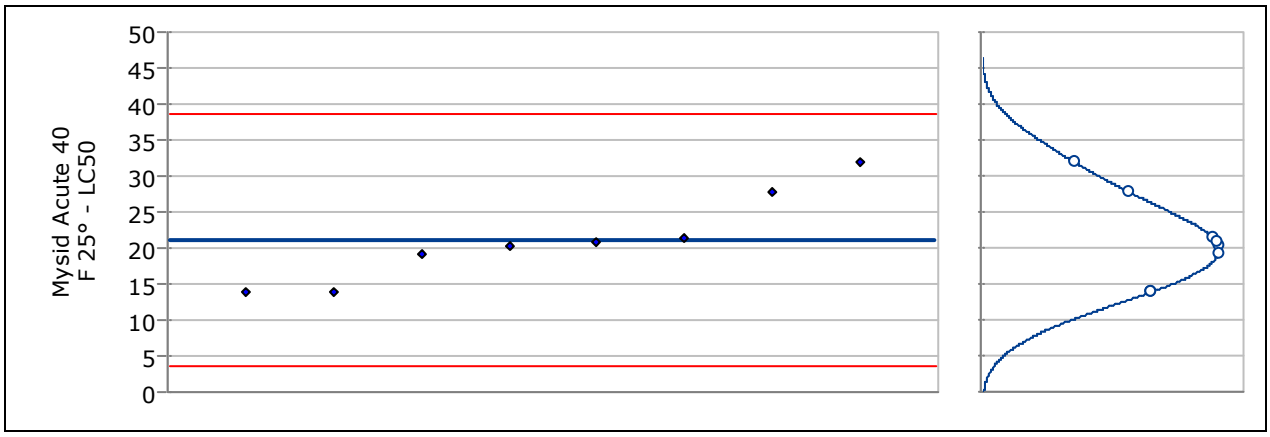


4.6 WET042-1EA Mysid Acute 40 Fathoms Seawater 25ºC / LRAC5797

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

4.6.1 Mysid Acute 40 F 25° - LC50

No. of participating laboratories (in total / with quant. data points only)	8 / 8
No. of data points (in total / quantitative)	8 / 8
Assigned value	21.2 %
Proficiency std. dev.	8.75 %
Acceptance window	3.67 - 38.7 %

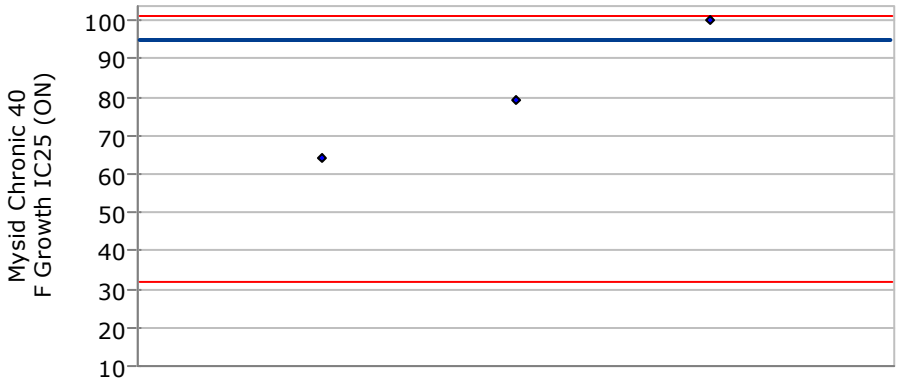


4.7 WET043-1EA Mysid Chronic 40 Fathoms Seawater / LRAC5798

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

4.7.1 Mysid Chronic 40 F Growth IC25 (ON)

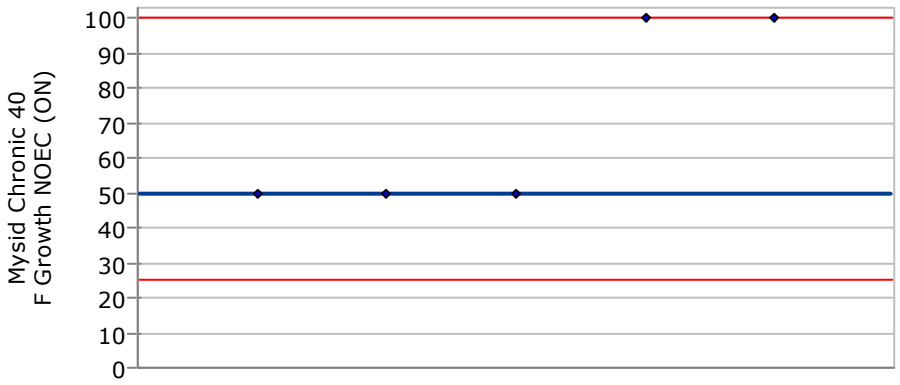
No. of participating laboratories (in total / with quant. data points only)	5 / 3
No. of data points (in total / quantitative)	5 / 3
Assigned value	95.0 %
Proficiency std. dev.	31.7 %
Acceptance window	31.7 - 101 %



Number of laboratories with quantitative data points is less than 8. A reliable kernel density estimation cannot be guaranteed.

4.7.2 Mysid Chronic 40 F Growth NOEC (ON)

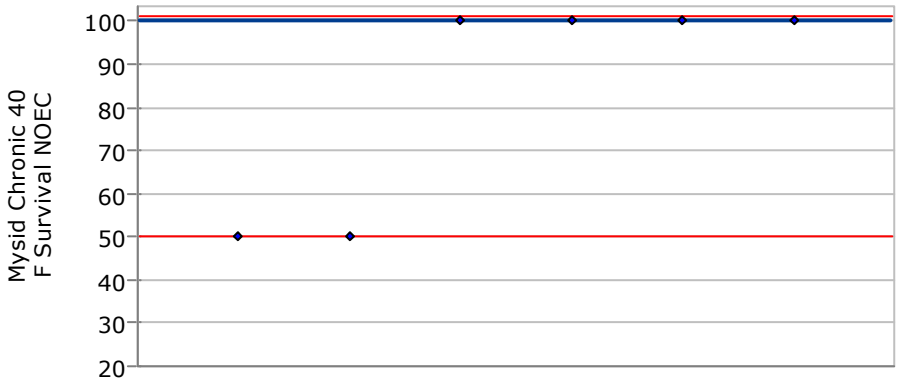
No. of participating laboratories (in total / with quant. data points only)	5 / 5
No. of data points (in total / quantitative)	5 / 5
Assigned value	50.0 %
Proficiency std. dev.	--- %
Acceptance window	25.0 - 100 %



Number of laboratories with quantitative data points is less than 8. A reliable kernel density estimation cannot be guaranteed.

4.7.3 Mysid Chronic 40 F Survival NOEC

No. of participating laboratories (in total / with quant. data points only)	6 / 6
No. of data points (in total / quantitative)	6 / 6
Assigned value	100 %
Proficiency std. dev.	--- %
Acceptance window	50.0 - 101 %



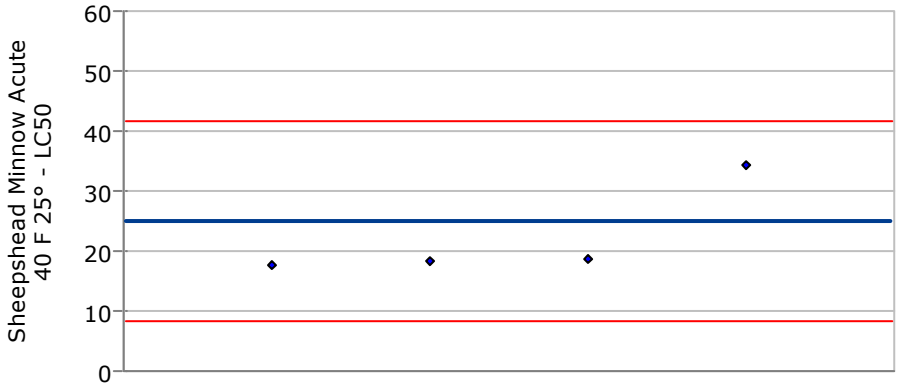
Number of laboratories with quantitative data points is less than 8. A reliable kernel density estimation cannot be guaranteed.

4.8 WET046-1EA Sheepshead Minnow Acute 40 Fathoms Seawater 25ºC / LRAC5799

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

4.8.1 Sheepshead Minnow Acute 40 F 25° - LC50

No. of participating laboratories (in total / with quant. data points only)	4 / 4
No. of data points (in total / quantitative)	4 / 4
Assigned value	25.0 %
Proficiency std. dev.	8.33 %
Acceptance window	8.34 - 41.7 %



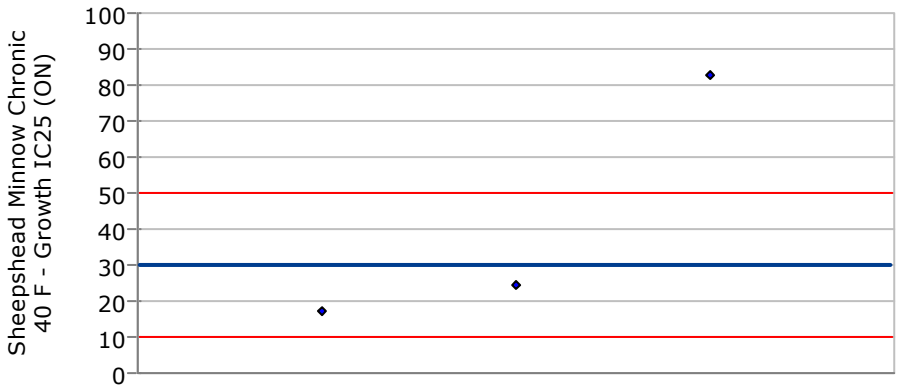
Number of laboratories with quantitative data points is less than 8. A reliable kernel density estimation cannot be guaranteed.

4.9 WET047-1EA Sheepshead Minnow Chronic 40 Fathoms Seawater / LRAC5800

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

4.9.1 Sheepshead Minnow Chronic 40 F - Growth IC25 (ON)

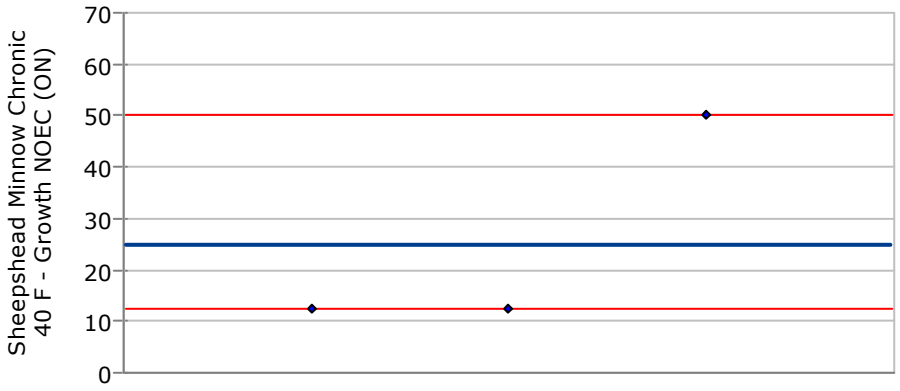
No. of participating laboratories (in total / with quant. data points only)	3 / 3
No. of data points (in total / quantitative)	3 / 3
Assigned value	30.0 %
Proficiency std. dev.	10.0 %
Acceptance window	10.0 - 50.0 %



Number of laboratories with quantitative data points is less than 8. A reliable kernel density estimation cannot be guaranteed.

4.9.2 Sheepshead Minnow Chronic 40 F - Growth NOEC (ON)

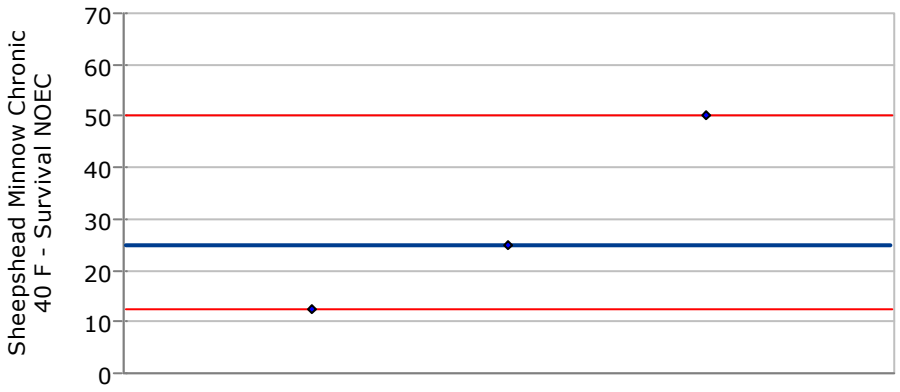
No. of participating laboratories (in total / with quant. data points only)	3 / 3
No. of data points (in total / quantitative)	3 / 3
Assigned value	25.0 %
Proficiency std. dev.	--- %
Acceptance window	12.5 - 50.0 %



Number of laboratories with quantitative data points is less than 8. A reliable kernel density estimation cannot be guaranteed.

4.9.3 Sheepshead Minnow Chronic 40 F - Survival NOEC

No. of participating laboratories (in total / with quant. data points only)	3 / 3
No. of data points (in total / quantitative)	3 / 3
Assigned value	25.0 %
Proficiency std. dev.	--- %
Acceptance window	12.5 - 50.0 %



Number of laboratories with quantitative data points is less than 8. A reliable kernel density estimation cannot be guaranteed.

5 Statistical Analysis

5.1 Definitions and Interpretation

Reported Value

The participant's result.

Assigned Value

Value attributed to a particular quantity and accepted, sometimes by convention, as having an uncertainty appropriate for a given purpose. See ISO/IEC 17043 for additional information. In general, the assigned value is the value used to assess proficiency and may or may not be the made to value (gravimetric value).

Acceptance Window

The range of values that constitute acceptable performance for a laboratory participating in this PT study.

z-score

A z-score shows how a single data point compares to normal data. A z-score says not only whether a point was above or below average, but how unusual the measurement is. Generally, a method result with a z-score less than |2| is considered to be in control and 'Acceptable'; a z-score between |2| and |3| is considered 'Questionable', but still within control and 'Acceptable' and a z-score greater than |3| is considered 'Not Acceptable' and the method is out of control. For WS studies, a z-score greater than |2| is not acceptable.

Calculated as z = (Reported Value - Assigned Value) / Proficiency Std. Dev.

A z-score cannot be provided

(1) for presence/absence data,
(2) for identification data and other categorial data,
(3) where the analyte is not present in the sample,
(4) for "less than" and "greater than" values,
(5) NOEC analytes (in the framework of WETT analysis).

In cases (1) to (3) the participant's result is only evaluated by "acceptable" if it matches with the assigned value. Otherwise the result is indicated as "not acceptable". In case the analyte is not present in the sample and a PTRL is available, the participant's result is indicated as "acceptable" as long the result is less than the PTRL.

In case (4) the following evaluation rules will be applied:

- "less than" values:
  - When the analyte is not present in the sample the result is always "acceptable".
  - When the analyte is truly present in the sample, the result is only "acceptable" if the "less than" value is greater than the lower limit of the acceptance window.
- "greater than" values:
  - When the analyte is not present in the sample the result is always "not acceptable".
  - When the analyte is truly present in the sample, the result is only "acceptable" if the "greater than" value is less than the upper limit of the acceptance window.

In case (5) the result is indicated as "acceptable" if it lies within the acceptance window, otherwise the result is indicated as "not acceptable".

Proficiency Std. Dev.

Standard deviation calculated based on Evaluation Criteria.

PTRL

Proficiency Testing Reporting Limit

Study Mean

Statistical study mean calculated using a robust statistical model. Robust statistical techniques are used to minimize the influence extreme results can have on estimates of the mean and standard deviation. NOTE - These techniques assign less weight to extreme results, rather than eliminate them from a data set.

Choice of statistical technique: In case of quantitative data points from at least 8 laboratories, Algorithm A (ISO 13528, Section C.3.1), and in case of quantitative data points of 4 to 7 laboratories, the Hampel estimator (ISO 13528, Section C.5.3) is applied. A study mean cannot be calculated in case there are quantitative data points from less than 4 laboratories available.

Study Std. Dev.

Standard deviation calculated from study data using robust statistics.

In case of quantitative data points from at least 8 laboratories, Algorithm A (ISO 13528, Section C.3.1), and in case of quantitative data points of 4 to 7 laboratories, the Q method (ISO 13528, Section C.5.2) is applied. A study standard deviation cannot be calculated in case there are quantitative data points from less than 4 laboratories available.

Gravimetric Value

The 'prepared to' value, determined by gravimetric means. The uncertainty associated with this value is the standard uncertainty and based on Sigma-Aldrich RTC's gravimetric tolerances.

Analytical Value

The measured value, determined after preparation. The uncertainty associated to this value is the standard uncertainty and based on the measurement process.

5.2 Evaluation Criteria

1 - Regression Equation

Acceptance windows based on TNI adopted equation of proficiency value +/- 3 proficiency standard deviations and check limits of proficiency value +/- 2 proficiency standard deviations. Proficiency value and proficiency standard deviation are calculated from gravimetric variables a, b, c & d as proficiency value = a * gravimetric + b and proficiency standard deviation = c * gravimetric + d.

2 - Study Robust Mean and c, d regression

Acceptance windows based on TNI adopted equation of proficiency value +/- 3 proficiency standard deviations and check limits of proficiency value +/- 2 proficiency standard deviations. Proficiency value and proficiency standard deviation calculated from robust study mean and variables c & d as proficiency value = robust mean and proficiency standard deviation = c *proficiency value + d.

3 - Fixed Limits

Acceptance windows based on span of gravimetric percentage from gravimetric as gravimetric +/- gravimetric * percentage.

4 - Adjustable Fixed Limits

Acceptance windows based on a span of gravimetric percentage from gravimetric as gravimetric +/- gravimetric * lowPercentage where gravimetric < break and gravimetric +/- gravimetric * highPercentage where gravimetric >= break.

5 - Study Statistics

Acceptance windows based on a number of standard deviations span from the study mean as study mean +/- (deviations * standard deviation).

6 - Log Transform Statistics

Acceptance windows based on lognormal distributed data. Acceptance windows = mean(lognormal) +/- span * standard deviation(lognormal).

7 - Regression Equation 2SD

Acceptance windows based on EPA equation of proficiency value +/- 2 proficiency standard deviations. Proficiency value and proficiency standard deviation are calculated from gravimetric variables a, b, c & d as proficiency value = a * gravimetric + b and proficiency standard deviation = c * gravimetric + d. Generally reserved for drinking water studies.

8 - Study Median and Dilution Levels

Acceptance windows based on study median ± 1 dilution. If the median falls between two test dilutions, then the assigned value is set at the higher value, and the lower acceptance limit is the second test dilution below the median, and the upper acceptance limit is the second test dilution above the median. Generally reserved for NOEC analytes (in the framework of WETT analysis).

9 - Fixed Limits based on Analytical Value

Acceptance windows based on span of analytical value from measurements.

6 Notes on the Interpretation of the Results

z score Overview

The z-scores are presented as colored triangles. For each item, the z-scores of all analytes of the current and the previous (up to three) scheduled studies of this study type. The z-scores depend on the lot, analytical method used, and analyst (if given). A red cross is shown if no z-score is available.

For the assessment of participants by means of z-scores according to ISO/IEC 17043:2010 [2], the triangles were colored as follows:

$\|z\| \leq 2$	green
$2 < \|z\| < 3$	yellow (WS studies, WETT samples: red)
$\|z\| \geq 3$	red.

For $|z| \geq 3$, the corresponding triangles are displayed as -3 or 3. For $|z| > 2$, the value of the z score is displayed next to the triangle (yellow or red). A z-score = 0 is shown as a light blue vertical bar.

Interpretation of the z-scores' overview:

A z-score < 0, i.e. the triangle points to the left, means that the measurement result is lower than the assigned value.

A z-score > 0, i.e. the triangle points to the right, means that the measurement result is higher than the assigned value.

A z-score = 0, i.e. a light blue vertical bar is shown, means that the measurement result coincides with the assigned value.

Figures per Combination of Item, Lot and Analyte

The *diagram on the left* shows the participant results by means of blue diamonds.

The horizontal blue line indicates the assigned value.

Both the acceptance and the check limits for the participant results are calculated based on z-scores.

The acceptance limits are displayed as solid lines and correspond to z-scores of ±3. For WS studies and non-NOEC analytes (in the framework of WETT analysis), the acceptance limits correspond to a z-score ±2. For NOEC analytes (in the framework of WETT analysis), the acceptance limits correspond to ±1 dilution.

The check limits are displayed as dashed lines and correspond to z-scores of ±2. They are only calculated if a rule is given by the evaluation criterion.

In case there are at least 8 laboratories with quantitative data points are available: The *diagram on the right* is a kernel density estimation of the distribution of the participant results. The measurement values are indicated as small circles. The kernel width is determined by the ISO 13528 formula from section 10.3.2 i) a).

7 Proficiency Test Item Preparation, Homogeneity and Stability Assessment

Sigma-Aldrich RTC uses proprietary and published methods for the manufacture, homogeneity and stability testing of proficiency test items. Sigma-Aldrich RTC's proficiency test materials meet the requirements of ISO 17034. For more information contact Sigma-Aldrich RTC. Additionally, Sigma-Aldrich RTC complies with the TNI Volume 3 'General Requirements for Environmental Proficiency Test Providers', EL-V3-2016, for all TNI Fields of Proficiency Testing analytes.

8 Metrological Traceability

All preparations are made using balances calibrated annually traceable to NIST standards. Where appropriate analytical measurements are traceable through an unbroken chain to NIST standards, or a Certified Reference Material manufactured under ISO 17034 in conjunction with ISO/IEC 17025.

9 Additional Information

Go to supelco-pt.com for additional information on summary statistics for specific methods, advice on the interpretation of the statistical analysis and additional comments/recommendations. Sigma-Aldrich RTC recommends that you contact your accreditation body for specific instruction.

10 References

[1] ISO 13528: Statistical methods for use in proficiency testing by interlaboratory comparison, August 2015

[2] ISO/IEC 17025:2017: General requirements for the competence of testing and calibration laboratories

[3] ISO/IEC 17043:2010: Conformity assessment - General requirements for proficiency testing, May 2010

[4] S. Uhlig und P. Henschel (1997): Limits of tolerance and z-scores in ring tests. Fresenius' J. Anal. Chem., Vol. 358, pp. 761-766.

[5] ISO 17034:2016: General requirements for the competence of reference material producers.

This section of the report is for informational purposes only. If you are unsure about specific accreditation requirements, please contact your state coordinator.

Unacceptable Analytes

WET015-1EA Fathead Minnow, 7Day, MHSF

Analyte	Method Number	Method Name
Fathead Minnow Chronic MHSF - Growth NOEC (ON)[1,2]	10214207	EPA 1000.0 - Fathead minnow, 7-day Chronic, daily renewal, MHSF 25°C (2002)

[1] TNI Compliant, covered by Sigma-Aldrich RTC's ANAB Proficiency Testing Provider accreditation, Cert. AP-1469

[2] ISO/IEC 17043 Accredited, covered by Sigma-Aldrich RTC's ANAB Proficiency Testing Provider accreditation, Cert AP-1469

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Coastal Bioanalysts, Inc.

Corrective Action Report DMR-QA Study 40, WET Method Code 015, Fathead Minnow Chronic (EPA Method 1000.0) Growth NOEC

While Maryland NPDES permits use the IC25 for evaluation of compliance with instream toxicity standards, the NOEC must also be reported for toxicity results (Attachment 1). The DMRQA instructions state "Laboratories should only report one endpoint for each DMR-QA WET test code required" and that the IC25 endpoint is the preferred endpoint to evaluate laboratory performance (Page 5 of instructions). Because of the requirements stated in the attached MDE guidance, we reported both the NOEC and IC25 values for chronic test methods. Thus, it should be noted that the IC25 value, used for compliance, was within acceptance limits for this method and this corrective action addresses only the ancillary NOEC value.

In response to the "Not Acceptable" result for the chronic Fathead minnow growth NOEC we performed a detailed review of all relevant data. Information evaluated included QA/QC data related to performance of all chronic Fathead minnow tests conducted during the period (i.e., the test performed with Study 40 toxicant, our in-house standard reference toxicant (SRT) tests and whole effluent toxicity (WET) tests), summary performance data provided by PT provider (Sigma-Aldrich RTC) for Study 40 as well as previous studies, and results for a graded re-test sample provided by Sigma-Aldrich RTC (RTC).

Because the absolute toxicity of any material is unknown, the "true" toxicity value of toxicants used in WET PT studies is typically based on the reported values of participating laboratories. Due to this basis for the "true" value and acceptance limits, an endpoint reported by an individual lab may fall out of limits for two reasons:

1. A **technical error** caused by incorrect methods, procedures or calculations related to a specific test or study, or a system-wide problem resulting in a low or high bias such as poor animal health or inadequate test dilution water.
2. A **statistical issue** inherent in any blind proficiency study that bases acceptance limits on the 95% probability limits of the mean for point estimates such as LC50 and IC25, or set limits based on hypothesis test endpoints such as the NOEC. It should be noted that in the latter case the hypothesis test is also based on 95% probability values. Thus, Type I statistical errors, wherein the true value is excluded, should occur 5% of the time for both hypothesis test endpoints (NOEC) and point estimates (LC50, IC25).

Evaluation for Technical Errors

1. No technical errors were identified in the review of test bench sheets related to the test (attachment 2).

VELAP# 460030
EPA# VA01116

2. No trends (i.e., bias) or other anomalies were identified in reference toxicant or WET test QC data. Standard reference toxicant (SRT) growth IC25 values were consistent over the 20-month period which included the previous PT study (which was within limits), this PT study, and the successful PT retest conducted this month (Figure 1). Note: As explained below, NOEC values cannot be evaluated by precision statistics. For this reason, growth IC25 values are presented to evaluate variability in growth as a toxic response.

3. Our intra-laboratory coefficient of variation (CV) for the growth IC25 (0.04-0.05) is much lower than that of most other labs nationally (Figure 2), as well as the (inter-laboratory) method CV for past studies by this PT provider (0.44; Table 1). These trends in IC25 and CV values indicate that neither normal intra-laboratory variability nor systemic bias was a cause of PT test failure.

4. For our laboratory the overall DMRQA acceptance rate for the growth NOEC endpoint for this method (this study included) is 96.6%, approximately equal to the theoretical acceptance rate based on 95% probability.

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Figure 1. IC25 Control chart (May 2019-December 2020)

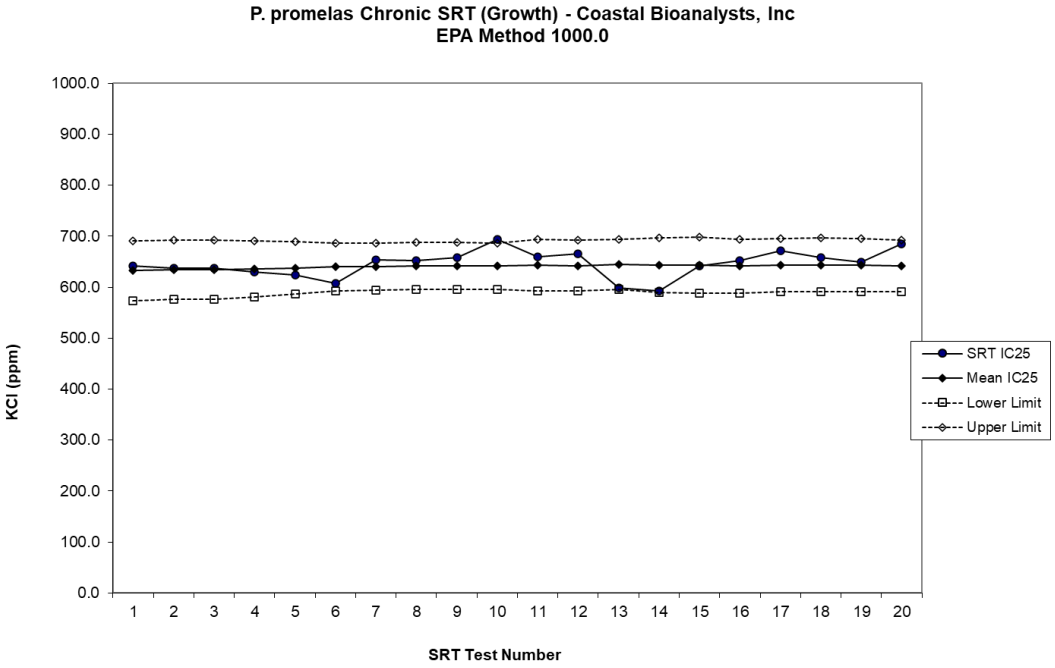

Figure 2. CBI SRT Coefficient of variation for IC25 (May 2019-December 2020)

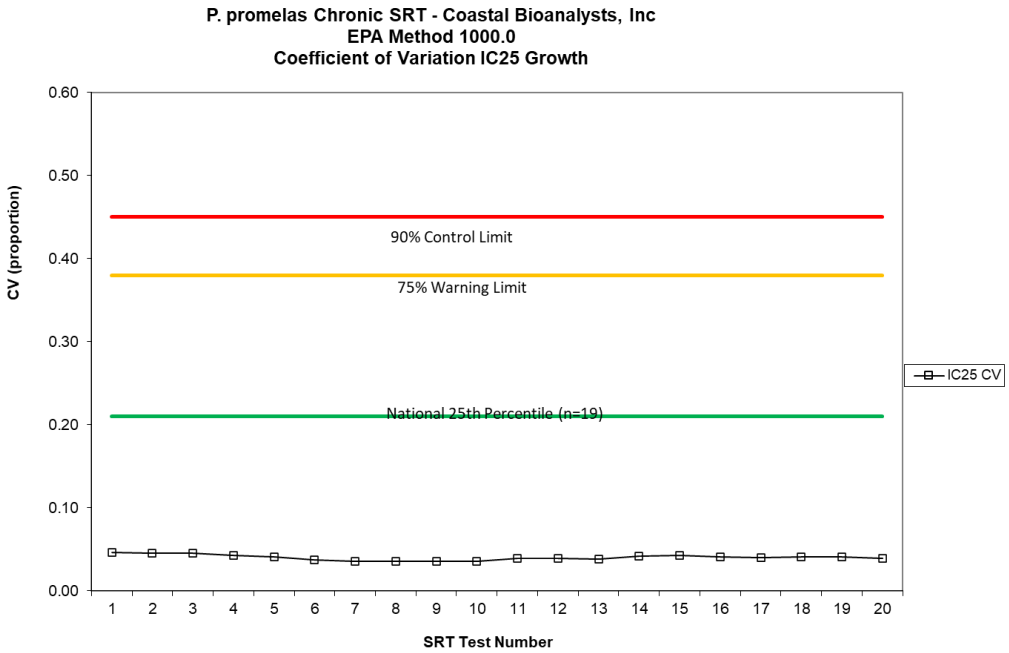

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Table. 1.

Method Coefficients of Variation for RTC PT Studies 2005-2019*

Species	Acute		Chronic	
	LC50	n	IC25	n
Ceriodaphnia dubia	0.49	13	0.53	13
Cyprinodon variegatus (Sheepshead minnow)	0.29	9	0.51	7
Daphnia magna	0.53	11	na	--
Mysidopsis bahia (Mysid shrimp)	0.35	11	0.37	9
Pimephales promelas (Fathead minnow)	0.32	14	0.44	12

*Studies for which study mean and standard deviation available and published in data reports. All freshwater methods are for moderately hard synthetic fresh water (as used by CBI).

Evaluation for Statistical Issues

1. Test Sensitivity. The NOEC (No Observable Effect Concentration) is defined as the highest test concentration at which there is no statistically significant reduction in growth or survival compared to the control group. An important consideration is test sensitivity. Tests with low variability among replicates can more readily detect a significant reduction in growth than tests with high variability. This sensitivity is expressed as the Percent Minimum Significant Difference (PMSD). For example, a test with a PMSD of 12% can detect a 12% reduction in growth compared to controls as statistically significant whereas a test with a PMSD of 30% can only detect a 30% or greater reduction in growth compared to controls as statistically significant. Fathead minnow tests with growth PMSD values greater than 30% are not considered to be adequately sensitive by EPA. This value is based on the 90th percentile in the WET Variability Study conducted by EPA (EPA, 2001); the 50th percentile value was 16%.

   Our value of 14% was relatively sensitive compared to that of the average lab participating in the study. Thus, our tests were more likely to “see” a reduction in growth as statistically significant. However, our test was not unusually sensitive based on the EPA 10th percentile PMSD value of 12% and historical data for our laboratory (Figure 3).

2. Acceptance limits for the NOEC endpoint are typically based on the median value +/- one test dilution (EPA 2002). EPA offers no justification for this convention and there is no statistical basis. As stated by EPA (EPA 2002, see 4.14.6), precision statistics cannot be applied to the NOEC and the true NOEC

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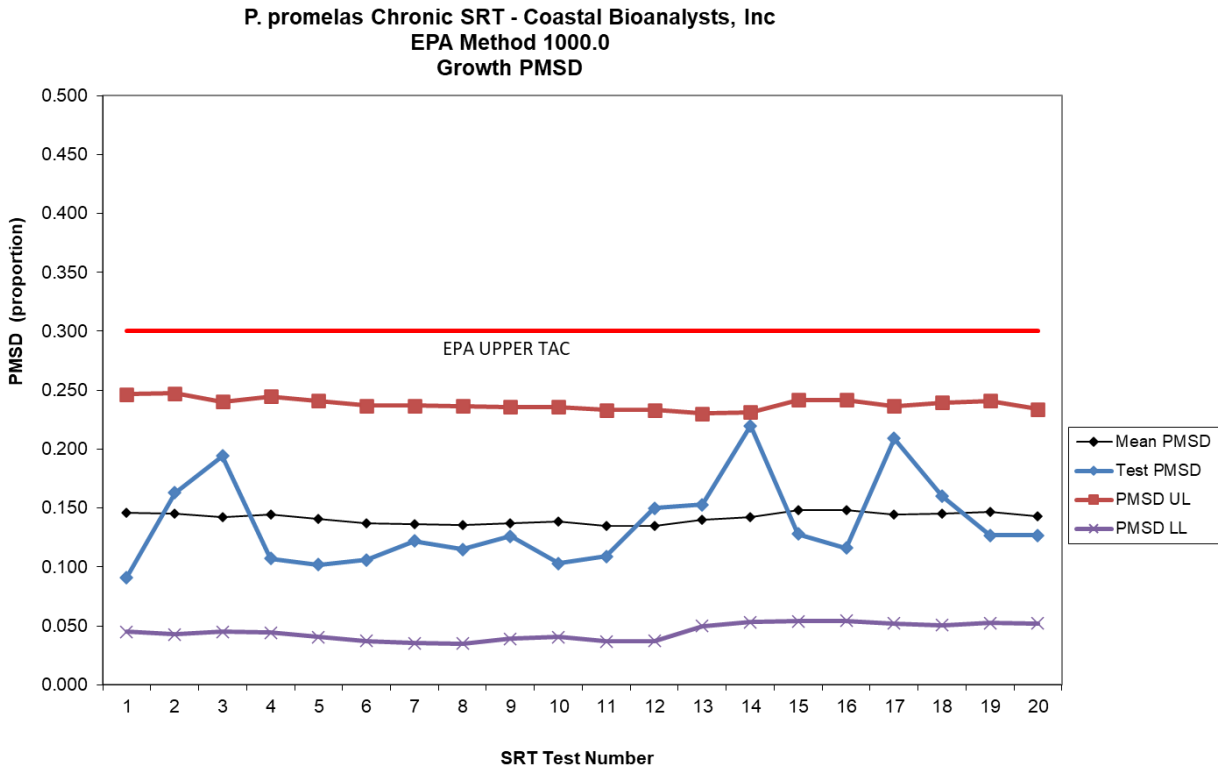
may lie anywhere within the range between the NOEC and lowest effect concentration (LOEC).

For PT studies a similar method for evaluation of NOECs is used but with guidance for situations where the “true” NOEC value appears split among participating labs (Attachment 3). It should be noted however that the guidance is flawed because, by definition, the median NOEC value (unlike the mean) cannot possibly lie between two concentrations if all labs are testing the same concentrations as required in these studies. As can be seen in the study data (Attachment 4), 9 of the 17 labs reported a NOEC of 25% while 7 reported a value of 12.5%. With such a small sample size one could argue that Evaluation Criterion 8 should identify an approximate median between 12.5% and 25%; the ratios of 9/17 and 7/17 are not statistically different. The lower acceptance limit under these conditions would be 6.25%, which would include our test results.

Figure 3. Control chart of growth PMSD.



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Retest of New PT Sample

A verification/re-test study was conducted on a graded "quick-turn" PT sample provided by RTC. Coincident with the re-test, a test was also performed with our SRT. Both the graded re-test (Attachment 5) and the SRT test were within acceptance limits.

Conclusions

There is always a 5% probability that the true value will be excluded from the study acceptance limits (a "Type I" statistical error). This is because statistical methods are used for setting limits (LC50, IC25) or evaluating the response in individual test concentrations (NOEC). For this reason, EPA recognizes that SRT test results will fall out of laboratory control chart limits 5% of the time "regardless of how well a laboratory performs" (EPA 2002). The same reasoning applies to PT studies. Because it is a random event occurring at a 5% frequency, it is theoretically impossible to conclude conclusively whether a specific data point is rejected due to a Type I error. Thus, a Type I error for a single data point can only be circumstantially implicated by demonstrating lack of technical errors and an overall acceptance rate of about 95% in DMRQA or similar QC programs. Our acceptance rate is well within these theoretical and expected limits.

In conclusion, an ambiguous estimate of the true growth NOEC value for the study, and possible Type 1 statistical error, are likely explanations for the out of limit growth NOEC value. Laboratory variability data, overall successful PT completion rate (96.6%) for the method endpoint, and successful testing of a new PT sample, indicate no deficiencies in the analysis of growth for EPA method 1000.0. Consequently, the only corrective action applicable is continued monitoring for potential Type 1 statistical errors.

Literature Cited:

EPA 2002. *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, 4th ed.* October 2002. EPA-821-R-02-013.

EPA 2001. *Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Vol. 2: Appendix*. September 2001 EPA 821-B-01-005

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Attachment 1

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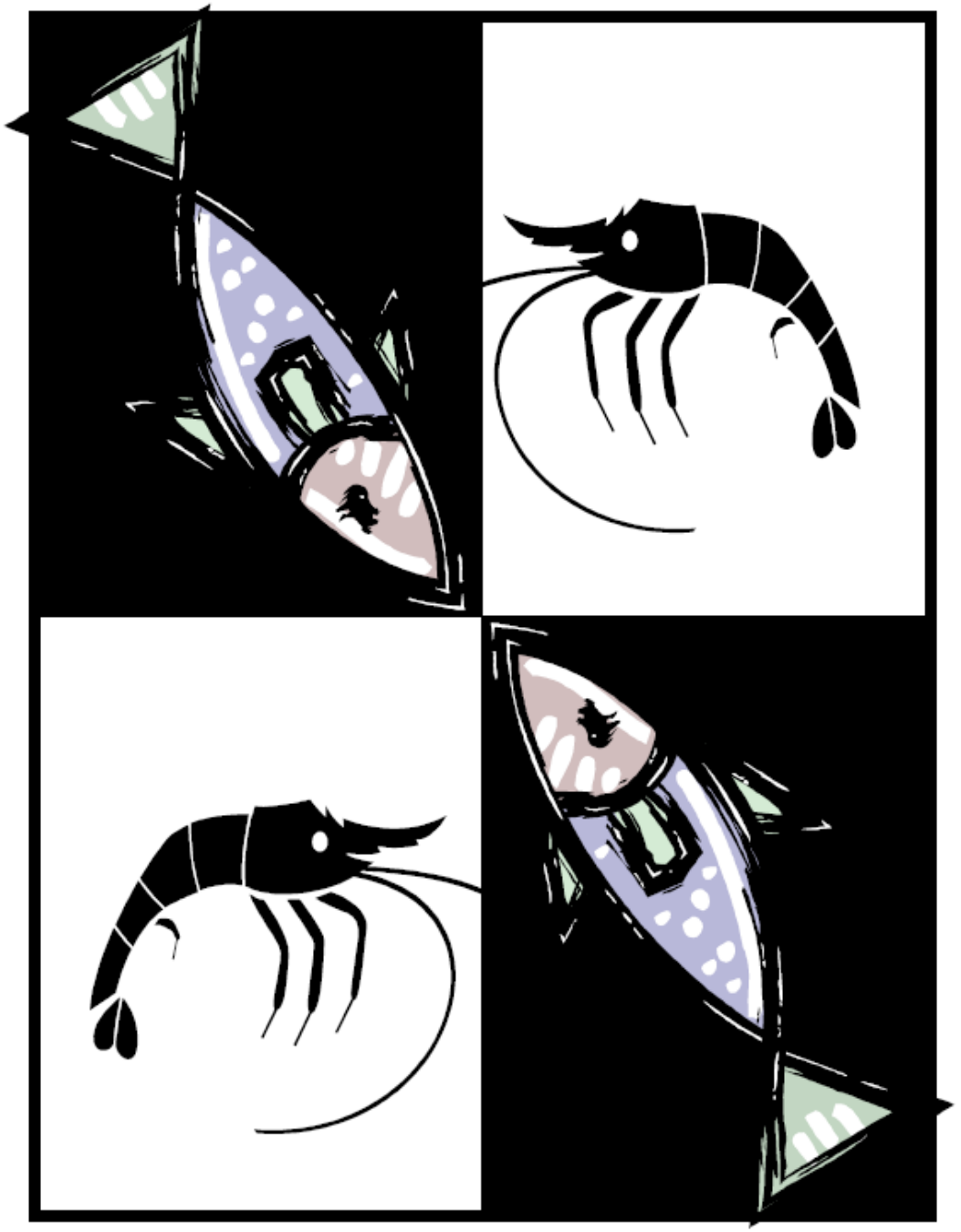
MARYLAND DEPARTMENT OF THE ENVIRONMENT
1800 Washington Boulevard • Baltimore MD 21230
410-537-3000 • 1-800-633-6101 • http://www.mde.state.md.us

WASTEWATER PERMITS PROGRAM

EFFLUENT BIOTOXICITY TESTING PROTOCOL FOR INDUSTRIAL AND MUNICIPAL EFFLUENTS



Revisions: 1/23/2019, 5/14/2018, 12/14/2012

The Department's biomonitoring program continues to evolve. As such, this document will be periodically updated to reflect changes in toxicity testing methodologies, toxicity reduction evaluation protocols, and other issues related to the control of toxic discharges.

I. PROGRAM DESCRIPTION

Since 1980, the Maryland Department of the Environment (MDE) has utilized whole effluent toxicity (WET) testing to assess acute and chronic toxicity in discharges to Maryland surface waters. In 1987 the emphasis greatly increased with the addition of the State Biomonitoring Laboratory. The current effort relies on toxicity testing of effluents performed by the permittee. In addition to these routine toxicity testing efforts, MDE may request dischargers to perform toxicity testing outside of the permit process. All tests consist of separate experiments using both a vertebrate (fish) and an invertebrate (crustacean) as the test species.

A finding of no toxicity in the effluent of a facility does not relieve the permittee from the obligation to provide best available treatment technology or to comply with water quality standards. In all cases, MDE reserves the authority to require additional biotoxicity testing and a toxicity reduction evaluation (TRE). This authority to require biomonitoring appears in COMAR 26.08.03.07 entitled "Control of the Discharge of Toxic Substances to Surfaces Waters". Specific provisions are found in sections A and D.

A. Permit Required Toxicity Testing

Biotoxicity testing is required in new or renewed discharge permits for all major and selected non-major dischargers. Maryland regulation (COMAR 26.08.03.07D(1)) specifically requires the following:

D. Applicability to Dischargers

(1) Dischargers Required to Conduct Monitoring for Toxic Substances. The Department shall require any permittee who has a discharge that falls into one of the following categories to perform biological or chemical monitoring for toxic substances:

(a) A POTW with a pretreatment program established in accordance with COMAR 26.08.08;

(b) An industrial discharger or POTW treatment plant with a wastewater flow greater than or equal to 1,000,000 gallons per day;

(c) A discharger whose discharge has demonstrated actual or potential toxicity; or

(d) A discharger whose discharge the Department has reason to believe may cause toxicity as determined by an evaluation of manufacturing processes, indirect discharges, treatment processes, effluent or receiving water data, or other relevant information.

Maryland regulation (COMAR 26.08.03.07D(2)) specifically requires the following:

(2) NPDES Permit Monitoring Requirements.

(a) A discharger identified in §D(1) of this regulation shall have requirements for toxic substance monitoring included in its permit at the time of permit issuance or reissuance.

(b) Modifications to these requirements may be allowed on a case-by-case basis if the:

(i) Specific conditions of the discharge suggest that a full scale toxics monitoring program is not necessary; or

(ii) Characteristics of the receiving water indicate that a full scale toxics monitoring program is not needed.

(c) Data submitted under any previous toxic substance monitoring program may be used to satisfy these requirements if the data is indicative of the current process and treatment conditions.

(d) Any toxic substance monitoring, including test protocols, shall be approved by the Department before initiation of the testing. All data generated shall be within the quality assurance and quality control specifications of the test protocol.

(e) Measurements below the minimum level may be reported as BML (below minimum level).

(f) If the Department determines through the monitoring described in §D(1) of this regulation, that a discharge causes or has the potential to cause the discharge of toxic substances or an impact on surface waters, the Department may modify the discharge permit to require the discharger to collect data to verify or rule out the existence of an impact from a toxic substance.

NPDES biotoxicity testing requirements for major facilities generally consist of four quarterly tests to be conducted during the first year of the permit for industrial facilities. As required by 40 CFR 122.21(j)(5)(iv), municipal facilities must submit (A) results of a minimum of four quarterly tests for a year, from the year preceding the permit application; or (B) results from four tests performed at least annually in the four and one half year period prior to the application (Appendices A & B). The Department has chosen option B as the standard permit requirement. Where the discharge flow is less than 10% of the receiving water flow, the permit requirements usually consist of three acute tests and one chronic test. Where the effluent flow is greater than 10% of the receiving water flow, chronic testing is emphasized. In estuarine waters where the discharge flow exceeds 10% of the receiving water flow, the permittee is required to use estuarine test organisms.

NPDES permit requirements for dischargers of lower concern where there is reason to believe a potential for toxicity exists generally consist of two quarterly acute tests to be conducted during the first year of the permit (Appendices C & D). Chronic, instead of acute, tests may be required in sensitive discharge situations such as discharges to intermittent streams.

Additional effluent toxicity testing beyond that specifically described in the permit may be

required by MDE of dischargers upon findings of toxicity or upon the performance of testing inconsistent with the permittee's approved biomonitoring study plan for that facility. A permittee will be required to repeat the permit required toxicity testing when initial findings of acute toxicity are not confirmed (COMAR 26.08.03.07E(4)f). The reporting of permittee test results must be consistent with MDE's document entitled "Reporting Requirements for Effluent Biomonitoring Data" (Appendix E). A toxicity reduction evaluation (TRE) is required when a review of the data indicates unacceptable toxicity.

The test organisms utilized in permittee toxicity testing are those recognized in federal guidance or local species approved by the Department (Appendix F).

II. INTERPRETATION OF BIOTOXICITY MONITORING RESULTS

Acute toxicity is broadly defined as the ability of a substance to cause deleterious effects to living organisms during a short-term exposure. In practice, acute toxicity testing of effluents involves the measurement of lethality or immobilization of aquatic organisms exposed to several effluent dilutions for time periods usually lasting up to 48 hours. The results of an acute toxicity test are expressed as an LC_{50} (effluent concentration at which 50% of the test organisms die during the test) or EC_{50} (effluent concentration at which 50% of the organisms are killed or disabled during the test). In order to calculate an LC_{50} (or EC_{50}), at least one of the test concentrations must cause more than 50% mortality (or immobilization) and at least one of the test concentrations must cause less than 50% mortality (or immobilization). The lower the LC_{50} or EC_{50}, the more toxic the effluent. For example, an LC_{50} (or EC_{50}) of greater than 100% means that full strength effluent (100%) did not kill (or immobilize) at least half the test organisms. An LC_{50} (or EC_{50}) of 50% means that half strength effluent (50%) killed (or immobilized) 50% of the test organisms.

Chronic toxicity testing is broadly defined as the ability of a substance to cause deleterious effects to living organisms during a long-term exposure. In practice, chronic toxicity testing of effluents usually involves the measurement of survival, growth, reproduction, and hatchability of aquatic organisms exposed to several effluent dilutions for time periods lasting up to 7 days. Generally, the "sub-lethal" endpoints of growth, reproduction, and hatchability are more sensitive indicators of chronic toxicity than survival. Because chronic toxicity tests involve the measurement of more sensitive endpoints over longer exposure periods compared to acute tests, chronic tests are considered to be more sensitive for measuring effluent toxicity.

The results of chronic toxicity testing are generally expressed as the NOEC (highest concentration at which no observable effect occurred), LOEC (the lowest concentration at which an observable effect occurred), Chronic Value (the geometric mean of the NOEC and LOEC) and the IC_{25} (effluent concentration which causes a 25% reduction in growth or reproduction and survival). In addition to these measures of chronic toxicity, acute toxicity data, in the form of LC_{50}s or EC_{50}s, can be gathered during the first 48 hours of chronic toxicity testing.

A. Acute Toxicity of Effluents

For purposes of determining the acute toxicity of effluents, the following criteria apply.

1. An effluent is considered to be acutely toxic when its 48-hour LC_{50} or EC_{50} (as determined from acute or chronic toxicity testing) is 100% or less.
2. An effluent is generally considered not acutely toxic when its 48-hour LC_{50} or EC_{50} (as determined from acute or chronic toxicity testing) is greater than 100%.

Upon consistent findings of acute toxicity, a permittee shall be required to conduct a TRE (see section III).

B. Chronic Toxicity of Effluents

For purposes of determining the chronic toxicity of effluents, the following criteria apply.

1. An effluent is considered to be chronically toxic when its IC_{25} is less than or equal to the in-stream waste concentration.[1]
2. An effluent is generally considered not chronically toxic when its IC_{25} is greater than the in-stream waste concentration.

Upon consistent findings of chronic toxicity, a permittee shall be required to perform a TRE (see Section III).

III. Toxicity Reduction Evaluation (TRE)

When effluent toxicity is confirmed, the discharger is required to perform a TRE. A TRE is an investigation conducted to identify the cause(s) of effluent toxicity or isolate the source(s) and determine the effectiveness of control options, implement the necessary control measures and then confirm the reduction in toxicity (see appendix H). TREs range widely in complexity. They may be as simple as the dechlorination of municipally supplied noncontact cooling water in response to measurements of toxic levels of chlorine. Alternatively, they may involve the performance of an in-depth investigation to determine the source or type of toxicity, evaluate control measures, and implement those selected. Guidance documents covering the various tiers, phases, and other aspects of a TRE are under continuous development by the EPA and its contractors (see Section V).

IV. Permit Limitations and Compliance Schedule

MDE will include a specific limitation for effluent toxicity and a compliance schedule for the elimination of the effluent toxicity in the facility's discharge permit as indicated below:

[1] $IWC = Q_D/(Q_D+Q_{RW})x100$ where $Q_{RW} = 30Q5$

Situations in which a Permit Limitation and Compliance Schedule will be included.

When issuing a NPDES permit renewal, MDE will include a permit limitation for effluent toxicity when toxicity testing demonstrates a reasonable potential for the discharge to cause or contribute to a violation of water quality standards. Reasonable potential is determined by the Department as at least one test from all current test results showing toxicity as defined in Sections II. A & B, unless there are a sufficient number of tests over time that provide for a statistical basis for concluding no reasonable potential. A compliance schedule could be considered, if necessary, which would outline the activities needed to eliminate the toxicity. During the compliance schedule period, the permittee is required to conduct a TRE as specified in Section III above, unless the basis of the schedule is to implement significant treatment system upgrades or major process modifications which are expected to address the toxicity. If the results of the TRE identify the chemical specific parameter(s) causing the toxicity, the whole effluent toxicity permit limit could be replaced with the chemical specific effluent limitation(s).

As described in the "Determination of discharge permit WET limitations" section below, the determination of discharge permit limitations may incorporate dilution resulting from mixing zone allowances in accordance with COMAR 26.08.02.05. However, when determining whether a TRE must take place to eliminate the toxicity under COMAR 26.08.03.07.E(4)(e), dilution is not considered when determining if an effluent is acutely toxic.

As indicted in I.A above, 40 CFR 122.21(j)(5)(iv) requires that municipal facilities must submit (A) results of a minimum of four quarterly tests for a year, from the year preceding the permit application; or (B) results from four tests performed at least annually in the four and one half year period prior to the application. Permit limitations expressed in toxic units and a compliance schedule will be included in a municipal facility's discharge permit when any of the four 40 CFR 122.21(j)(5)(iv) required tests show toxicity. A component of the compliance schedule will require eighteen months of quarterly whole effluent toxicity testing resulting in six individual test that are the same type of test that determined the original toxicity. If none of the six tests show toxicity, the permittee may request a permit modification to remove the permit limit and the compliance schedule. If any of the six tests are toxic, the permit limit will go into effect and a TRE will begin to discover the source of the toxicity and explore solutions to remove that toxicity.

To address federal NPDES requirements for WET testing and limits, MDE shall implement permit limits in a new or renewal permit when a WET test result shows reasonable potential for toxicity unless it can be demonstrated that the source of toxicity has been eliminated, inappropriate test procedures were utilized, or the source has been controlled via a chemical specific permit limitation. Where reasonable potential has been assumed based on one test result, the permit shall include a WET limit effective within the term of the permit unless the effluent shows no toxicity in six follow-up quarterly tests.

Determination of discharge permit WET limitations

The determination of discharge permit limits may incorporate dilution resulting from mixing zone allowances in accordance with COMAR 26.08.02.05.

Acute Conditions

To protect aquatic life against acute effects, the ambient toxicity should be less than 1.0 acute toxic unit (TU_a) where a TU_a is defined as 100 divided by the LC_{50} value resulting from the first 48 hours of a valid acute or chronic toxicity test.

Chronic Conditions

To protect aquatic life against chronic effects, the effluent's IC_{25} shall be greater than the in-stream waste concentration (IWC).

Using the formula for IWC shown in footnote 1 and the requirement that the effluent's IC_{25} shall be greater than the in-stream waste concentration (IWC) the below relationship for allowable effluent toxicity can be expressed in chronic toxic units (TU_c) where a TU_c is defined as 100 divided by the IC_{25} value resulting from a valid chronic toxicity test.

For effluent not to be chronically toxic

$IC_{25} > IWC$

$100/TU_c > IWC$

$100/[(TU_c)(IWC)] > 1$

$100/[\{TU_c\}\{Q_D/(Q_D + Q_{RW})\}\{100\}] > 1$

Therefore Allowable Effluent Chronic Toxicity = $TUc < (Q_D + Q_{RW})/Q_D$

Example WET Limit Calculations - for discharge situations when 1/3 of the receiving stream flow is the limiting factor for determination of the mixing zone for the effluent.

Acute WET Limit

Using the below mass balance equation

$C_R = [(C_D)(Q_D) + (C_{RW})(Q_{RW})]/[Q_D + Q_{RW}]$

Where:

Facility Flow = Q_D = 6.8 MGD = 10.52 cfs
Facility Toxicity = C_D
Upstream Receiving Stream 7Q10 flow = Q_{RW} = 45.81 cfs
Allowable Acute Mixing Zone flow = (1/3)(Q_{RW}) = (1/3)(45.81 cfs) = 15.27 cfs
Assumed Upstream Receiving Stream Toxicity = C_{RW} = 0 TU_a
Downstream Receiving Stream Toxicity = C_R
Allowable C_R < 1.0 TU_a

Assuming Allowable In-Stream Toxicity = 0.9999 TU_a

$0.9999\ TU_a = [(C_D)(Q_D) + (C_{RW})(Q_{RW})]/[Q_D + Q_{RW}]$

$0.9999\ TU_a = [(C_D)(10.52\ cfs) + (0\ TU_a)(15.27\ cfs)]/[10.52\ cfs + 15.27\ cfs]$

Permit Acute WET Limit = C_D < 2.45 TU_a

Chronic WET Limit

Using the below equation for allowable effluent chronic toxicity

Allowable Effluent Chronic Toxicity = TUc < $(Q_D + Q_{RW})/Q_D$

Where:

Facility Flow = Q_D = 6.8 MGD = 10.52 cfs
Facility Toxicity = C_D

Upstream Receiving Stream 30Q5 flow = Q_{RW} = 23.75 cfs
Assumed Upstream Receiving Stream Toxicity = C_{RW} = 0 TU_c

Permit Chronic WET Limit = C_D = TUc < $(Q_D + Q_{RW})/Q_D$

Permit Chronic WET Limit = C_D = TUc < (10.52 cfs + 23.75 cfs)/10.52 cfs

Permit Chronic WET Limit = C_D < 3.26 TUc

V. Relevant Guidance Documents

- Maryland Department of the Environment, Water Management Administration. "Reporting Requirements for Effluent Biomonitoring Data." 3/21/03

- Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, EPA-821-R-02-012, October 2002

- Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms Fourth Edition, EPA-821-R-02-013, October, 2002

- Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, EPA-821-R-02-014, October 2002

- Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations (TREs). EPA/600/2-88/070. USEPA, March 1989

- Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants. EPA/833B-99/002. USEPA, Office of Wastewater Management, Washington DC

- Methods for Aquatic Toxicity Identification Evaluations - Phase I Toxicity Characterization Procedures. EPA-600/6-91/003. USEPA, Second Edition, February 1991

- Methods for Aquatic Toxicity Identification Evaluations - Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA/600/R-92/080. USEPA, September 1993

- Methods for Aquatic Toxicity Identification Evaluations - Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA-600/R-92/081. USEPA, September 1993

- Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I. EPA/600/6-91/005. USEPA, June 1991

Appendix A

BIOMONITORING PROGRAM (Significant concern and effluent flow is greater than 10% of the receiving water low flow)

1. Within three months of the effective date of the permit, the permittee shall submit to the Department for approval a study plan to evaluate wastewater toxicity at Outfall by using biomonitoring. The study plan should include a discussion of:
   a. wastewater and production variability
   b. sampling & sample handling
   c. source & age of test organisms
   d. source of dilution water
   e. testing procedures/experimental design
   f. data analysis
   g. quality assurance/quality control
   h. report preparation
   i. testing schedule

2. For industrial facilities:
   The testing program shall consist of definitive quarterly chronic testing for one year. This testing shall be initiated no later than three months following the Department's acceptance of the study plan.

   For municipal facilities:
   The testing program shall consist of four quarters of definitive annual chronic testing. The testing events shall be conducted annually during January or February of each of the first four years after approval of the study plan. This testing shall be initiated no later than the January or February following the Department's acceptance of the study plan. If results from any of the required annual tests show toxicity in the effluent, the permittee shall repeat the required chronic test within 30 days as a follow-up test. If toxicity is observed from the results of the follow-up test, the permittee shall be subject to the requirements specified in Special Condition
   II. D.10.

   For Freshwater Receiving Stream

   Each annual testing event shall include the Ceriodaphnia survival and reproduction test and the fathead minnow larval survival and growth test.

   For Estuarine Receiving Stream

   Testing shall include the sheepshead minnow (*Cyprinodon* variegatus) or inland silverside (*Menidia beryllina*) larval survival and growth tests and mysid shrimp (*Americamysis bahia* AKA *Mysidopsis bahia*) survival, growth, and fecundity tests. Testing must include one vertebrate species and one invertebrate species. Test results shall be expressed as NOEC, LOEC, ChV, and IC_{25}.

3. The samples used for biomonitoring shall be collected at the same time and location as the samples analyzed for the effluent limitations and monitoring requirements for this outfall. For chlorinated effluents, samples shall be collected after dechlorination. The permittee shall collect 24-hour flow–proportioned composite samples unless the Department has given prior approval of an alternative sampling type.

4. The following EPA document discusses the appropriate methods:

   For Freshwater Receiving Stream

   Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms Fourth Edition, EPA-821-R-02-013, October, 2002

   For Estuarine Receiving Stream

   Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, EPA-821-R-02-014, October 2002

5. Test results shall be submitted to the Department within one month of completion of each set of tests.

6. Test results shall be reported in accordance with MDE/WMA "Reporting Requirements for Effluent Biomonitoring Data," revised 11/2/2018.

7. As a minimum, the reported chronic results shall be expressed as NOEC, LOEC, ChV, and IC_{25}.

8. The 48-hour LC_{50} shall be calculated and reported along with the chronic results

9. If testing is not performed in accordance with MDE-approved study plan, additional testing may be required by the Department.

10. If the test results of any two consecutive valid toxicity tests show acute or chronic toxicity (LC_{50} equal to or less than 100% for acute tests and an IC_{25} equal to or less than the in-stream waste concentration for chronic tests), the permittee shall repeat the test within 30 days to confirm the findings of acute or chronic toxicity. Intermittent toxicity or other concerns may require additional testing or limits. If acute and/or chronic toxicity is confirmed, the permittee shall:

    a. Eliminate the source of toxicity through operational changes as soon as possible but in any case not longer than within three months, or

    b. Perform a TRE. If the permittee repeats the toxicity testing as stated above and the results of the repeat test do not confirm the acute or chronic toxicity, the

Department will require the permittee to repeat the toxicity testing as stated above to reconfirm a finding of no acute or chronic toxicity. After reconfirmation, the permittee shall complete any remaining quarterly testing required.

11. If the permittee completes a TRE in accordance with II.E.10.b and unacceptable toxicity is confirmed, a Whole Effluent Toxicity (WET) permit limit and a compliance schedule will be required.

12. To address federal NPDES requirements for WET testing and limits, MDE shall implement permit limits in a new or renewal permit when a WET test result shows reasonable potential for toxicity unless it can be demonstrated that the source of toxicity has been eliminated, inappropriate test procedures were utilized, or the source has been controlled via a chemical specific permit limitation. Where reasonable potential has been assumed based on one test result, the permit shall include a WET limit effective within three years unless the effluent shows no toxicity in six follow-up quarterly tests. The permit may be modified to remove the WET limit if the six follow-up quarterly tests show no toxicity.

13. If plant processes or operations change so that there is a significant change in the nature of the wastewater, the Department may require the permittee to conduct a new set of tests.

*14. If a significant industrial user locates within the service area so that significant change in the nature of the wastewater might be anticipated, MDE may require the permittee to conduct a new set of tests.

15. The biomonitoring program study plan, WET test results and related materials shall be submitted electronically to the Department if the permittee has already been approved for the NetDMR tool. The material shall be attached as separate single files and labeled as "Biomonitoring Program Study Plan" and "WET Test Results" in the NetDMR tool. Otherwise, the permittee shall submit all pertinent physical documents to:

Attention: Whole Effluent Toxicity Coordinator
Compliance Program
Water and Science Administration
Maryland Department of the Environment
Montgomery Park Business Center
1800 Washington Boulevard, Suite 420
Baltimore, MD 21230-1708

The permittee shall notify the Department at the above address or via email at mde.biomonitoring@maryland.gov immediately upon electronic submission of the biomonitoring program study plan, WET test results and associated material through NetDMR tool.

*omit for industrial facilities

Appendix B

BIOMONITORING PROGRAM (Significant concern and effluent flow is less than 10% of the receiving water low flow)

1. Within three months of the effective date of the permit, the permittee shall submit to the Department for approval a study plan to evaluate wastewater toxicity at Outfall by using biomonitoring. The study plan should include a discussion of:

   a. wastewater and production variability
   b. sampling & sample handling
   c. source & age of test organisms
   d. source of dilution water
   e. testing procedures/experimental design
   f. data analysis
   g. quality control/quality assurance
   h. report preparation
   i. testing schedule

2. For industrial facilities:
   The testing program shall consist of definitive quarterly testing for one year. Three of the quarters shall have acute testing and one of the quarters shall have chronic testing. This testing shall be initiated no later than three months following the Department's acceptance of the study plan.

   For municipal facilities:
   The testing program shall consist of definitive testing for four annual testing events. Three of the events shall have acute testing and one of the events shall have chronic testing. The testing events shall be conducted annually during January or February of each of the first four years after approval of the study plan. One of these first two testing events shall include the chronic tests. This testing shall be initiated no later than January or February following the Department's acceptance of the study plan. If results from any of the required annual tests show toxicity in the effluent, the permittee shall repeat the required test within 30 days as a follow-up test. If toxicity is observed from the results of the follow-up test, the permittee shall be subject to the requirements specified in Special Condition II (D)10.

   For Freshwater Receiving Stream

   a. The acute testing shall consist of 48-hour static renewal tests using fathead minnow and the 48-hour static renewal tests using a daphnid.

   b. The chronic testing shall include the Ceriodaphnia survival and reproduction test and the fathead minnow larval survival and growth test.

   c. Acute test results shall be expressed as LC_{50}. Chronic test results shall be

expressed as NOEC, LOEC, ChV, and IC_{25}.

For Estuarine Receiving Stream

a. The acute testing shall consist of 48-hour static renewal tests using either sheepshead minnows (*Cyprinodon* variegatus), silversides (*Menidia beryllina* , *Menidia menidia* , *Menidia peninsulae*) and mysid shrimp (*Americamysis bahia* A.K.A. *Mysidopsis bahia*). Testing must include one vertebrate species and one invertebrate species

b. The chronic testing shall include the sheepshead minnow (*Cyprinodon* variegatus) or inland silverside (*Menidia beryllina*) larval survival and growth tests and mysid shrimp (*Americamysis bahia* AKA *Mysidopsis bahia*) survival, growth, and fecundity tests. Testing must include one vertebrate species and one invertebrate species

c. Acute test results shall be expressed as LC_{50}. Chronic test results shall be expressed as NOEC, LOEC, ChV, and IC_{25}.

3. The samples used for biomonitoring shall be collected at the same time and location as the samples analyzed for the effluent limitations and monitoring requirements for this outfall. For chlorinated effluents, samples shall be collected after dechlorination. The permittee shall collect 24-hour flow–proportioned composite samples unless the Department has given prior approval of an alternative sampling type.

4. The following EPA documents discuss the appropriate methods:

For Freshwater Receiving Stream

a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, EPA-821-R-02-012, October 2002

b. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms Fourth Edition, EPA-821-R-02-013, October, 2002

For Estuarine Receiving Stream

a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, EPA-821-R-02-012, October 2002

b. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, EPA-821-R-

02-014, October 2002

5. Test results shall be submitted to the Department within one month of completion of each set of tests.

6. Test results shall be reported in accordance with MDE/WMA "Reporting Requirements for Effluent Biomonitoring Data," revised 11/2/2018.

7. As a minimum, the reported chronic results shall be expressed as NOEC, LOEC, ChV, and IC_{25}.

8. The 48-hour LC_{50} shall be calculated and reported along with the chronic results.

9. If testing is not performed in accordance with MDE-approved study plan, additional testing may be required by the Department.

10. If the test results of any two consecutive valid toxicity tests show acute or chronic toxicity (LC_{50} equal to or less than 100% for acute tests and an IC_{25} equal to or less than the in-stream waste concentration for chronic tests), the permittee shall repeat the test within 30 days to confirm the findings of acute or chronic toxicity. Intermittent toxicity or other concerns may require additional testing or limits. If acute and/or chronic toxicity is confirmed, the permittee shall:

    a. Eliminate the source of toxicity through operational changes as soon as possible but in any case not longer than within three months, or

    b. Perform a TRE. If the permittee repeats the toxicity testing as stated above and the results of the repeat test do not confirm the acute or chronic toxicity, the Department will require the permittee to repeat the toxicity testing as stated above to reconfirm a finding of no acute or chronic toxicity. After reconfirmation, the permittee shall complete any remaining quarterly testing required.

11. If the permittee completes a TRE in accordance with II.E.10.b and unacceptable toxicity is confirmed, a Whole Effluent Toxicity (WET) permit limit and a compliance schedule will be required.

12. To address federal NPDES requirements for WET testing and limits, MDE shall implement permit limits in a new or renewal permit when a WET test result shows reasonable potential for toxicity unless it can be demonstrated that the source of toxicity has been eliminated, inappropriate test procedures were utilized, or the source has been controlled via a chemical specific permit limitation. Where reasonable potential has been assumed based on one test result, the permit shall include a WET limit effective within three years unless the effluent shows no toxicity in six follow-up quarterly tests. The permit may be modified to remove the WET limit if the six follow-up quarterly tests show no toxicity.

13. If plant processes or operations change so that there is a significant change in the nature of the wastewater, the Department may require the permittee to conduct a new set of tests.

*14. If a significant industrial user locates within the service area so that significant change in the nature of the wastewater might be anticipated, MDE may require the permittee to conduct a new set of tests.

15. The biomonitoring program study plan, WET test results and related materials shall be submitted electronically to the Department if the permittee has already been approved for the NetDMR tool. The material shall be attached as separate single files and labeled as "Biomonitoring Program Study Plan" and "WET Test Results" in the NetDMR tool. Otherwise, the permittee shall submit all pertinent physical documents to:

Attention: Whole Effluent Toxicity Coordinator
Compliance Program
Water and Science Administration
Maryland Department of the Environment
Montgomery Park Business Center
1800 Washington Boulevard, Suite 420
Baltimore, MD 21230-1708

The permittee shall notify the Department at the above address or via email at mde.biomonitoring@maryland.gov immediately upon electronic submission of the biomonitoring program study plan, WET test results and associated material through NetDMR tool.

*omit for industrial facilities

Appendix C

BIOMONITORING PROGRAM (Lower concern and effluent flow is greater than 10% of the receiving water low flow)

1. Within three months of the effective date of the permit, the permittee shall submit to the Department for approval a study plan to evaluate wastewater toxicity at Outfall by using biomonitoring. The study plan should include a discussion of:

   a. wastewater and production variability
   b. sampling & sample handling
   c. source & age of test organisms
   d. source of dilution water
   e. testing procedures/experimental design
   f. data analysis
   g. quality control/quality assurance
   h. report preparation
   i. testing schedule

2. The testing program shall consist of two definitive acute testing events, three months apart. This testing shall be initiated no later than three months following the Department's acceptance of the study plan.

   For Freshwater Receiving Stream

   Each of the two testing events shall include a 48-hour static renewal test using fathead minnow and a 48-hour static renewal test using a daphnid species.

   For Estuarine Receiving Stream

   a. The testing shall consist of 48-hour static renewal tests using either sheepshead minnows (*Cyprinodon* variegatus), silversides (*Menidia beryllina , Menidia menidia* , *Menidia peninsulae*) and mysid shrimp (*Americamysis bahia* A.K.A. *Mysidopsis bahia*). Testing must include one vertebrate species and one invertebrate species

   b. Test results shall be expressed as LC_{50}.

3. The samples used for biomonitoring shall be collected at the same time and location as the samples analyzed for the effluent limitations and monitoring requirements for this outfall. For chlorinated effluents, samples shall be collected after dechlorination. The permittee shall collect 24-hour flow–proportioned composite samples unless the Department has given prior approval of an alternative sampling type.

4. Testing shall be conducted in accordance with the procedures described in Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and

Marine Organisms, Fifth Edition, EPA-821-R-02-012, October 2002

5. Test results shall be submitted to the Department within one month of completion of each set of tests.

6. Test results shall be reported in accordance with MDE/WMA "Reporting Requirements for Effluent Biomonitoring Data," revised 11/2/2018.

7. If testing is not performed in accordance with MDE-approved study plan, additional testing may be required by the Department.

8. If the test results of any two consecutive valid toxicity tests conducted within any 12-month period show acute toxicity (LC_{50} equal to or less than 100%) the permittee shall repeat the test within 30 days to confirm the findings of acute toxicity. If acute toxicity is confirmed, the permittee shall:

    a. Eliminate the source of toxicity through operational changes as soon as possible but in any case not longer than within three months, or

    b. Perform a TRE. If the permittee repeats the toxicity testing as stated above and the results of the repeat test do not confirm the acute toxicity, the Department will require the permittee to repeat the toxicity testing as stated above to reconfirm a finding of no acute toxicity. After reconfirmation, the permittee shall complete any remaining quarterly testing required.

    .

9. If the permittee completes a TRE in accordance with II.E.8.b and unacceptable toxicity is confirmed, a Whole Effluent Toxicity (WET) permit limit and a compliance schedule will be required.

10. To address federal NPDES requirements for WET testing and limits, MDE shall implement permit limits in a new or renewal permit when a WET test result shows reasonable potential for toxicity unless it can be demonstrated that the source of toxicity has been eliminated, inappropriate test procedures were utilized, or the source has been controlled via a chemical specific permit limitation. Where reasonable potential has been assumed based on one test result, the permit shall include a WET limit effective within three years unless the effluent shows no toxicity in six follow-up quarterly tests. The permit may be modified to remove the WET limit if the six follow-up quarterly tests show no toxicity.

11. If plant processes or operations change so that there is a significant change in the nature of the wastewater, the Department may require the permittee to conduct a new set of tests.

*12. If a significant industrial user locates within the service area so that significant change in the nature of the wastewater might be anticipated, MDE may require the permittee to

conduct a new set of tests.

13. The biomonitoring program study plan, WET test results and related materials shall be submitted electronically to the Department if the permittee has already been approved for the NetDMR tool. The material shall be attached as separate single files and labeled as "Biomonitoring Program Study Plan" and "WET Test Results" in the NetDMR tool. Otherwise, the permittee shall submit all pertinent physical documents to:

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Compliance Program
Water and Science Administration
Maryland Department of the Environment
Montgomery Park Business Center
1800 Washington Boulevard, Suite 420
Baltimore, MD 21230-1708

The permittee shall notify the Department at the above address or via email at mde.biomonitoring@maryland.gov immediately upon electronic submission of the biomonitoring program study plan, WET test results and associated material through NetDMR tool.

*omit for industrial facilities

Appendix D

BIOMONITORING PROGRAM (Lower concern and effluent flow is less than 10% of the receiving water low flow)

1. Within three months of the effective date of the permit, the permittee shall submit to the Department for approval a study plan to evaluate wastewater toxicity at Outfall by using biomonitoring. The study plan should include a discussion of:

   a. wastewater and production variability
   b. sampling & sample handling
   c. source & age of test organisms
   d. source of dilution water
   e. testing procedures/experimental design
   f. data analysis
   g. quality control/quality assurance
   h. report preparation
   i. testing schedule

2. The testing program shall consist of two definitive acute testing events, three months apart. This testing shall be initiated no later than three months following the Department's acceptance of the study plan.

   For Freshwater Receiving Stream

   Each of the two testing events shall include a 48-hour static renewal test using fathead minnow and a 48-hour static renewal test using a daphnid species.

   For Estuarine Receiving Stream

   a. Each of the two testing events shall include a 48-hour static renewal test using fathead minnow and a 48-hour static renewal test using a daphnid species.

   b. The permittee may substitute 48-hour static renewal tests using either sheepshead minnows (*Cyprinodon* variegatus), silversides (*Menidia beryllina , Menidia menidia* , *Menidia peninsulae*) and mysid shrimp (*Americamysis bahia* A.K.A. *Mysidopsis bahia*) for the above tests. Testing must include one vertebrate species and one invertebrate species

   c. Test results shall be expressed as LC_{50}

3. The samples used for biomonitoring shall be collected at the same time and location as the samples analyzed for the effluent limitations and monitoring requirements for this outfall. For chlorinated effluents, samples shall be collected after dechlorination. The permittee shall collect 24-hour flow–proportioned composite samples unless the Department has given prior approval of an alternative sampling type.

4. Testing shall be conducted in accordance with the procedures described in Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, EPA-821-R-02-012, October 2002

5. Test results shall be submitted to the Department within one month of completion of each set of tests.

6. Test results shall be reported in accordance with MDE/WMA "Reporting Requirements for Effluent Biomonitoring Data," revised 11/2/2018.

7. If testing is not performed in accordance with MDE-approved study plan, additional testing may be required by the Department.

8. If the test results of any two consecutive valid toxicity tests conducted within any 12-month period show acute toxicity (LC_{50} equal to or less than 100%), the permittee shall repeat the test within 30 days to confirm the findings of acute toxicity. If acute toxicity is confirmed, the permittee shall:

    a. Eliminate the source of toxicity through operational changes as soon as possible but in any case not longer than within three months, or

    b. Perform a TRE. If the permittee repeats the toxicity testing as stated above and the results of the repeat test do not confirm the acute toxicity, the Department will require the permittee to repeat the toxicity testing as stated above to reconfirm a finding of no acute toxicity. After reconfirmation, the permittee shall complete any remaining quarterly testing required.

9. If the permittee completes a TRE in accordance with II.E.8.b and unacceptable toxicity is confirmed, a Whole Effluent Toxicity (WET) permit limit and a compliance schedule will be required.

10. To address federal NPDES requirements for WET testing and limits, MDE shall implement permit limits in a new or renewal permit when a WET test result shows reasonable potential for toxicity unless it can be demonstrated that the source of toxicity has been eliminated, inappropriate test procedures were utilized, or the source has been controlled via a chemical specific permit limitation. Where reasonable potential has been assumed based on one test result, the permit shall include a WET limit effective within three years unless the effluent shows no toxicity in six follow-up quarterly tests. The permit may be modified to remove the WET limit if the six follow-up quarterly tests show no toxicity.

11. If plant processes or operations change so that there is a significant change in the nature of the wastewater, the Department may require the permittee to conduct a new set of tests.

*12 If a significant industrial user locates within the service area so that significant change in the nature of the wastewater might be anticipated, MDE may require the permittee to conduct a new set of tests.

13. The biomonitoring program study plan, WET test results and related materials shall be submitted electronically to the Department if the permittee has already been approved for the NetDMR tool. The material shall be attached as separate single files and labeled as "Biomonitoring Program Study Plan" and "WET Test Results" in the NetDMR tool. Otherwise, the permittee shall submit all pertinent physical documents to:

Attention: Whole Effluent Toxicity Coordinator
Compliance Program
Water and Science Administration
Maryland Department of the Environment
Montgomery Park Business Center
1800 Washington Boulevard, Suite 420
Baltimore, MD 21230-1708

The permittee shall notify the Department at the above address or via email at mde.biomonitoring@maryland.gov immediately upon electronic submission of the biomonitoring program study plan, WET test results and associated material through NetDMR tool.

*omit for industrial facilities

Appendix E

SAMPLING AND REPORTING REQUIREMENTS FOR EFFLUENT BIOMONITORING DATA

BIOMONITORING SAMPLING REQUIREMENTS

Samples for all WET testing should be planed and collected during periods that best represent the facility's routine operations, that is, times when the effluent sample matrix is representative of the operational waste streams associated with the facility.

MARYLAND DEPARTMENT OF THE ENVIRONMENT

BACKGROUND

The Maryland Department of the Environment has compiled the following guidelines for reporting toxicity data from biomonitoring tests. These guidelines were formulated in an effort to standardize evaluations of toxicity data submitted to the Department.

BIOMONITORING REPORTING REQUIREMENTS

The results from biomonitoring toxicity tests shall be reported in a concise, easily understood manner. Each test report, in addition to an overall summary of the results, shall include the following documentation.

1. Chain of Custody Forms: A chain of custody form should accompany each individual sample collected. Each form shall include the following information.

    - Facility name
    - Sample collection date, time, and location (start and finish)
    - Sampling Method (grab or composite)
    - Volume of sample
    - Type of test (Acute or Chronic)
    - Sampler's signature and date
    - Description of sample storage during transportation
    - The signatures of all persons receiving custody of sample prior to use in testing, dates and times of receipt
    - Comments (as appropriate)

2. Effluent Quality Measurements: These data shall be reported for each effluent sample either at the time of collection or upon receipt by the toxicity testing laboratory.

    Date and time of measurements Conductivity and Salinity

Temperature	Hardness
pH	Alkalinity
Dissolved Oxygen	Visual Description
Total Residual Chlorine*(TRC)	Comments (as appropriate)

- *If the TRC exceeds 0.02 mg/l, the samples are dechlorinated in the laboratory, prior to heir use in toxicity tests.*

3. Toxicity Test Data:

A. Dilution Water.
   (1) Source of the dilution water
   (2) Manipulation steps (if any)

B. Test Organisms.
   (1) Source of the test organisms
   (2) Age of test organisms
   (3) Any acclimation steps
   (4) Disease treatment (if applicable)
   (5) Reference toxicant test data*
      (a) Reference toxicant identity
      (b) Test date(s)
      (c) A complete copy of the monthly in-house SRT test report associated with the WET test including bench sheets notes and all statistical data.
      (d) Summary of test results (48-hr LC_{50} with 95% confidence limits for acute tests; NOEC, LOEC, ChV. PMSD & IC_{25} for chronic tests)**
      (e) Plotted control charts along with the applicable upper and lower control limits should be submitted for each test species for each report. Only the last 20 data points can be used to determine QA acceptance criteria.

**When in-house organisms are used, monthly test data from the previous 5 months shall be reported. When organisms from an outside source are used, reference toxicant data from a test performed concurrently with the effluent test shall be reported, unless the test organism supplier provides control chart data from at least the last five monthly toxicity tests. Regardless of the source of test organisms (in-house cultures or purchased from external suppliers), the testing laboratory must perform at least one acceptable reference toxicant test per month for each toxicity test method conducted in that month. If a test method is conducted only monthly, or less frequently, a reference toxicant test must be performed concurrently with each effluent toxicity test.*

***Tests with values that are not within the control limits must be investigated by the laboratory's QA manager and documented. Repeat testing should be conducted*

based on the outcome of the laboratory's investigation

C. Effluent Toxicity Tests. The organisms utilized shall be clearly identified in the reporting of the following information for each effluent toxicity test.

(1) Test results.

(a) For both acute and chronic tests, the LC_{50} value, with 95% confidence limits, from the first 48 hours of the test.

(b) For chronic tests, the values for NOEC, LOEC, ChV and IC_{25} (based on biomass with 95% confidence limits). The PMSD results for reproduction, growth and if applicable, fecundity*** must be reported with the summary of the endpoints. A test with a PMSD that exceeds the upper bounds specified by the method manual is not acceptable and must be repeated unless the effluent is identified as being toxic.

****The fecundity endpoint is an optional but required endpoint. It is in many cases the most sensitive measure of toxicity. Laboratories should optimize temperature, feeding and organism densities during pre-test holding and testing periods to ensure achieving the criteria (egg production by 50 % or more of the control females) necessary to determine the fecundity endpoint. If the test organisms are purchased, the WET testing laboratory should make the necessary arrangements with the supplier to ensure that pre-test holding conditions are optimized to successfully achieve the fecundity endpoint. See Section 14.6.13.2.11 in Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, October 2002.*

(2) Water quality measurements.

(a) Daily measurements (before and after renewal) of temperature, DO****, and pH for all dilutions.

(b) Daily measurements of conductivity, alkalinity, and hardness for 100% and 0% dilutions.

(c) A summary (mean and range) of the data described in (a) and (b) above.

*****If DO is below 40% saturation (3.3 mg/l at 25^{o}C), samples are to be aerated gently before toxicity testing. The report shall indicate if aeration is necessary.*

(3) Initial test measurements and set up.

(a) Number of replicates.

(b) Number of organisms in each replicate.

(c) Volume of solution and the size of test chambers.

(d) Daily diet or lack of feeding.

(e) Randomization performed and documented.

(f) *Ceriodaphnia dubia* blocking procedures performed and documented.

(4) Daily mortality data, and for chronic reproduction tests, daily brood production.
(5) For chronic growth tests, final weight data for all organisms remaining at test conclusion.
(6) Summarized mortality, and for chronic tests, growth and reproduction data.
(7) Statistical calculations, including tests on assumptions (e.g., normality, homogeneity of variance). The statistical method and data used shall be clearly identified.
(8) Any test method deviations.
(9) Relevant observations on test organisms or conditions.
(10) Randomization template records or documentation that randomization procedures were properly followed for both test species.
(11) Documentation of *Ceriodaphnia dubia* blocking procedures for each testing event
(12) Sample manipulation steps (if any) shall be reported.
(13) A complete copy of the monthly in-house SRT test report associated with the WET test including bench sheets, notes and statistical data.

EFFLUENT TOXICITY TEST PROCEDURES GUIDANCE

On October 16, 1995, the EPA published its final rule in the Federal Register establishing whole effluent toxicity test methods at 40 CFR Part 136. These test methods are described in the following manuals. All WET testing required to be conducted for discharge permits issued under the National Pollutant Discharge Elimination System must conform to these methods.

EPA Effluent Toxicity Test Manuals:

Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, EPA-821-R-02-012, October 2002

Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms Fourth Edition, EPA-821-R-02-013, October, 2002

Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, EPA-821-R-02-014, October 2002

Appendix F – Whole Effluent Toxicity Tests to be Employed by Permittees

Test methods utilized by permittees for whole effluent toxicity testing must conform to the test methods found in *Table IA—List of Approved Biological Methods for Wastewater and Sewage Sludge* found in the latest edition of 40 CFR Part 136

freshwater

acute - 48 hour or 96 hour static renewal assays for lethality or immobility utilizing:

fathead minnow (*Pimephales promelas*), Bannerfin *shiner* (*Cyprinella leedsi*), Rainbow Trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*)
and *Daphnia magna*, *Daphnia pulex*, or *Ceriodaphnia dubia*

chronic - *Ceriodaphnia dubia* survival & reproduction
larval fathead minnows (*Pimephales promelas*) survival & growth

estuarine/marine

acute - 48-hour or 96 hour static renewal assays for lethality or immobility utilizing:

sheepshead minnows (*Cyprinodon variegatus*), inland silversides (*Menidia beryllina*), Atlantic silverside (*Menidia menidia*), tidewater silverside (*Menidia peninsulae*) mysid shrimp (*Americamysis bahia*, *f*ormerly Mysidopsis *bahia*

chronic - sheepshead minnows (*Cyprinodon variegatus*) larval survival & growth
inland silversides (*Menidia beryllina*) larval survival & growth
mysid shrimp (*Americamysis bahia*, *formerly Mysidopsis bahia*) survival, growth & fecundity

Appendix G TOXICITY REDUCTION EVALUATION

The permittee shall conduct a Toxicity Reduction Evaluation (TRE) when a review of toxicity test data by the Department indicates unacceptable acute or chronic effluent toxicity. A TRE is an investigation conducted to identify the causative agents of effluent toxicity, isolate the source(s), determine the effectiveness of control options, implement the necessary control measures and then confirm the reduction in toxicity.

1. Within 90 days of notification by the Department that a TRE is required, the permittee shall submit for approval by the Department a plan of study, schedule and completion date for conducting a TRE. The permittee shall conduct the TRE study consistent with the submitted plan and schedule.

for industrials: 2. This plan should follow the framework presented in Generalized Methods for Conducting Industrial Toxicity Reduction Evaluations (EPA/600/2-88/070) March 1989.

for municipals: 2. This plan should follow the framework presented in Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants (EPA/833B-99/002) August 1999.

Additional Guidance documents on the TRE process are shown below:

Methods for Aquatic Toxicity Identification Evaluations Phase I Toxicity Characterization Procedures Second Edition United States Environmental Protection Agency Office of Research and Development Washington, DC 20460
EPA/600/6-9 l/003 February 1991

Methods for Aquatic Toxicity Identification Evaluations Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity, United States Environmental Protection Agency Office of Research and Development EPA/600/R-92/080 September 1993 Washington DC 20460

Methods for Aquatic Toxicity Identification Evaluations Phase Ill Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity, United States Environmental Protection Agency Office of Research and Development Washington DC 20460 EPA /600/R-92/08 1 September 1993

Clarifications Regarding Toxicity Reduction and Identification Evaluations in the National Pollutant Discharge Elimination System Program, March 27, 2001, U.S. Environmental Protection Agency, Office of Wastewater Management , Office of Regulatory Enforcement, Washington, DC 20460

3. Beginning 60 days from the date of the Department's acceptance of the TRE study plan and every 60 days thereafter, the permittee shall submit progress reports including all

relevant test data to the Department. This shall continue until completion of the toxicity reduction confirmation.

4. Within 60 days of completion of the toxicity identification or the source identification phase of the TRE, the permittee shall submit to the Department a plan, schedule and completion date for implementing those measures necessary to eliminate acute toxicity, an LC_{50} greater than 100%, and/or eliminate chronic toxicity, an IC_{25} greater than the in-stream waste concentration (IWC). The implementation of these measures shall begin immediately upon submission of this plan.

5. Within 60 days of completing the implementation of the control measures to eliminate or reduce toxicity, the permittee shall submit to the Department for approval a study plan to confirm the elimination or reduction of toxicity by using biomonitoring.

6. If, for any reason, the implemented measures do not result in compliance with the Department's toxicity limitations, the permittee shall continue the TRE and a Whole Effluent Toxicity (WET) permit limit and a compliance schedule will be required.

7. All TRE-related materials shall be submitted electronically to the Department if the permittee has already been approved for the NetDMR tool. The material shall be attached as a separate single file with the file name "TRE" in the NetDMR tool. Otherwise, the permittee shall submit all pertinent physical documents to:

   Attention: Whole Effluent Toxicity Coordinator
   Compliance Program
   Water and Science Administration
   Maryland Department of the Environment
   Montgomery Park Business Center
   1800 Washington Boulevard, Suite 420
   Baltimore, MD 21230-1708

   The permittee shall notify the Department at the above address or via email at mde.biomonitoring@maryland.gov immediately upon electronic submission of TRE material through the NetDMR tool

Attachment 2

VELAP# 460030
EPA# VA01116

Fathead minnow test set up bench sheet (EPA METHOD 1000.0) Template version CPP5TRT061013

Test chamber:	500 ml plastic:	✓	Illumination & photoperiod:	50-100 ft-c 16L:8D	
	Other:		Number of replicates/treatment:	4	
Test solution vol. (250 ml min):	500 ml:		Initial number animals/replicate:	10	
	Other (ml):	250			
CHANGES & NOTES (INITIALS, DATE, SPECIFIC CHANGE MADE	1 ampule in 3000 ml = 100% simulated effluent				

SPECIES:	Pimephales promelas	
ACCLIMATION WATER:	Mod. Hard Synthetic Freshwater	
FEEDING PRIOR TO TEST:	Artemia nauplii (<24 h old) ad libitum	
FEEDING DURING TEST:	Artemia nauplii (<24 h old, ~0.15 ml) 2x/day	
SOURCE:	CBI Stock cultures	
ACCLIMATION TEMP (o C):	25	
HATCH START DATE & TIME:	5/25/20 17:00	
HATCH END DATE & TIME:	5/26/20 10:00	
DATE/TIME WATER ADDED:	5/26/20 11:01	
DATE/TIME ANIMALS ADDED:	5/26/20 11:17	
ANIMAL AGE WINDOW:	17h 0m	
MAX AGE AT TEST START (TAC 24 h MAX):	18h 18m	
TEST SET UP BY:	AK	
TEST ID:	RTC2019-CPP	
PEER REVIEW BY (Initial/Date):	LT PB	6/3/20 11:10
RTC2019-CPP		

Effluent and Dilution Water Log (Freshwater Tests). FWEFFL061013

											SUMMARY WATER QUALITY DATA				
Initial sample characterization	**Bottle(1):**	B1									**MEAN**	**S.D.**	**MIN.**	**MAX.**	**PARAMETER**
	Arrival Temp. (oC, from CoC):	NA											0	0	**Arrival Temp.**
	TRC (mg/l)(2):	<DL													
	TRC Corrected(2):	☐	☐	☐	☐	☐	☐	☐	☐						
	Hardness (mg/l):	80									80		80	80	**Hardness (mg/l)**
	Alkalinity (mg/l):	62									62		62	62	**Alkalinity (mg/l)**
	NH3-N (mg/l):	<1.0													
	Color/Appearance(3):	C													
	Obvious odor?	NO													
	Date & Time:	5/26/20 10:59													
	Initials:	AK													
Sample prep measure-ments	**Test Day:**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**		**MEAN**	**S.D.**	**MIN.**	**MAX.**	
	Bottle(s):	B1	B2	B3	B4	B5	B6	B7							
	Prep. Temp. (oC):	25	25	25	25	25	25	25			25	0.0	25	25	**Temp. (oC)**
	D.O. (mg/l) After Warming:	8.2	8.2	8.2	8.2	8.2	8.2	8.2							
	Aeration Time (min):	0	0	0	0	0	0	0							
	Adjusted D.O. (mg/l):	8.2	8.2	8.2	8.2	8.2	8.2	8.2			8.2	0.0	8.2	8.2	**D.O. (mg/l)**
	Final pH (S.U.):	7.67	7.69	7.92	7.93	7.63	7.74	7.73			7.76	0.12	7.63	7.93	**pH (S.U.)**
	Conductivity (uS/cm)(4):	297	300	311	308	310	308	312			307	5.8	297	312	**Cond. (uS/cm)**
	Final TRC (mg/l)(5):	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.							
	Sample Filtered (60 um)?	☐	☐	☐	☐	☐	☐	☐	☐	☐					
	Date & Time:	5/26/20 11:00	5/27/20 10:39	5/28/20 15:50	5/29/20 13:07	5/30/20 12:27	5/31/20 11:30	6/1/20 14:04							
	Initials:	AK	AK	LT	LT	LT	CPV	AK							
Dilution water	**Test Day:**	**Day 0**	**Day 1**	**Day 2**		**Day 3**	**Day 4**	**Day 6**	**Day 7**		**MEAN**	**S.D.**	**MIN.**	**MAX.**	
	Vat Number:	1	3	3	3	2	2	2							
	Temperature (oC):	25	25	25	25	25	25	25			25	0.0	25	25	**Temp. (oC)**
	Conductivity (uS/cm):	311	315	319	315	300	301	296			308	9.0	296	319	**Cond. (uS/cm)**
	D.O. (mg/l):	8.2	8.2	8.2	8.2	8.2	8.2	8.2			8.2	0.0	8.2	8.2	**D.O. (mg/l)**
	pH (S.U.):	7.75	7.76	7.79	7.73	7.69	7.75	7.81			7.75	0.04	7.69	7.81	**pH (S.U.)**
	Hardness (mg/l):	80	90	90	90	86	86	86			87	3.6	80	90	**Hardness (mg/l)**
	Alkalinity (mg/l):	58	57	57	57	60	60	60			58	1.5	57	60	**Alkalinity (mg/l)**
	Date & Time:	5/26/20 8:10	5/27/20 8:10	5/28/20 8:15	5/29/20 7:50	5/30/20 7:10	5/31/20 7:45	6/1/20 8:15							
	Initials:	LT	LT	LT	LT	LT	CPV	CPV							
	Changes & Notes (Initials, date, specific change or notes)	1 ampule each day in 3000 ml = 100% simulated effluent. Lot #: LRAC5790. Exp. Mar/25													
	Peer review Initial/Date:	PB	6/3/20 15:44	DILUTION WATER TYPE:	Mod. Hard Synthetic Freshwater (EPA)	ND=Not Determined/Measured, NA=Not Applicable. 1) Ninth character of lab sample ID on chain of custody AND bottle number in collection series. Together with Project ID constitutes entire sample bottle ID. 2) TRC MDL 0.02 mg/l; QL 0.22 mg/l. Corrected value if Mn, Cr potential positive interference. Corrected using KI and NaAsO2. 3) C-clear, O-opaque, T-turbid, S-solids (Sl-slight, M-moderate, H-heavy), Y-yellow, B-brown, Bl-black, G-green, P-pink, Gr-grey, Or-orange. 4) Measured on first use of sample only. 5) Final TRC measured only if chlorine present in initial characterization.					
PROJECT ID:	**RTC2019-CPP**	ADDITIONAL EFFLUENT TREATMENT:													

Fathead minnow daily water quality bench sheet (EPA METHOD 1000.0) Template version CPP5TRT061013

		Day 0	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	SUMMARY WATER QUALITY DATA			
	TRTMNT	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	MEAN	S.D.	MIN.	MAX.
pH (S.U.)	C	7.86	7.65	7.79	7.64	8.01	7.67	7.65	7.54	7.78	7.67	7.78	7.63	7.84	7.37	7.71	0.15	7.37	8.01
	1	7.86	7.63	7.85	7.61	8.00	7.66	7.67	7.51	7.75	7.65	7.81	7.60	7.85	7.37	7.70	0.16	7.37	8.00
	2	7.85	7.59	7.88	7.62	7.98	7.64	7.69	7.47	7.74	7.62	7.85	7.56	7.85	7.36	7.69	0.17	7.36	7.98
	3	7.84	7.59	7.86	7.59	7.97	7.63	7.70	7.47	7.75	7.60	7.82	7.55	7.87	7.34	7.68	0.18	7.34	7.97
	4	7.76	7.55	7.80	7.55	7.93	7.58	7.70	7.40	7.75	7.58	7.79	7.51	7.82	7.32	7.65	0.17	7.32	7.93
	5	7.69	7.52	7.70	7.56	7.91	7.54	7.70	7.40	7.75	7.53					7.63	0.15	7.40	7.91
Temp. (o C)	C	25	24	25	24	25	24	25	24	25	24	25	24	25	24	25	0.5	24	25
	1	25	24	25	24	25	24	25	24	25	24	25	24	25	24	25	0.5	24	25
	2	25	24	25	24	25	24	25	24	25	24	25	24	25	24	25	0.5	24	25
	3	25	24	25	24	25	24	25	24	25	24	25	24	25	24	25	0.5	24	25
	4	25	24	25	24	25	24	25	24	25	24	25	24	25	24	25	0.5	24	25
	5	25	24	25	24	25	24	25	24	25	24					25	0.5	24	25
Diss. Oxygen (mg/l)	C	8.2	8.0	8.1	7.8	7.9	7.6	8.0	7.8	8.0	7.7	8.1	7.4	8.0	7.8	7.9	0.2	7.4	8.2
	1	8.2	8.0	8.0	7.8	8.0	7.7	7.9	7.7	8.1	7.7	8.0	7.4	8.1	7.7	7.9	0.2	7.4	8.2
	2	8.2	8.0	8.1	7.9	8.1	7.6	8.0	7.7	8.2	7.6	8.1	7.3	8.0	7.7	7.9	0.3	7.3	8.2
	3	8.2	7.9	8.1	7.9	7.9	7.7	8.0	7.7	8.2	7.7	8.1	7.2	8.1	7.7	7.9	0.3	7.2	8.2
	4	8.2	7.9	8.1	7.8	7.9	7.7	8.0	7.7	8.2	7.7	8.1	7.5	8.1	7.7	7.9	0.2	7.5	8.2
	5	8.2	7.8	8.1	7.9	8.0	7.7	8.1	7.6	8.1	7.8					7.9	0.2	7.6	8.2
Cond. (uS/cm)	C	292		304		303		299		298		294		292		297	5.0	292	304
	1	297		300		302		302		293		303		283		297	7.2	283	303
	2	296		299		316		299		307		302		284		300	9.8	284	316
	3	297		298		301		303		302		302		288		299	5.2	288	303
	4	297		299		302		302		303		303		288		299	5.4	288	303
	5	298		302		298		299		298						299	1.7	298	302
Replicate measured		D	D	A	B	C	A	D/A	B/A	C/A	A	B	C	D	A				
Initials		AK	LT	AK	LT	LT	LT	LT	LT	LT	CPV	CPV	CPV	AK	AK				
Changes & Notes (Initials, date, specific change or notes)		1 ampule in 3000 ml = 100% simulated effluent																	

	Test Aerated?	No			D.O. Highest conc. @ aeration:			TRT ID:	1	2	3	4	5		
RTC2019-CPP	Date & Time Air Start:				Total live highest conc.@ aeration			CONC(%):	6.25%	12.5%	25.0%	50.0%	100%		

Fathead minnow daily biological measurements bench sheet (EPA METHOD 1000.0) Template version CPP5TRT061013

TRTMNT	Rep	#Live Day 0	#Live Day 1	#Live Day 2	#Live Day 3	#Live Day 4	#Live Day 5	#Live Day 6	#Live Day 7	Total Dry Wt (mg)	Tare Wt (mg)	Wt Count	Pan Number
C	A	10	10	10	10	10	10	10	10	23.78	16.72	10	1
	B	10	10	10	10	10	9	9	9	21.43	14.44	10	2
Lab	C	10	10	10	10	10	10	10	10	22.16	15.94	10	3
Control	D	10	10	10	10	10	10	10	10	21.51	15.68	10	4
#1	A	10	10	10	10	10	10	10	10	20.94	13.86	10	5
6.25%	B	10	10	10	10	9	9	9	9	21.45	15.49	10	6
Vol. Effl:	C	10	10	10	10	10	10	10	10	23.77	16.25	10	7
62.5 ml	D	10	10	10	10	10	10	10	10	24.85	17.22	10	8
# 2	A	10	10	10	10	10	10	10	10	20.76	15.18	10	9
12.5%	B	10	10	10	10	10	10	10	10	20.27	15.56	10	10
Vol. Effl:	C	10	10	10	10	10	10	10	10	18.18	13.28	10	11
125 ml	D	10	10	10	10	10	10	10	10	21.95	16.77	10	12
# 3	A	10	10	10	10	10	10	9	8	18.41	14.99	10	13
25.0%	B	10	10	10	10	6	6	6	6	18.53	15.42	10	14
Vol. Effl:	C	10	10	9	9	7	7	7	7	21.35	19.26	10	15
250 ml	D	10	10	8	8	8	8	8	8	19.65	16.32	10	16
# 4	A	10	10	10	7	5	3	3	3	16.87	15.58	10	17
50.0%	B	10	8	8	8	5	5	5	5	15.88	14.98	10	18
Vol. Effl:	C	10	10	10	6	3	3	3	3	15.69	14.76	10	19
500 ml	D	10	9	5	5	4	2	2	2	16.02	15.66	10	20
# 5	A	10	2	1	1	1	0	0	0	0.00	14.48	10	21
100%	B	10	1	0	0	0	0	0	0	0.00	18.51	10	22
Vol. Effl:	C	10	0	0	0	0	0	0	0	0.00	14.89	10	23
1000 ml	D	10	1	0	0	0	0	0	0	0.00	16.84	10	24
INITIALS:		AK	AK	LT	LT	LT	CPV	AK	LT	LT	KG	See ToxCalc printout for summary survival & biomass data	
DATE & TIME:		5/26/20 11:17	5/27/20 10:42	5/28/20 16:01	5/29/20 14:01	5/30/20 12:32	5/31/20 12:38	6/1/20 14:18	6/2/20 11:19	6/3/20 11:28	6/1/20 10:04		
SAMPLE USED:		B1	B2	B3	B4	B5	B6	B7	50 mg wt ck:	50.00	50.01	Test Duration:	7d 0h 2m
CHANGES & NOTES (INITIALS, DATE, SPECIFIC CHANGE MADE		1 ampule in 3000 ml = 100% simulated effluent											
		MEAN % CONTROL SURVIVAL (TAC 80% MIN):				97.5	AVG. DRY WT. PER SURV. CONTROL (TAC 0.25 mg):				0.669		
RTC2019-CPP													

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:		Test ID:	RTC2019CPP	Sample ID:	
End Date:		Lab ID:	CBI	Sample Type:	
Sample Date:		Protocol:	EPAF 94-EPA Freshwater	Test Species:	PP-Pimephales promelas
Comments:	DATA ENTERED BY PB				

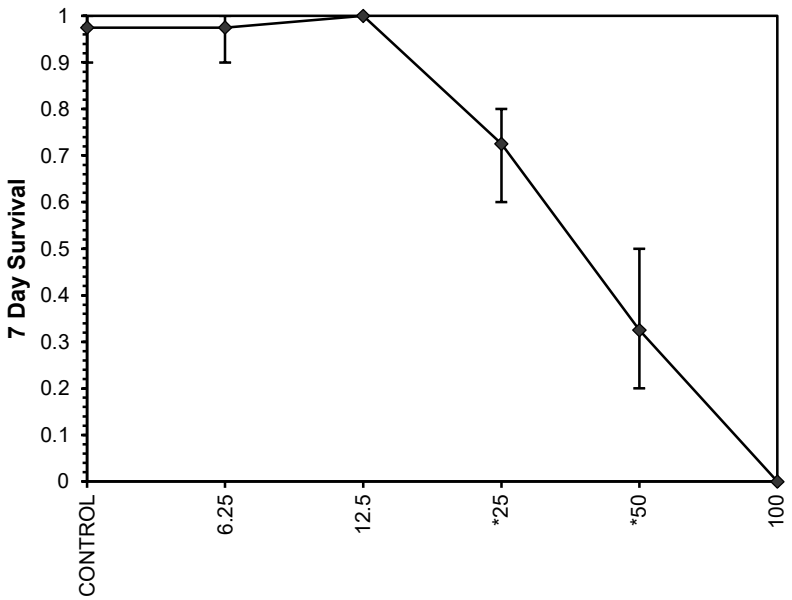
Conc-%	1	2	3	4
CONTROL	1.0000	0.9000	1.0000	1.0000
6.25	1.0000	0.9000	1.0000	1.0000
12.5	1.0000	1.0000	1.0000	1.0000
25	0.8000	0.6000	0.7000	0.8000
50	0.3000	0.5000	0.3000	0.2000
100	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
CONTROL	0.9750	1.0000	1.3713	1.2490	1.4120	5.942	4		
6.25	0.9750	1.0000	1.3713	1.2490	1.4120	5.942	4	18.00	10.00
12.5	1.0000	1.0256	1.4120	1.4120	1.4120	0.000	4	20.00	10.00
*25	0.7250	0.7436	1.0229	0.8861	1.1071	10.396	4	10.00	10.00
*50	0.3250	0.3333	0.6021	0.4636	0.7854	22.237	4	10.00	10.00
100	0.0000	0.0000	0.1588	0.1588	0.1588	0.000	4		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91661	0.868	-0.12304	0.32795
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	12.5	25	17.6777	8

Dose-Response Plot



pd

ToxCalc v5.0.23

Reviewed by:_____

Larval Fish Growth and Survival Test-7 Day Biomass

Start Date:		Test ID:	RTC2019CPP	Sample ID:	
End Date:		Lab ID:	CBI	Sample Type:	
Sample Date:		Protocol:	EPAF 94-EPA Freshwater	Test Species:	PP-Pimephales promelas
Comments:	DATA ENTERED BY PB				

Conc-%	1	2	3	4
CONTROL	0.7060	0.6990	0.6220	0.5830
6.25	0.7080	0.5960	0.7520	0.7630
12.5	0.5580	0.4710	0.4900	0.5180
25	0.3420	0.3110	0.2090	0.3330
50	0.1290	0.0900	0.0930	0.0360
100	0.0000	0.0000	0.0000	0.0000

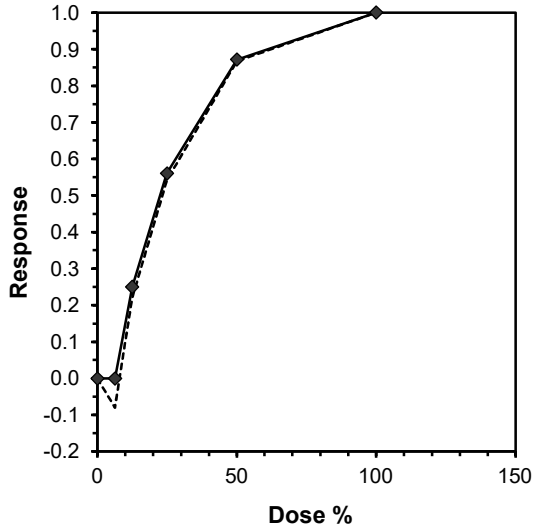
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
CONTROL	0.6525	1.0000	0.6525	0.5830	0.7060	9.189	4				0.6786	1.0000
6.25	0.7048	1.0801	0.7048	0.5960	0.7630	10.826	4	-1.229	2.180	0.0927	0.6786	1.0000
*12.5	0.5093	0.7805	0.5093	0.4710	0.5580	7.423	4	3.369	2.180	0.0927	0.5093	0.7504
25	0.2988	0.4579	0.2988	0.2090	0.3420	20.497	4				0.2988	0.4402
50	0.0870	0.1333	0.0870	0.0360	0.1290	44.070	4				0.0870	0.1282
100	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90202	0.805	-0.70529	-0.40621
Bartlett's Test indicates equal variances (p = 0.55)	1.19491	9.21035		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	6.25	12.5	8.83883	16	0.09268	0.14204	0.04098	0.00361	0.00348	2, 9

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	7.502	0.400	5.597	8.220	-3.8604
IC10	8.754	0.427	7.371	10.190	-0.0947
IC15	10.006	0.567	8.455	12.161	0.6407
IC20	11.258	0.727	9.521	14.224	0.7758
IC25	12.517	0.972	10.335	16.273	0.6871
IC40	18.561	1.203	14.568	22.335	-0.0817
IC50	22.591	1.498	18.225	27.102	0.0344



Attachment 3

VELAP# 460030
EPA# VA01116

5.2 Evaluation Criteria

1 - Regression Equation

Acceptance windows based on TNI adopted equation of proficiency value +/- 3 proficiency standard deviations and check limits of proficiency value +/- 2 proficiency standard deviations. Proficiency value and proficiency standard deviation are calculated from gravimetric variables a, b, c & d as proficiency value = a * gravimetric + b and proficiency standard deviation = c * gravimetric + d.

2 - Study Robust Mean and c, d regression

Acceptance windows based on TNI adopted equation of proficiency value +/- 3 proficiency standard deviations and check limits of proficiency value +/- 2 proficiency standard deviations. Proficiency value and proficiency standard deviation calculated from robust study mean and variables c & d as proficiency value = robust mean and proficiency standard deviation = c *proficiency value + d.

3 - Fixed Limits

Acceptance windows based on span of gravimetric percentage from gravimetric as gravimetric +/- gravimetric * percentage.

4 - Adjustable Fixed Limits

Acceptance windows based on a span of gravimetric percentage from gravimetric as gravimetric +/- gravimetric * lowPercentage where gravimetric < break and gravimetric +/- gravimetric * highPercentage where gravimetric >= break.

5 - Study Statistics

Acceptance windows based on a number of standard deviations span from the study mean as study mean +/- (deviations * standard deviation).

6 - Log Transform Statistics

Acceptance windows based on lognormal distributed data. Acceptance windows = mean(lognormal) +/- span * standard deviation(lognormal).

7 - Regression Equation 2SD

Acceptance windows based on EPA equation of proficiency value +/- 2 proficiency standard deviations. Proficiency value and proficiency standard deviation are calculated from gravimetric variables a, b, c & d as proficiency value = a * gravimetric + b and proficiency standard deviation = c * gravimetric + d. Generally reserved for drinking water studies.

8 - Study Median and Dilution Levels

Acceptance windows based on study median ± 1 dilution. If the median falls between two test dilutions, then the assigned value is set at the higher value, and the lower acceptance limit is the second test dilution below the median, and the upper acceptance limit is the second test dilution above the median. Generally reserved for NOEC analytes (in the framework of WETT analysis).

9 - Fixed Limits based on Analytical Value

Acceptance windows based on span of analytical value from measurements.

Attachment 4

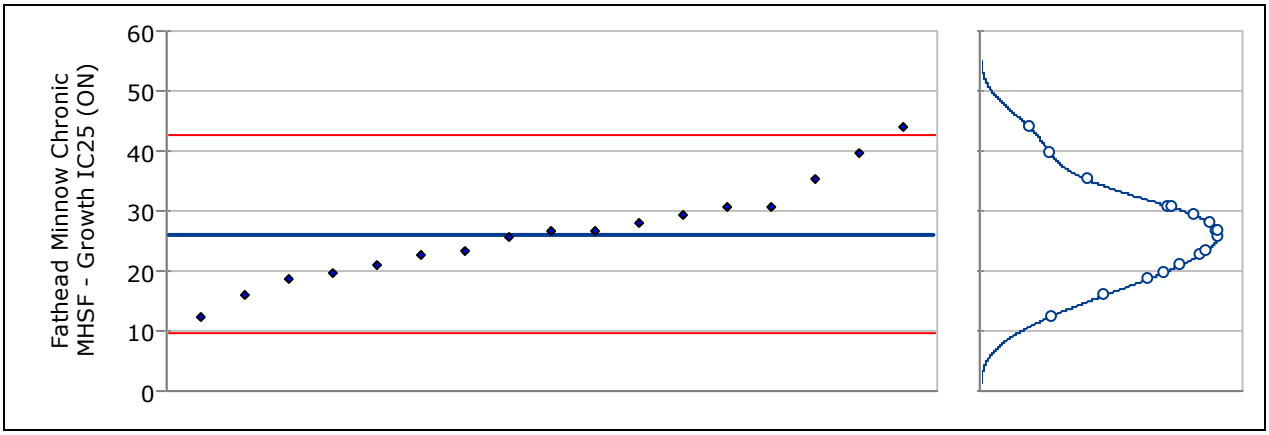
VELAP# 460030
EPA# VA01116

4.5 WET015-1EA Fathead Minnow, 7Day, MHSF / LRAC5790

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

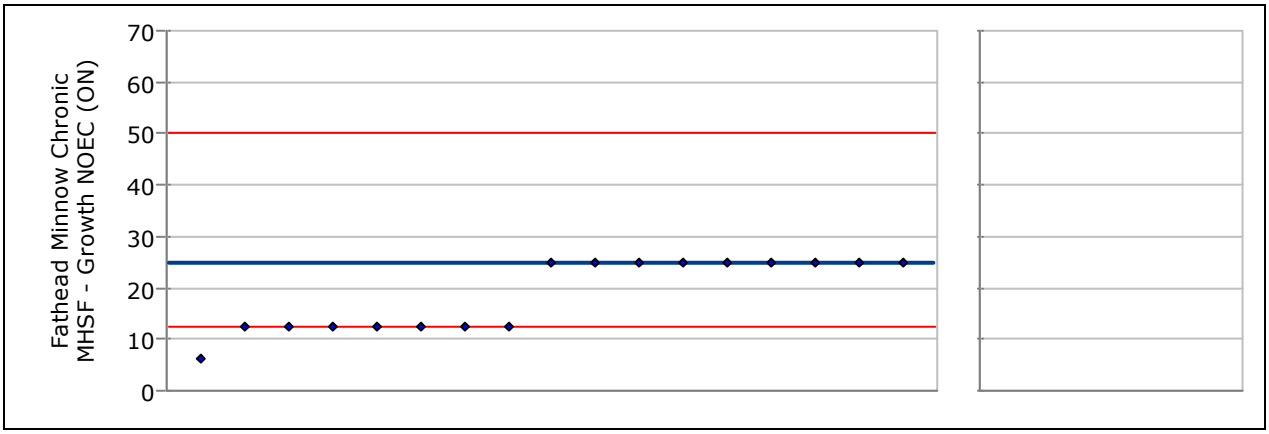
4.5.1 Fathead Minnow Chronic MHSF - Growth IC25 (ON)

No. of participating laboratories (in total / with quant. data points only)	17 / 17
No. of data points (in total / quantitative)	17 / 17
Assigned value	26.2 %
Proficiency std. dev.	8.26 %
Acceptance window	9.64 - 42.7 %



4.5.2 Fathead Minnow Chronic MHSF - Growth NOEC (ON)

No. of participating laboratories (in total / with quant. data points only)	17 / 17
No. of data points (in total / quantitative)	17 / 17
Assigned value	25.0 %
Proficiency std. dev.	--- %
Acceptance window	12.5 - 50.0 %



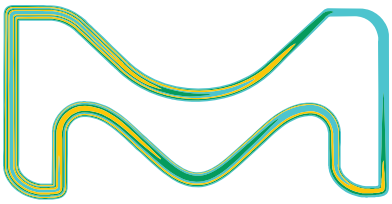
Attachment 5

VELAP# 460030
EPA# VA01116

PROFICIENCY TESTING

Evaluation Report

Quick Turn	**QT-0029550**
Study Type	WPCHEM_MICRO
Open Date	2020-11-02
Close Date	2020-12-10
Report Generated	2020-12-11
Laboratory	Coastal Bioanalysts, Inc Pete DeLisle 6400 Enterprise Court Gloucester VA 23061 US
Account Number	49480494
US EPA Lab Code	VA01116



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All batch numbers of proficiency testing samples, including microbiological materials, are manufactured and tested in accordance with ISO/IEC 17043 requirements. For further information on proficiency testing samples, please check the PT product code information on each product detail page located on our website.

Accreditors

Evaluations of this study will be sent to the accreditor(s) listed below. If any of the information listed below is not correct, please contact Sigma-Aldrich RTC immediately.

Accrediting Agency

Commonwealth of Virginia DGS-DCLS

Agency lab code: 00067

Lab Certification
600 North 5th St.
Richmond VA 23219-3691 US

Accrediting Agency

Kentucky DEP

Agency lab code: VA01116

Laboratory Certification
300 Sower Blvd. 3rd floor
Frankfort KY 40601 US

Accrediting Agency

Maryland Department of the Environment

Agency lab code: VA01116

Ron Wicks
MDE - Water Supply Program
1800 Washington Blvd., Ste 450
Water Supply Program
Baltimore MD 21230-1708 US

1 Laboratory Performance Evaluation Summary

Summary Results for QT-0029550

WET015-1EA Fathead Minnow, 7Day, MHSF

LRAC2197

This proficiency testing sample was produced in accordance with ISO/IEC 17043:2010.

Analyte	Reported Value	Assigned Value	Acceptance Window	z-score*
EPA 1000.0 - Fathead minnow, 7-day Chronic, daily renewal, MHSF 25°C (2002) 10214207				
Test Code 15 / EPA Method 1000				
Fathead Minnow Chronic MHSF - Survival NOEC[1,2] 756 Analyst: LT Analysis Date: 2020-12-01	12.5 %	25.0 % *Evaluation Criteria – 8** *Parameters*: ± 1 dilution*	12.5 - 50.0 %	Acceptable
Fathead Minnow Chronic MHSF - Growth IC25 (ON)[1,2] 808 Analyst: LT Analysis Date: 2020-12-01	19.0 %	27.0 % *Evaluation Criteria – 5** *Parameters*: deviations:2*	8.92 - 45.0 %	**-0.9** Acceptable
Fathead Minnow Chronic MHSF - Growth NOEC (ON)[1,2] 810 Analyst: LT Analysis Date: 2020-12-01	12.5 %	25.0 % *Evaluation Criteria – 8** *Parameters*: ± 1 dilution*	12.5 - 50.0 %	Acceptable
Group Analysis Summary	Acceptable: 3/3			**Score: 100% - Acceptable**

* Evaluation parameters used for the statistical analysis: explanation at the end of report; a yellow highlighted results is acceptable but to be checked.

** Unable to calculate a study mean due to <4 data points being received, therefore an effective evaluation could not be performed.

[1] TNI Compliant, covered by Sigma-Aldrich RTC's ANAB Proficiency Testing Provider accreditation, Cert. AP-1469

[2] ISO/IEC 17043 Accredited, covered by Sigma-Aldrich RTC's ANAB Proficiency Testing Provider accreditation, Cert AP-1469

2 Sample Information

WET015-1EA Fathead Minnow, 7Day, MHSF
LRAC2197

Analyte	Unit	Gravimetric Value	PTRL	Study Mean*	Study Std. Dev.*
Fathead Minnow Chronic MHSF - Survival NOEC 756	%	25.0	---	25.0	0
Fathead Minnow Chronic MHSF - Growth IC25 (ON) 808	%	22.8	---	27.0	9.02
Fathead Minnow Chronic MHSF - Growth NOEC (ON) 810	%	25.0	---	18.8	8.74

* Study mean and Study Std. Dev. from the latest scheduled study within this scheme. If not available, this is indicated by "---".

3 Statistical Analysis

3.1 Definitions and Interpretation

Reported Value

The participant's result.

Assigned Value

Value attributed to a particular quantity and accepted, sometimes by convention, as having an uncertainty appropriate for a given purpose. See ISO/IEC 17043 for additional information. In general, the assigned value is the value used to assess proficiency and may or may not be the made to value (gravimetric value).

Acceptance Window

The range of values that constitute acceptable performance for a laboratory participating in this PT study.

z-score

A z-score shows how a single data point compares to normal data. A z-score says not only whether a point was above or below average, but how unusual the measurement is. Generally, a method result with a z-score less than |2| is considered to be in control and 'Acceptable'; a z-score between |2| and |3| is considered 'Questionable', but still within control and 'Acceptable' and a z-score greater than |3| is considered 'Not Acceptable' and the method is out of control. For WS studies, a z-score greater than |2| is not acceptable.

Calculated as z = (Reported Value - Assigned Value) / Proficiency Std. Dev.

A z-score cannot be provided

(1) for presence/absence data,
(2) for identification data and other categorial data,
(3) where the analyte is not present in the sample,
(4) for "less than" and "greater than" values,
(5) NOEC analytes (in the framework of WETT analysis).

In cases (1) to (3) the participant's result is only evaluated by "acceptable" if it matches with the assigned value. Otherwise the result is indicated as "not acceptable". In case the analyte is not present in the sample and a PTRL is available, the participant's result is indicated as "acceptable" as long the result is less than the PTRL.

In case (4) the following evaluation rules will be applied:

- "less than" values:
  - When the analyte is not present in the sample the result is always "acceptable".
  - When the analyte is truly present in the sample, the result is only "acceptable" if the "less than" value is greater than the lower limit of the acceptance window.
- "greater than" values:
  - When the analyte is not present in the sample the result is always "not acceptable".
  - When the analyte is truly present in the sample, the result is only "acceptable" if the "greater than" value is less than the upper limit of the acceptance window.

In case (5) the result is indicated as "acceptable" if it lies within the acceptance window, otherwise the result is indicated as "not acceptable".

Proficiency Std. Dev.

Standard deviation calculated based on Evaluation Criteria.

PTRL

Proficiency Testing Reporting Limit

Study Mean

Statistical study mean calculated using a robust statistical model. Robust statistical techniques are used to minimize the influence extreme results can have on estimates of the mean and standard deviation. NOTE - These techniques assign less weight to extreme results, rather than eliminate them from a data set.

Choice of statistical technique: In case of quantitative data points from at least 8 laboratories, Algorithm A (ISO 13528, Section C.3.1), and in case of quantitative data points of 4 to 7 laboratories, the Hampel estimator (ISO 13528, Section C.5.3) is applied. A study mean cannot be calculated in case there are quantitative data points from less than 4 laboratories available.

Study Std. Dev.

Standard deviation calculated from study data using robust statistics.

In case of quantitative data points from at least 8 laboratories, Algorithm A (ISO 13528, Section C.3.1), and in case of quantitative data points of 4 to 7 laboratories, the Q method (ISO 13528, Section C.5.2) is applied. A study standard deviation cannot be calculated in case there are quantitative data points from less than 4 laboratories available.

Gravimetric Value

The 'prepared to' value, determined by gravimetric means. The uncertainty associated with this value is the standard uncertainty and based on Sigma-Aldrich RTC's gravimetric tolerances.

3.2 Evaluation Criteria

1 - Regression Equation

Acceptance windows based on TNI adopted equation of proficiency value +/- 3 proficiency standard deviations and check limits of proficiency value +/- 2 proficiency standard deviations. Proficiency value and proficiency standard deviation are calculated from gravimetric variables a, b, c & d as proficiency value = a * gravimetric + b and proficiency standard deviation = c * gravimetric + d.

2 - Study Robust Mean and c, d regression

Acceptance windows based on TNI adopted equation of proficiency value +/- 3 proficiency standard deviations and check limits of proficiency value +/- 2 proficiency standard deviations. Proficiency value and proficiency standard deviation calculated from robust study mean and variables c & d as proficiency value = robust mean and proficiency standard deviation = c *proficiency value + d.

3 - Fixed Limits

Acceptance windows based on span of gravimetric percentage from gravimetric as gravimetric +/- gravimetric * percentage.

4 - Adjustable Fixed Limits

Acceptance windows based on a span of gravimetric percentage from gravimetric as gravimetric +/- gravimetric * lowPercentage where gravimetric < break and gravimetric +/- gravimetric * highPercentage where gravimetric >= break.

5 - Study Statistics

Acceptance windows based on a number of standard deviations span from the study mean as study mean +/- (deviations * standard deviation).

6 - Log Transform Statistics

Acceptance windows based on lognormal distributed data. Acceptance windows = mean(lognormal) +/- span * standard deviation(lognormal).

7 - Regression Equation 2SD

Acceptance windows based on EPA equation of proficiency value +/- 2 proficiency standard deviations. Proficiency value and proficiency standard deviation are calculated from gravimetric variables a, b, c & d as proficiency value = a * gravimetric + b and proficiency standard deviation = c * gravimetric + d. Generally reserved for drinking water studies.

8 - Study Median and Dilution Levels

Acceptance windows based on study median ± 1 dilution. If the median falls between two test dilutions, then the assigned value is set at the higher value, and the lower acceptance limit is the second test dilution below the median, and the upper acceptance limit is the second test dilution above the median. Generally reserved for NOEC analytes (in the framework of WETT analysis).

9 - Fixed Limits based on Analytical Value

Acceptance windows based on span of analytical value from measurements.

4 Proficiency Test Item Preparation, Homogeneity and Stability Assessment

Sigma-Aldrich RTC uses proprietary and published methods for the manufacture, homogeneity and stability testing of proficiency test items. Sigma-Aldrich RTC's proficiency test materials meet the requirements of ISO 17034. For more information contact Sigma-Aldrich RTC. Additionally Sigma-Aldrich RTC complies with the TNI Volume 3 'General Requirements for Environmental Proficiency Test Providers', EL-V3-2016, for all TNI Fields of Proficiency Testing analytes.

5 Metrological Traceability

All preparations are made using balances calibrated annually traceable to NIST standards. Where appropriate analytical measurements are traceable through an unbroken chain to NIST standards, or a Certified Reference Material manufactured under ISO 17034 in conjunction with ISO/IEC 17025.

6 Additional Information

Go to supelco-pt.com for additional information on summary statistics for specific methods, advice on the interpretation of the statistical analysis and additional comments/recommendations. Sigma-Aldrich RTC recommends that you contact your accreditation body for specific instruction.

7 References

[1] ISO 13528: Statistical methods for use in proficiency testing by interlaboratory comparison, August 2015

[2] ISO/IEC 17025:2017: General requirements for the competence of testing and calibration laboratories

[3] ISO/IEC 17043:2010: Conformity assessment - General requirements for proficiency testing, May 2010

[4] S. Uhlig und P. Henschel (1997): Limits of tolerance and z-scores in ring tests. Fresenius' J. Anal. Chem., Vol. 358, pp. 761-766.

[5] ISO 17034:2016: General requirements for the competence of reference material producers.

This section of the report is for informational purposes only. If you are unsure about specific accreditation requirements, please contact your state coordinator.

Unacceptable Analytes

No unacceptable analytes

[1] TNI Compliant, covered by Sigma-Aldrich RTC's ANAB Proficiency Testing Provider accreditation, Cert. AP-1469
[2] ISO/IEC 17043 Accredited, covered by Sigma-Aldrich RTC's ANAB Proficiency Testing Provider accreditation, Cert AP-1469

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