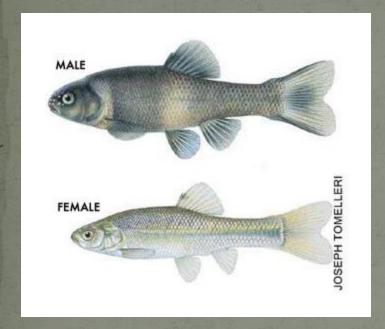
WET Testing Common Concerns

AWW & WEA Conference
April 27, 2015
Hot Springs, AR

Fathead Minnow (P. promelas)







Water fleas C. dubia & D. pulex





Test Containers





WET Testing Overview

- Acute
 - 48 hours
 - Survival only
 - 3 Liters (~1 gallon) of water needed
- Pimephales promelas (Fathead minnow)
 - 1-14 days; ≤ 24-hour range in age
 - 20 organisms per concentration
- Daphnia pulex (water flea)
 - < 24 hours</p>
 - 20 organisms per concentration

WET Testing Overview

- Chronic
 - 7 days
 - 24.5 Liters (~6.5 gallons) of water needed
- Pimephales promelas (Fathead minnow)
 - < 24 hours old; ≤ 24-hour range in age</p>
 - 40 per concentration
 - Sub-lethal = growth of fish
- Ceriodaphnia dubia (water flea)
 - < 24 hour old; ≤ 8-hour range in age
 - 10 per concentration
 - Sub-lethal = number of neonates reproduced

Concern – Timing of Sampling

- All samples must be collected completely within the reporting period.
- Cannot finish into the next period
 - e.g., cannot start sampling at end of March and finish sampling during first of April and report the test as 1st quarter
- Cannot alter required sampling scheme to fit into reporting period.
 - I.E. cannot use a single grab sample when 3 composite samples are required

Concern – Temperature of Samples

- Ice down the samples
 - Samples must be collected & stored at 0 to 6 degrees C
 - Includes shipping
- Exception
 - If sample collection ends and samples are delivered to laboratory in less than 4 to 6 hours and
 - If its summer and samples are over 6 degrees C
 - ADEQ will take into consideration the time it takes samples to cool down after collection

Concern – Holding Time Excursions

- Holding Time
 - Begins when sampling is completed
 - Sample collection to first use of sample must not exceed
 36 hours
 - Sample may also be used to prepare test solutions for renewal at 24 hours, 48 hours, and/or 72 hours after first use

Concern – Holding Time Excursions

- Notify ADEQ by phone and/or e-mail
- EPA's chronic method manual section 8.5.4
 - EPA manual allows for variance in sample holding time
 - No more than 72 h should elapse between collection and first use of the sample
- ADEQ will make a determination if the circumstance warrants a holding time variance

Concern – How's a Test Doing?

- Lab cannot report results prior to completion
- EPA memo November 15, 2007
- Any communication of sample, organism, or test performance during the test apply to effluent samples used for purposes of performing TIE.
- For all other WET testing for NPDES permits there should be no communications between labs and clients during a WET test.
 - Only exception missing samples

Concern – Result Reporting

- Full report submission ALL Wet Tests
 - Passing tests, failing tests, re-tests, invalid tests
 - NetDMR, E-mail, or mail
- NetDMR Layne Pemberton
 - 501-682-0664
 - pemberton@adeq.state.ar.us

		QUANTITY OR LOADING		QUALITY OR CONCENTRATION			NO.	FREQUENCY	SAMPLE		
PARAMETER		VALUE	VALUE	UNITS	VALUE	VALUE	VALUE	UNITS	EX	OF ANALYSIS	TYPE
Pass/Fall Static Renewal 7 Day	SAMPLE	******	******	******		******					
Chronic Ceriodaphnia	MEASUREMENT										
TGP3B 1 0	PERMIT	*****	******		Reg. Mon.	******		pass=0/fall		Quarterly	COMPOS
Effluent Gross	REQUIREMENT				7 DA MIN			-1		,	
Pass/Fall Statre 7Day Chronic	SAMPLE	******	******	******		******		T			
Pimephales Prometas	MEASUREMENT										
TGP6C 1 0	PERMIT	******	******	******	Req. Mon.	******	******	pass=0/fall		Quarterty	COMPOS
Effluent Gross	REQUIREMENT				7 DA MIN			-1		,	
Low Flow Pass/Fall Survival Test	SAMPLE	******	******			******					
Static Renewal 7 Day Chronic	MEASUREMENT										
TLP3B 1 0	PERMIT	******	******	******	Req. Mon.	******	******	pass=0/fall		Quarterty	COMPOS
Effluent Gross	REQUIREMENT				7 DA MIN			-1		,	
Low Flow Pass/Fall Survival Test	SAMPLE	******	******			******	******				
Static Renewal 7 Day Chronic	MEASUREMENT										
TLP6C 1 0	PERMIT	******	******	******	Req. Mon.	******	******	pass=0/fall		Quarterty	COMPOS
Effluent Gross	REQUIREMENT				7 DA MIN			-1			
NOEC Lethal Static Renewal 7 Day	8AMPLE	******	******			******	******				
Chronic Ceriodaphnia dubia	MEASUREMENT										
TOP3B 1 0	PERMIT	******	******	******	Req. Mon.	******	******	%		Quarterty	COMPOS
Effluent Gross	REQUIREMENT				7 DA MIN						
NOEC Lethal Static Renewal 7 Day	SAMPLE	******	******			******	******				
Chronic Pimephales promelas	MEASUREMENT										
TOP6C 1 0	PERMIT	******	******	******	Req. Mon.	******	******	%		Quarterty	COMPOS
Effluent Gross	REQUIREMENT				7 DA MIN					,	
NOEC Sub-Lethal Static Renewal 7	SAMPLE	******	******			******	******				
Day Chronic Ceriodaphnia dubia	MEASUREMENT										
TPP3B 1 0	PERMIT	******	******	******	Req. Mon.	******	******	%		Quarterly	COMPOS
Effluent Gross	REQUIREMENT				7 DA MIN						

NAME/TITLE PRINCIPAL EXECUTIVE OFFICER	I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel property gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the		TELEP	HONE	DATE
	system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my indicated and belief, ince, accurate, and complete. I am aware that there are significant-penalize for submitting take information, including the penalistiny of fine and imprisonment for individing submitted.	8IGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR			
TYPED OR PRINTED		AUTHORIZED AGENT	AREA Code	NUMBER	MMDDMYYY

SUMMARY REPORTING FORMS FOR CHRONIC BIOMONITORING FATHEAD MINNOW LARVAE GROWTH AND SURVIVAL Pimephales promelas

1.	Dunnett's procedure or Steel's Many-One Rank Test as appropriate: Is the mean survival at 7 days significantly different (p=0.05) than the control survival for:		
	a) LOW FLOW OR CRITICAL DILUTION, (30%) YES NOX		
2.	Dunnett's Procedure Is the mean dry weight (growth) at 7 days significantly different (p=0.05) than the control's dry weight (growth) for:		
	a) LOW FLOW OR CRITICAL DILUTION, (30%) YES NOX		
3.	If NO was answered to 1.a) enter [0] otherwise enter [1] (parameter TLP6C):0		
4.	If NO was answered to 2.a) enter [0] otherwise enter [1] (parameter TGP6C):0		
5.	Enter percentage corresponding to each parameter below:		
	a) NOEC survival (parameter TOP6C)= 40 % effluent		
	b) NOEC growth (parameter TPP6C)=		
	c) Coefficient of variation (parameter TQP6C)=6.40%		

I. Ceriodaphnia dubia	Response
(A) If the NOEC for survival is less than the critical dilution, enter a "1"; otherwise, enter a "0". Parameter No. TLP3B.	0
(B)) If the NOEC for reproduction is less than the critical dilution, enter a "1"; otherwise, enter a "0". Parameter No. TGP3B.	0
(C) Report the NOEC value for survival, Parameter No. TOP3B.	80%
(D) Report the NOEC value for reproduction, Parameter No. TPP3B.	80%
(E) Report the higher (critical dilution or control) Coefficient of Variation, Parameter No. TQP3B.	6.80%
II. Pimephales promelas (fathead minnow)	Response
,	Response 0
A) If the No Observed Effect Concentration (NOEC) for survival is less than the critical dilution, enter a "1"; otherwise, enter a "0". Parameter No. TLP6C.	
 A) If the No Observed Effect Concentration (NOEC) for survival is less than the critical dilution, enter a "1"; otherwise, enter a "0". Parameter No. TLP6C. B) If the No Observed Effect Concentration (NOEC) for growth is less than the critical dilution, enter a "1"; otherwise, enter a "0". Parameter No. TGP6C. 	0
Parameter No. TLP6C. B) If the No Observed Effect Concentration (NOEC) for growth is less than the critical dilution, enter a "1"; otherwise, enter a "0".	0

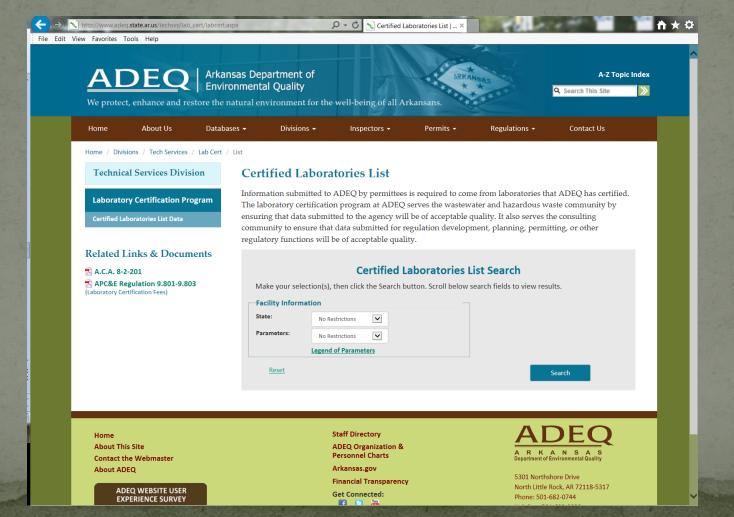
Fa	thead Minnow Larvae (Pimephales promelas) Survival and Growth
1.	FISHER'S EXACT TEST: Is the mean survival for the critical dilution (100%) at 7 days significantly different (p=0.05) than the control survival? Yes X_No
2.	DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST AS APPROPRIATE: Is the mean growth by P. promelas in the critical dilution (100%) significantly different (p=0.05) than the growth in control exposures? YesX_No
3.	If the NOEC for survival is less than the critical dilution, enter [1], otherwise enter [0] for parameter #TGP6C:0_
4.	If the NOEC for growth is less than the critical dilution, enter [1], otherwise enter [0] for parameter #TLP6C:0_
5.	Report the NOEC value for survival, Parameter #TOP6C: NOEC survival 100 % effluent
6.	Report the NOEC value for growth, Parameter #TPP6C: NOEC growth 100 % effluent
7.	Report the % coefficient of variation (largest of low flow and control dilutions), Parameter #TQP6C: CV % growth 9.3% (control)

Concern – Analysis Not Conducted

- A valid test for each species must be reported on the DMR during each reporting period
- A routine test conducted in a later reporting period can not serve as a test for the previous reporting period
- Potential Factors Resulting in Analysis Not Conducted
 - Invalid Test
 - Testing Frequency Reduction Expiring
 - Weather Related Shipping Issues
 - Sampling Issues due to Flooding, etc.
 - Lab lacking appropriate # of organisms
 - Lab certification lapse
- Suggest sampling early in the monitoring period
 - Allow Time for an additional test

Concern – Lab Certification

http://www.adeq.state.ar.us/techsvs/lab_cert/labcert.aspx



Concern – Invalid Test

- A test is invalid if these QA requirements are not satisfied:
 - Control survival ≥ 80%
 - Average # of offspring produced by surviving control C. $dubia \ge 15$
 - 60% of the surviving control *C. dubia* must produce 3 broods
 - Average dry weight of surviving Fathead minnow control ≥ 0.25 mg per larva
 - Percent coefficient of variation (%CV) between replicates shall be
 ≤40% in the control for: the sub-lethal endpoints
 - For passing endpoints: the % CV between replicates shall be ≤40% in the critical dilution for: the sub-lethal endpoints and Fathead minnow lethal endpoint
 - PMSD for *C. dubia* reproduction < 47
 - PMSD for Fathead minnow growth < 30

Concern – Invalid Test

- A test is invalid if ADEQ reviews a test with atypical results according to EPA's TSD and deems it invalid
- Invalid tests are **not** recorded as failures
- Invalid tests must have a re-test conducted

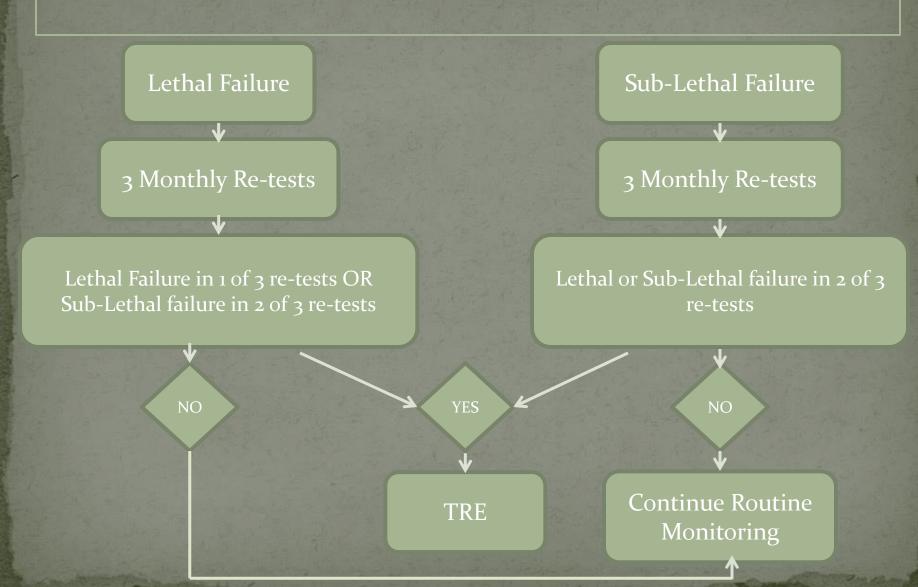
Concern – Testing Frequency Reduction

- New facility –12 quarters (3 years)
- Existing facility 4 quarters (1 year)
 - Begins on renewed permit effective date
- Send letter or e-mail requesting reduction
- Reduction expires when permit expires
 - Frequency reverts back to original frequency
 - Cannot be extended

Concern – Re-tests

- Limits
 - Monthly until passing 3 consecutive months
- Report Only
 - 3 re-tests for the species that demonstrate significant toxic effects at or below the critical dilution
 - Monthly for the next three consecutive months
 - Full report should be submitted for each re-test
 - Copy a blank DMR
 - White out and revise the test dates for the month
 - Write at the top "Re-test 1"
 - If all 3 retests fall with in a single quarter, one retest will serve as the quarterly test

Concern –Toxicity Reduction Evaluation



Concern – Toxicity Reduction Evaluation

- Within 90 days submit a TRE Action Plan & Schedule Specific approach
 - Sampling Plan
 - Reviewing Tasks
 - Quality Assurance Plan
 - Project Organization
- Submit a quarterly TRE Activities Report
- Submit a Final Report no later than 28 months from confirming toxicity in the re-tests
 - Specific corrective action schedule for implementing the selected control mechanism

Concern – Toxicity Identification

Test	Potential Toxicant Class
pH Adjustment (pH 3 & 11)	
Aeration	Volatile or oxidizable compound
Filtration	Suspended particulate phase or soluble fraction
Aeration, Filtration, & pH Adjustments	Volatility & solubility of ammonia, hydrogen sulfide and metals
C18 SPE Treatment	Non-polar organic compounds
Sodium Thiosulfate	Oxidants and certain metals
EDTA Additions	Cationic metal toxicity
Graduated pH Adjustments	Cationic metal toxicity

Concern – Toxicity Reduction Evaluation

- Goal is to determine what is causing failures
- How to remedy the situation
- When the remedy will be completed



Any Questions?

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