



Ecotoxicology Research Facility



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August 25, 2016

Jonathan Kopp  
Walnut Ridge Wastewater Treatment Plant  
216 Southwest 4<sup>th</sup> Street  
Walnut Ridge, AR 72476

Dear Jon,

Please find enclosed the results of the 7-day chronic tests using water collected from Walnut Ridge's plant facilities during the week of August 15, 2016. No lethal or sublethal effects were measured in *Pimephales promelas* or *Ceriodaphnia dubia* exposed to the critical flow concentration (100%) or other treated dilutions from this outfall.

All test conditions and acceptability criteria as suggested by our laboratory and the US EPA were met during these tests.

Please call if you have any questions regarding this particular test series or any other tests conducted in the past.

Sincerely,

Jennifer L. Bouldin, PhD  
Director Ecotoxicology Research Facility  
PO Box 847  
Arkansas State University  
State University, AR 72467

**Arkansas State University Ecotoxicology Research Facility**

**Laboratory Report**

Facility Director / Lab Contact: Jennifer Bouldin  
 Phone: (870) 972-2570

Client: Walnut Ridge Wastewater Treatment Plant  
 216 Southwest 4th Street  
 Walnut Ridge, AR 72476

Contact: Jon Kopp  
 870-866-2312

NPDES Permit #: AR0046566 AFIN#: 38-00040

Effluent Sampling Point/Type: 24hr Composite

Samples Collected:

Sample #	Sampling Times	Received	Arrival Temp
1	08/14/16 0900 hrs to 08/15/16 0900 hrs	08/15/16 1130 hrs	3.0 °C
2	08/16/16 0900 hrs to 08/17/16 0900 hrs	08/17/16 1210 hrs	3.5 °C
3	08/18/16 0900 hrs to 08/19/16 0900 hrs	08/19/16 1240 hrs	0.7 °C

**Test Methods:**

7-Day Chronic Toxicity, Static renewal, Cladoceran, EPA 821/R-02/013, Section 13  
 7-Day Chronic Toxicity, Static renewal, Fathead minnow, EPA 821/R-02/013, Section 11

Organisms: *C. dubia* <24hrs, *P. promelas* <24hrs

Culture Source: ASU Ecotox

Dilutions: 0%, 32%, 42%, 56%, 80%, 100%

Critical Dilution: 100%

Statistical Method: Toxcalc 5.0.25

<i>C. dubia</i>			<i>P. promelas</i>		
whole effluent toxicity			whole effluent toxicity		
	lethality	sublethality		lethality	sublethality
DMR Code	22414 10	22414 P0	DMR Code	22414 10	22414 P0
Result	100%	100%	Result	100%	100%
	lethality	sublethality		lethality	sublethality
DMR Code	TGP3B	TLP3B	DMR Code	TGP6C	TLP6C
Result	0	0	Result	0	0
	NOEC lethality	NOEC sublethal		NOEC lethality	NOEC sublethal
DMR Code	TOP3B	TPP3B	DMR Code	TOP6C	TPP6C
Result	100%	100%	Result	100%	100%
	CV%			CV%	
DMR Code	TQP3B		DMR Code	TQP6C	
Result	22.7%		Result	7.4%	
	control survival	control mean reproduction		control survival	control mean weight
	100%	22.8		100%	0.5352
	critical dil. survival	critical mean reproduction		critical dil. survival	critical mean weight
	100%	29.0		100%	0.5777
				MSDp	
				0.1038	

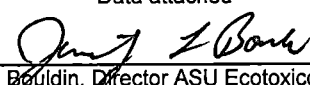
**Results Summary:**

No lethal or sublethal effects were measured in *C. dubia* or *P. promelas*.

**QA/Reference Testing:**

Data attached

**Reviewed By:**

  
 Jennifer L. Bouldin, Director ASU Ecotoxicology Research Facility

Toxicity Test Performed: 7-day *Ceriodaphnia dubia* Survival and Reproduction  
 Effluent Sampling Point: Walnut Ridge WWT Plant  
 Date Test Started: 08/15/16 *C. dubia*  
 Time Test Started: 1445 *C. dubia*  
 Date Test Terminated: 08/22/16 *C. dubia*  
 Time Test Terminated: 1345 *C. dubia*  
 Laboratory Analyst: Cooper/Sweeney

Toxicity Test Performed: 7-day *Pimephales promelas* Survival and Growth  
 Effluent Sampling Point: Walnut Ridge WWT Plant  
 Date Test Started: 08/15/16 *P. promelas*  
 Time Test Started: 1450 *P. promelas*  
 Date Test Terminated: 08/22/16 *P. promelas*  
 Time Test Terminated: 1445 *P. promelas*  
 Laboratory Analyst: McCauley/Moland

## I. Test Methods

### A. Physical and Chemical Testing - APHA, Standard Methods for the Examination of Water and Wastewater; Vol. 21, 2005.

<u>Test</u>	<u>Method</u>
Alkalinity	2320B
Conductivity	2510B
Dissolved Oxygen (mg/L, DO)	4500-O-G
Hardness (mg/L CaCO <sub>3</sub> )	2340C
pH	4500-H <sup>+</sup> B
Temperature (°C)	2550B

### B. Toxicity Testing – EPA 821/R-02/013: Short Term Methods for Estimating the Chronic Toxicity of Effluents to Freshwater Organisms

<u>Test</u>	<u>Method</u>
Cladoceran Survival and Reproduction	Section 13
Fathead Minnow Survival and Growth	Section 11

## II. Test Organisms

A. Name: *Ceriodaphnia dubia* (Cladoceran)  
 Source: Laboratory Culture  
 Age: <24 hours  
 Life Stage: Neonate

- B. Name: *Pimephales promelas* (Fathead minnow)  
Source: Laboratory Culture  
Age: <24 hours  
Life Stage: Larval

### III. External Factors

#### A. Incubator

Temperature (°C)

Average: 25.0

Range: 25.0-25.2

Light Cycle: 16 hours light/ 8 hours dark

Light Intensity: 100 footcandles

Control Water: Moderately Hard Synthetic Water (#MH 973)

#### B. *Ceriodaphnia dubia*

Test Chambers: 30 ml Solo cups

Volume per Chamber: 15-20 ml

Number of Organisms per Chamber: 1

Number of Replicates per Concentration: 10

Acclimation: Laboratory control water was added to cultures until >50% of the culture water consisted of control water.

Food: Cladocera were fed *Selenastrum* (#ABS 080716) and yeast/cereal/trout chow mix (#YCT 060716) one hour prior to test setup and once daily thereafter.

#### C. *Pimephales promelas*

Test Chambers: 250 ml storage dishes

Volume per Chamber: 200 ml

Number of Organisms per Chamber: 8

Number of Replicates per Concentration: 5

Acclimation: Laboratory control water was added to cultures until >50% of the culture water consisted of control water.

Food: Larval fish were fed 0.15ml of laboratory-cultured *Artemia* brine shrimp one hour prior to test setup and then 3X daily thereafter.

## IV. Quality Assurance

## A. Standard Toxicant: Sodium Chloride

B. Organism: *Ceriodaphnia dubia*

Date and time of Reference Toxicant Test

Start: 08/15/16

Terminated: 08/22/16

Time of Reference Toxicant Test

Start: 1520

Terminated: 1500

Laboratory Analyst: McCauley

Dilution Water Used: Moderately Hard Synthetic Water #973

Results: Survival and Reproduction were within control limits

Survival

LOEC: 2.60 g/L NaCl

EC50: 2.04 g/L NaCl

Reproduction

LOEC: 0.62 g/L NaCl

IC25: 0.30 g/L NaCl

C. Organism: *Pimephales promelas*

Date of Reference Toxicant Test

Start: 08/08/16

Terminated: 08/15/16

Time of Reference Toxicant Test

Start: 1410

Terminated: 1515

Laboratory Analyst: McCauley

Dilution Water Used: Moderately Hard Synthetic Water #973

Results: Survival and Growth were within control limits.

Survival

LOEC: 5.63 g/L NaCl

EC50: 5.47 g/L NaCl

Growth

LOEC: &gt;5.63 g/L NaCl

IC25: &gt;5.63 g/L NaCl

V. Physical and Chemical Data - See Attached

VI. Survival and Growth Data - See Attached

VII. Statistical Methods - See Attached

VIII. NPDES Permit Results - See Attached

**SUMMARY REPORTING FORM**  
**WET Testing**  
*Ceriodaphnia dubia* Survival and Reproduction

Permittee: Walnut Ridge WWT Plant

NPDES No.: AR0046566

		<u>Time</u>	<u>Date</u>	to	<u>Time</u>	<u>Date</u>
Composite 1:	Collected from	0900	08/14/16		0900	08/15/16
Composite 2:	Collected from	0900	08/16/16		0900	08/17/16
Composite 3:	Collected from	0900	08/18/16		0900	08/19/16

Test Initiated: 1445  
 Time Terminated: 1345  
 Dilution H<sub>2</sub>O: MH 973

Date: 08/15/16

Date: 08/22/16

**PERCENT SURVIVAL**

Percent Effluent

<u>Time of Reading</u>	<u>Control</u>	<u>32%</u>	<u>42%</u>	<u>56%</u>	<u>80%</u>	<u>100%</u>
24h	100	100	100	100	100	100
48h	100	100	100	100	100	100
7 day	100	100	100	100	90	100

**NUMBER OF YOUNG/FEMALE @ 7 DAYS**

Percent Effluent

<b>REP</b>	<u>0%</u>	<u>32%</u>	<u>42%</u>	<u>56%</u>	<u>80%</u>	<u>100%</u>
A	25	32	24	35	37	30
B	24	22	34	33	30	34
C	22	30	30	36	33	33
D	26	27	34	33	33	15
E	23	31	31	34	X/O	27
F	21	35	31	25	34	37
G	21	32	30	28	33	33
H	28	30	31	28	30	32
I	28	33	27	31	35	25
J	10	31	32	38	32	24
<b>Mean</b>	<b>22.8</b>	<b>30.3</b>	<b>30.4</b>	<b>32.1</b>	<b>33.0</b>	<b>29.0</b>
<b>CV%*</b>	<b>22.7</b>	<b>11.9</b>	<b>10.0</b>	<b>12.7</b>	<b>6.8</b>	<b>22.2</b>

\*Coefficient of Variation% = Standard Deviation x 100/Mean

### ***Ceriodaphnia dubia* Survival and Reproduction**

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  No
  
2. DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST AS APPROPRIATE:  
Is the mean number of young produced per female by the critical dilution (100%) significantly different ( $p=0.05$ ) than the control's number of young per female?  
 Yes  No
  
3. If the NOEC for survival is less than the critical dilution, enter [1], otherwise enter [0] for parameter #TGP3B:   0
  
4. If the NOEC for reproduction is less than the critical dilution, enter [1], otherwise enter [0] for parameter #TLP3B:   0
  
5. Report the NOEC value for survival, Parameter #TOP3B:  
NOEC survival  100%  effluent
  
6. Report the NOEC value for reproduction, Parameter #TPP3B:  
NOEC reproduction  100%  effluent
  
7. Report the % coefficient of variation (largest of critical and control dilutions), Parameter #TQP3B:  
CV % reproduction  22.7  % (control)

### **Whole Effluent Lethality Values for *Ceriodaphnia dubia***

1. Report the Whole Effluent Lethality values for the 30-Day average minimum, Parameter #22414:  
Daily Average Minimum NOEC:  100%
  
2. Report the Whole Effluent Lethality values for the 7-day minimum, Parameter #22414:  
7-Day Minimum NOEC:  100%

**WET Testing Summary Form**  
***Ceriodaphnia dubia* (Cladoceran)**  
**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant

Sample No. 1 Collected Ending Date: 08/15/16 Time: 0900

NPDES No.: AR0046566

Sample No. 2 Collected Ending Date: 08/17/16 Time: 0900

Contact: Jon Kopp

Sample No. 3 Collected Ending Date: 08/19/16 Time: 0900

Analyst: Cooper/Sweeney

Test Begin: Date: 08/15/16 Time: 1445 Test End: Date: 08/22/16 Time: 1345

Initial Water Chemistry for Chronic Tests								
Project: Walnut Ridge – <i>C. dubia</i>								
Test day		0	1	2	3	4	5	6
Date		8/15/2016	8/16/2016	8/17/2016	8/18/2016	8/19/2016	8/20/2016	8/21/2016
H <sub>2</sub> O #		MH973	MH973	MH973	MH973	MH973	MH973	MH973
Temp (°C)	Control	21.5	23.5	22.8	22.9	22.8	23.8	23.5
	32%	21.8	24.0	22.6	22.8	22.8	23.5	23.3
	42%	22.8	23.6	22.6	22.8	22.8	23.7	23.2
	56%	22.0	23.7	23.0	22.9	22.9	23.9	23.5
	80%	22.1	23.7	23.0	23.0	23.0	23.9	23.4
	100%	22.4	23.7	23.2	23.0	23.1	23.9	23.4
pH (Standard Units)	Control	8.16	7.97	8.12	8.13	8.16	8.15	7.81
	32%	8.18	8.15	8.18	8.18	8.27	8.25	7.72
	42%	8.16	8.12	8.19	8.16	8.26	8.25	7.71
	56%	8.15	8.11	8.19	8.17	8.28	8.23	7.71
	80%	8.17	8.21	8.21	8.14	8.32	8.29	7.67
	100%	8.16	8.22	8.22	8.09	8.34	8.27	7.91
DO (mg/L)	Control	8.7	8.8	8.7	8.9	8.8	8.7	8.4
	32%	8.6	8.7	8.7	8.9	8.8	8.8	8.6
	42%	8.5	8.6	8.6	8.7	8.6	8.8	8.6
	56%	8.5	8.6	8.5	8.7	8.7	8.6	8.5
	80%	8.4	8.3	8.5	8.6	8.6	8.6	8.6
	100%	8.3	8.1	8.4	8.5	8.5	8.6	8.7
Cond (µS/cm)	Control	310	307	307	305	308	305	318
	32%	262	262	258	258	321	319	334
	42%	247	247	243	243	325	325	341
	56%	225	225	221	220	332	332	349
	80%	188	187	183	182	345	344	363
	100%	158	161	150	150	359	357	375
Alk (mg/L)	Control	57		57		57		
	100%	94		80		206		
Hard (mg/L)	Control	80		80		80		
	100%	150		100		230		



**WET Testing Summary Form**  
***Ceriodaphnia dubia* (Cladoceran)**  
**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant      Sample No. 1 Collected    Ending Date: 08/15/16    Time: 0900  
 NPDES No.: AR0046566                              Sample No. 2 Collected    Ending Date: 08/17/16    Time: 0900  
 Contact: Jon Kopp                                      Sample No. 3 Collected    Ending Date: 08/19/16    Time: 0900  
 Analyst: Cooper/Sweeney                          Test Begin: Date: 08/15/16    Time: 1445    Test End: Date: 08/22/16 Time: 1345

<b>Final Water Chemistry for Chronic Tests</b>								
Project: Walnut Ridge - <i>C. dubia</i>								
Test day		1	2	3	4	5	6	7
Date:		8/16/2016	8/17/2016	8/18/2016	8/19/2016	8/20/2016	8/21/2016	8/22/2016
H <sub>2</sub> O #		MH973	MH973	MH973	MH973	MH973	MH973	MH973
Temp (°C)	Control	23.3	23.0	23.1	23.8	23.2	23.8	24.5
	32%	23.7	23.3	23.3	23.4	23.2	23.3	24.4
	42%	23.7	23.4	23.0	23.0	23.1	23.4	24.3
	56%	23.8	23.4	23.0	23.3	23.2	23.8	24.1
	80%	23.8	23.4	23.2	23.0	23.2	23.3	24.0
	100%	23.9	23.5	23.2	23.1	23.2	23.4	24.0
pH (Standard Units)	Control	8.29	8.49	8.17	8.38	8.41	8.41	8.24
	32%	8.40	8.58	8.29	8.56	8.64	8.60	8.49
	42%	8.41	8.63	8.34	8.58	8.66	8.69	8.55
	56%	8.46	8.65	8.35	8.65	8.74	8.71	8.63
	80%	8.46	8.76	8.36	8.73	8.76	8.81	8.66
	100%	8.50	8.79	8.37	8.73	8.79	8.84	8.72
DO (mg/L)	Control	8.8	8.8	8.9	9.0	8.8	9.1	8.8
	32%	8.5	8.8	8.6	8.8	8.8	8.9	8.8
	42%	8.7	8.8	8.6	8.9	8.9	9.1	8.8
	56%	8.5	8.8	8.6	9.0	8.8	9.1	8.8
	80%	8.6	8.9	8.6	9.1	8.7	9.0	8.8
	100%	8.6	8.9	8.5	9.1	8.8	9.2	8.8

**SUMMARY REPORTING FORM**

**WET Testing**

**Fathead Minnow Larvae (*Pimephales promelas*) Survival and Growth**

Permittee: Walnut Ridge WWT Plant

NPDES No.: AR0046566

		<u>Time</u>	<u>Date</u>	to	<u>Time</u>	<u>Date</u>
Composite 1:	Collected from	0900	08/14/16		0900	08/15/16
Composite 2:	Collected from	0900	08/16/16		0900	08/17/16
Composite 3:	Collected from	0900	08/18/16		0900	08/19/16

Test Initiated: 1450

Date: 08/15/16

Time Terminated: 1445

Date: 08/22/16

Dilution H<sub>2</sub>O: MH 973

**DATA TABLE FOR SURVIVAL**

Effluent Conc. %	% Survival in Replicate Chambers					Mean % Survival			CV%
	A	B	C	D	E	24h	48h	7 days	
Control	100	100	100	100	100	100	100	100	0.0
32	100	100	87.5	100	100	100	100	97.5	6.1
42	100	100	100	100	100	100	100	100	0.0
56	100	87.5	100	100	100	100	100	97.5	6.1
80	87.5	100	100	87.5	100	100	100	95	7.6
100	100	100	100	100	100	100	100	100	0.0

**DATA TABLE FOR GROWTH**

Effluent Conc %	Average Dry Weight in Replicate Chambers (mg)					Mean Dry Weight (mg)	CV%
	A	B	C	D	E		
Control	0.5400	0.5863	0.5575	0.4912	0.5012	0.5352	7.4
32	0.5475	0.5400	0.5929	0.5113	0.5625	0.5508	5.4
42	0.5950	0.5387	0.4975	0.6188	0.5087	0.5518	9.6
56	0.5950	0.5900	0.5962	0.6363	0.5525	0.5940	5.0
80	0.5986	0.5725	0.5588	0.6029	0.5488	0.5763	4.1
100	0.5775	0.6237	0.5938	0.5787	0.5150	0.5777	6.9

Coefficient of Variation = Standard Deviation x 100/Mean

## Fathead 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  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?

Yes  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  7.4%  (control)

## Whole Effluent Lethality Values

1. Report the Whole Effluent Lethality values for the 30-Day average minimum, Parameter #22414:

Daily Average Minimum NOEC:  100% 

2. Report the Whole Effluent Lethality values for the 7-day minimum, Parameter #22414:

7-Day Minimum NOEC:  100%

**WET Testing Summary Form**  
**Fathead Minnow Larvae (*Pimephales promelas*)**

**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant      Sample No. 1 Collected Ending Date: 08/15/16 Time: 0900  
 NPDES No.: AR0046566      Sample No. 2 Collected Ending Date: 08/17/16 Time: 0900  
 Contact: Jon Kopp      Sample No. 3 Collected Ending Date: 08/19/16 Time: 0900  
 Analyst: McCauley/Moland      Test Begin: Date: 08/15/16 Time: 1450 Test End: Date: 08/22/16 Time: 1620

Initial Water Chemistry for Chronic Tests								
Project: Walnut Ridge – <i>P. promelas</i>								
Test day		1	2	3	4	5	6	7
Date		8/15/2016	8/16/2016	8/17/2016	8/18/2016	8/19/2016	8/20/2016	8/21/2016
H <sub>2</sub> O #		MH973	MH973	MH973	MH973	MH973	MH973	MH973
Temp (°C)	Control	21.5	23.5	22.8	22.9	22.8	23.8	23.5
	32%	21.8	24.0	22.6	22.8	22.8	23.5	23.3
	42%	22.8	23.6	22.6	22.8	22.8	23.7	23.2
	56%	22.0	23.7	23.0	22.9	22.9	23.9	23.5
	80%	22.1	23.7	23.0	23.0	23.0	23.9	23.4
	100%	22.4	23.7	23.2	23.0	23.1	23.9	23.4
pH (Standard Units)	Control	8.16	7.97	8.12	8.13	8.16	8.15	7.81
	32%	8.18	8.15	8.18	8.18	8.27	8.25	7.72
	42%	8.16	8.12	8.19	8.16	8.26	8.25	7.71
	56%	8.15	8.11	8.19	8.17	8.28	8.23	7.71
	80%	8.17	8.21	8.21	8.14	8.32	8.29	7.67
	100%	8.16	8.22	8.22	8.09	8.34	8.27	7.91
DO (mg/L)	Control	8.7	8.8	8.7	8.9	8.8	8.7	8.4
	32%	8.6	8.7	8.7	8.9	8.8	8.8	8.6
	42%	8.5	8.6	8.6	8.7	8.6	8.8	8.6
	56%	8.5	8.6	8.5	8.7	8.7	8.6	8.5
	80%	8.4	8.3	8.5	8.6	8.6	8.6	8.6
	100%	8.3	8.1	8.4	8.5	8.5	8.6	8.7
Cond (µS/cm)	Control	310	307	307	305	308	305	318
	32%	262	262	258	258	321	319	334
	42%	247	247	243	243	325	325	341
	56%	225	225	221	220	332	332	349
	80%	188	187	183	182	345	344	363
	100%	158	161	150	150	359	357	375
Alk (mg/L)	Control	57		57		57		
	100%	94		80		206		
Hard (mg/L)	Control	80		80		80		
	100%	150		100		230		

**WET Testing Summary Form**  
**Fathead Minnow Larvae (*Pimephales promelas*)**  
**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant      Sample No. 1 Collected Ending Date: 08/15/16    Time: 0900  
 NPDES No.: AR0046566                              Sample No. 2 Collected Ending Date: 08/17/16    Time: 0900  
 Contact: Jon Kopp                                      Sample No. 3 Collected Ending Date: 08/19/16    Time: 0900  
 Analyst: McCauley/Moland    Test Begin: Date: 08/22/16    Time: 1450    Test End: Date: 08/22/16    Time: 1445

Final Water Chemistry for Chronic Tests								
Project: Walnut Ridge – <i>P. promelas</i>								
Test day		1	2	3	4	5	6	7
Date		8/16/2016	8/17/2016	8/18/2016	8/19/2016	8/20/2016	8/21/2016	8/22/2016
H <sub>2</sub> O #		MH973	MH973	MH973	MH973	MH973	MH973	MH973
Temp (°C)	Control	21.9	22.5	22.9	23.0	22.8	22.5	23.4
	32%	22.8	22.8	22.9	22.5	23.0	22.5	23.5
	42%	22.9	22.9	23.0	23.5	23.0	22.8	23.3
	56%	22.9	23.0	23.0	24.0	23.0	22.8	23.4
	80%	22.9	22.5	23.0	24.0	23.0	22.8	23.4
	100%	22.5	23.5	23.0	24.0	23.2	22.8	23.3
pH (Standard Units)	Control	7.97	7.80	7.76	7.77	7.77	7.67	7.68
	32%	8.00	7.82	7.81	7.74	7.94	7.84	7.96
	42%	8.00	7.85	7.87	7.81	8.04	7.97	7.94
	56%	8.00	7.84	7.88	7.77	8.02	7.60	8.08
	80%	8.02	7.94	7.88	7.80	8.08	8.10	8.20
	100%	8.05	8.00	7.93	7.82	8.20	8.22	8.23
DO (mg/L)	Control	8.2	7.6	7.7	7.4	7.9	7.7	7.3
	32%	8.0	7.2	7.5	7.3	7.6	7.4	7.1
	42%	7.8	7.1	7.7	7.0	7.3	7.7	6.7
	56%	7.8	7.0	7.9	6.9	7.3	7.5	7.3
	80%	7.7	7.2	7.9	6.7	7.2	7.3	7.6
	100%	7.8	7.4	7.7	6.7	7.2	7.5	6.9

**Ceriodaphnia Survival and Reproduction Test-7 Day Survival**

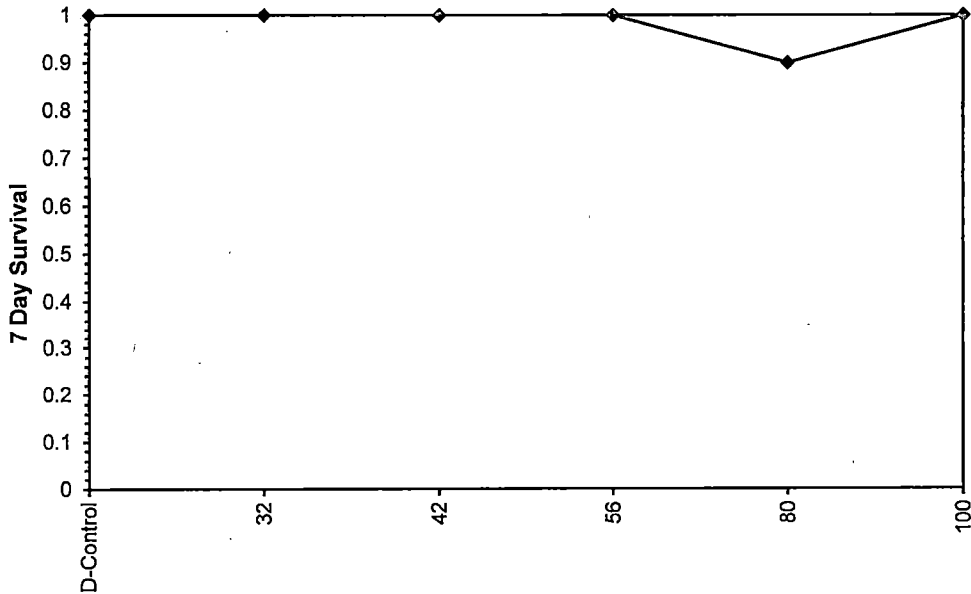
Start Date: 8/15/2016 14:45    Test ID: W. Ridge    Sample ID: AR0046566-NPDES Permit #  
 End Date: 8/22/2016 13:45    Lab ID: ASU ERF    Sample Type: EFF1-POTW  
 Sample Date:    Protocol: EPAF 02-EPA Freshwater    Test Species: CD-Ceriodaphnia dubia  
 Comments:

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
32	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
42	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
56	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
80	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical
D-Control	1.0000	1.0000	0	10	10	10		
32	1.0000	1.0000	0	10	10	10	1.0000	0.0500
42	1.0000	1.0000	0	10	10	10	1.0000	0.0500
56	1.0000	1.0000	0	10	10	10	1.0000	0.0500
80	0.9000	0.9000	1	9	10	10	0.5000	0.0500
100	1.0000	1.0000	0	10	10	10	1.0000	0.0500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	100	>100		1

**Dose-Response Plot**



**Ceriodaphnia Survival and Reproduction Test-Reproduction**

Start Date: 8/15/2016 14:45    Test ID: W. Ridge    Sample ID: AR0046566-NPDES Permit #  
 End Date: 8/22/2016 13:45    Lab ID: ASU ERF    Sample Type: EFF1-POTW  
 Sample Date:    Protocol: EPAF 02-EPA Freshwater    Test Species: CD-Ceriodaphnia dubia  
 Comments:

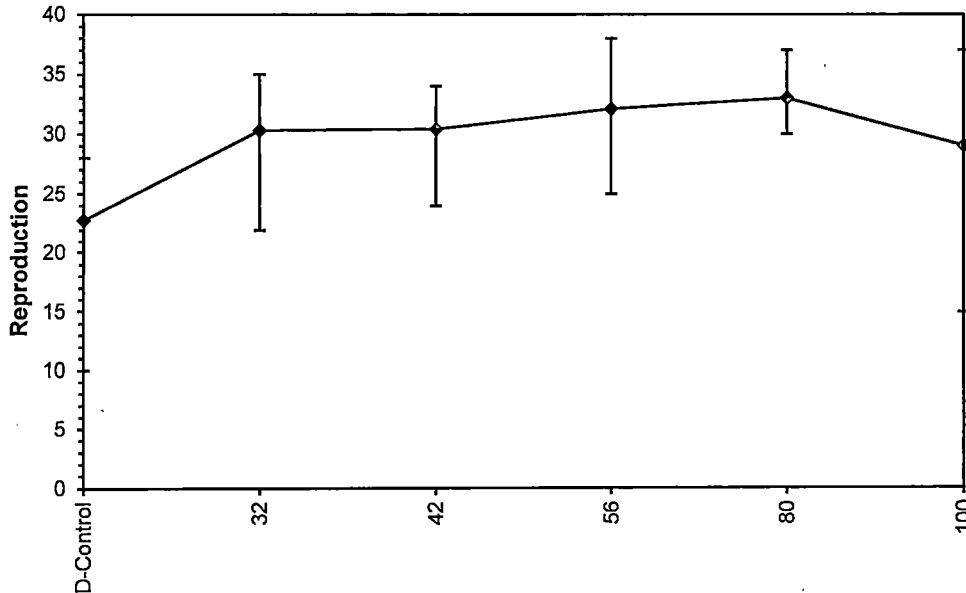
Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	25.000	24.000	22.000	26.000	23.000	21.000	21.000	28.000	28.000	10.000
32	32.000	22.000	30.000	27.000	31.000	35.000	32.000	30.000	33.000	31.000
42	24.000	34.000	30.000	34.000	31.000	31.000	30.000	31.000	27.000	32.000
56	35.000	33.000	36.000	33.000	34.000	25.000	28.000	28.000	31.000	38.000
80	37.000	30.000	33.000	33.000	34.000	33.000	30.000	35.000	32.000	
100	30.000	34.000	33.000	15.000	27.000	37.000	33.000	32.000	25.000	24.000

Conc-%	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%	N		
D-Control	22.800	1.0000	22.800	10.000	28.000	22.724	10		
32	30.300	1.3289	30.300	22.000	35.000	11.854	10	146.50	74.00
42	30.400	1.3333	30.400	24.000	34.000	9.953	10	148.50	74.00
56	32.100	1.4079	32.100	25.000	38.000	12.671	10	149.50	74.00
80	33.000	1.4474	33.000	30.000	37.000	6.776	9	135.00	61.00
100	29.000	1.2719	29.000	15.000	37.000	22.169	10	136.00	74.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.08644	1.035	-1.19225	2.34206
Bartlett's Test indicates equal variances (p = 0.05)	11.0704	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test	100	>100		1

**Dose-Response Plot**



**CHRONIC TEST DATA SHEET**  
*Ceriodaphnia dubia*

Project: Walnut Ridge Beginning Date: 081516 Time: 1445 Test Species: C. dubia  
Dilution H<sub>2</sub>O: MH973 Ending Date: 082216 Time: 1345 Age: < 24 hours

Test Type: ( \* )Static Renewal ( ) Flowthrough Toxicant/Effluent:

Conc.	Rep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Neonates
Control	1	0	0	0	4	0	7	14	25
	2			0	4	0	8	12	24
	3			0	3	0	6	13	22
	4			0	5	0	8	13	20
	5			0	1	0	9	13	23
	6			3	0	9	9	0	21
	7			0	3	0	7	11	21
	8				5	0	11	12	28
	9				4	0	9	15	28
	10	✓	✓	✓	4	0	6	0	10
32%	1	0	0	0	4	0	10	18	32
	2				4	0	7	11	22
	3				3	0	12	15	30
	4				3	1	10	13	27
	5				4	0	11	16	31
	6				3	0	13	19	35
	7				3	1	11	17	32
	8				3	0	10	17	30
	9				4	0	11	18	33
	10	✓	✓	✓	4	0	12	15	31
Date		0816/16	0817/16	0818/16	0819/16	8/20/16	0821/16	0822/16	0824/16
Initials		HGS	GS	GS	RLC	JP	RLC	RLC	ALN



**CHRONIC TEST DATA SHEET**  
*Ceriodaphnia dubia*

Project: Walnut Ridge Beginning Date: 081516 Time: 1445 Test Species: C. dubia  
Dilution H<sub>2</sub>O: MHA73 Ending Date: 082216 Time: 1345 Age: <24 hours

Test Type: (  ) Static Renewal (  ) Flowthrough Toxicant/ Effluent

Conc.	Rep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Neonates
42%	1	0	0	0	4	0	10	10	24
	2	↓	↓	↓	3	0	14	17	34
	3	↓	↓	↓	3	1	11	16	30
	4	↓	↓	↓	4	0	13	17	34
	5	↓	↓	↓	4	0	9	18	31
	6	↓	↓	↓	5	0	10	16	31
	7	↓	↓	↓	3	3	9	15	30
	8	↓	↓	↓	4	0	9	18	31
	9	↓	↓	↓	3	0	9	15	27
	10	↓	↓	↓	3	0	13	16	32
56%	1	0	0	0	4	2	10	19	35
	2	↓	↓	↓	4	0	12	17	33
	3	↓	↓	↓	6	0	14	16	36
	4	↓	↓	↓	2	0	15	16	33
	5	↓	↓	↓	7	0	14	13	34
	6	↓	↓	↓	4	0	6	15	25
	7	↓	↓	↓	3	0	11	14	28
	8	↓	↓	↓	3	0	10	15	28
	9	↓	↓	↓	4	0	9	18	31
	10	↓	↓	↓	6	0	13	19	38
Date		081616	081716	081816	081916	082016	082116	082216	082416
Initials		GS	GS	GS	RIC	GS	RIC	RIC	Dem

**CHRONIC TEST DATA SHEET**  
*Ceriodaphnia dubia*

Project: Walnut Ridge Beginning Date: 081516 Time: 1445 Test Species: C. dubia  
Dilution H<sub>2</sub>O: MH973 Ending Date: 082216 Time: 1345 Age: < 24 hours

Test Type: ( \* )Static Renewal ( ) Flowthrough Toxicant/Effluent:

Conc.	Rep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Neonates
80%	1	0	0	0	3	0	16	18	37
	2				4	0	11	15	30
	3				3	1	11	18	33
	4				4	0	13	16	33
	5				5	1	X/0		6
	6				5	0	11	18	<del>34</del>
	7				4	0	13	16	33
	8				5	0	15	20	30
	9				4	0	12	19	35
	10		↓	↓	↓	4	0	11	17
100%	1	0	0	0	4	0	10	16	30
	2				4	0	12	18	34
	3				4	0	11	18	33
	4				<del>3</del> 0	6	0	9	15
	5				0	0	13	14	27
	6				5	0	15	17	37
	7				4	1	9	19	33
	8				3	0	11	18	32
	9				4	0	10	11	25
	10		↓	↓	↓	0	10	14	0
Date		081616	081716	081816	081916	8/20/16	082116	082216	082416
Initials		gs	gs	gs	RLC	gs	RLC	RLC	den

**Larval Fish Growth and Survival Test-7 Day Survival**

Start Date: 8/15/2016 14:50	Test ID: W. Ridge	Sample ID: AR0046566-NPDES Permit #
End Date: 8/22/2016 14:45	Lab ID: ASU ERF	Sample Type: EFF1-POTW
Sample Date:	Protocol: EPAF 02-EPA Freshwater	Test Species: PP-Pimephales promelas

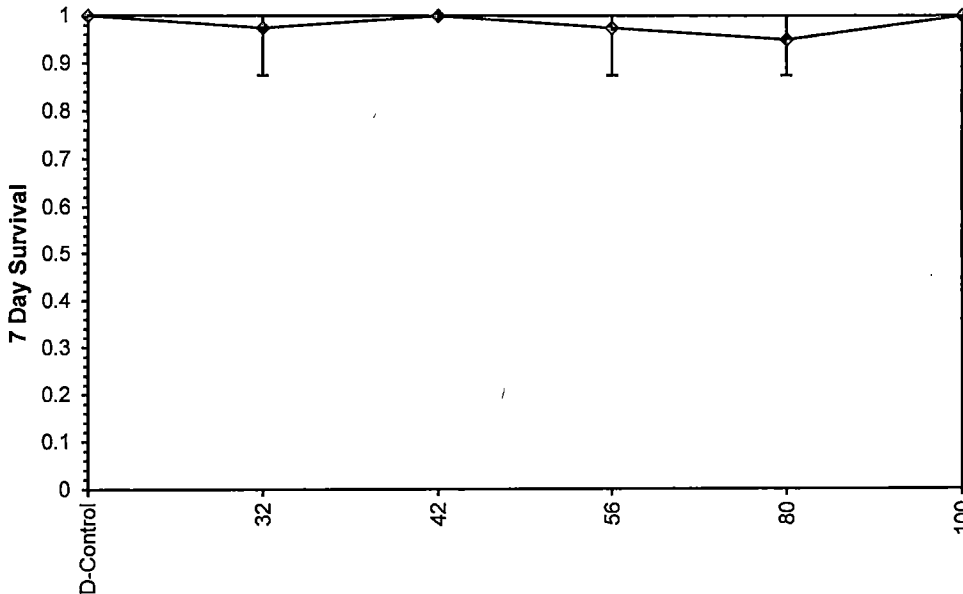
Comments:

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
32	1.0000	1.0000	0.8750	1.0000	1.0000
42	1.0000	1.0000	1.0000	1.0000	1.0000
56	1.0000	0.8750	1.0000	1.0000	1.0000
80	0.8750	1.0000	1.0000	0.8750	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%	N		
D-Control	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5		
32	0.9750	0.9750	1.3564	1.2094	1.3931	6.055	5	25.00	16.00
42	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	16.00
56	0.9750	0.9750	1.3564	1.2094	1.3931	6.055	5	25.00	16.00
80	0.9500	0.9500	1.3196	1.2094	1.3931	7.623	5	22.50	16.00
100	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	16.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.76012	0.9	-1.47777	1.97749
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1

**Dose-Response Plot**



**Larval Fish Growth and Survival Test-7 Day Growth**

Start Date: 8/15/2016 14:50	Test ID: W. Ridge	Sample ID: AR0046566-NPDES Permit #
End Date: 8/22/2016 14:45	Lab ID: ASU ERF	Sample Type: EFF1-POTW
Sample Date:	Protocol: EPAF 02-EPA Freshwater	Test Species: PP-Pimephales promelas

Comments:

Conc-%	1	2	3	4	5
D-Control	0.5400	0.5863	0.5575	0.4912	0.5012
32	0.5475	0.5400	0.5929	0.5113	0.5625
42	0.5950	0.5387	0.4975	0.6188	0.5087
56	0.5950	0.5900	0.5962	0.6363	0.5525
80	0.5986	0.5725	0.5588	0.6029	0.5488
100	0.5775	0.6237	0.5938	0.5787	0.5150

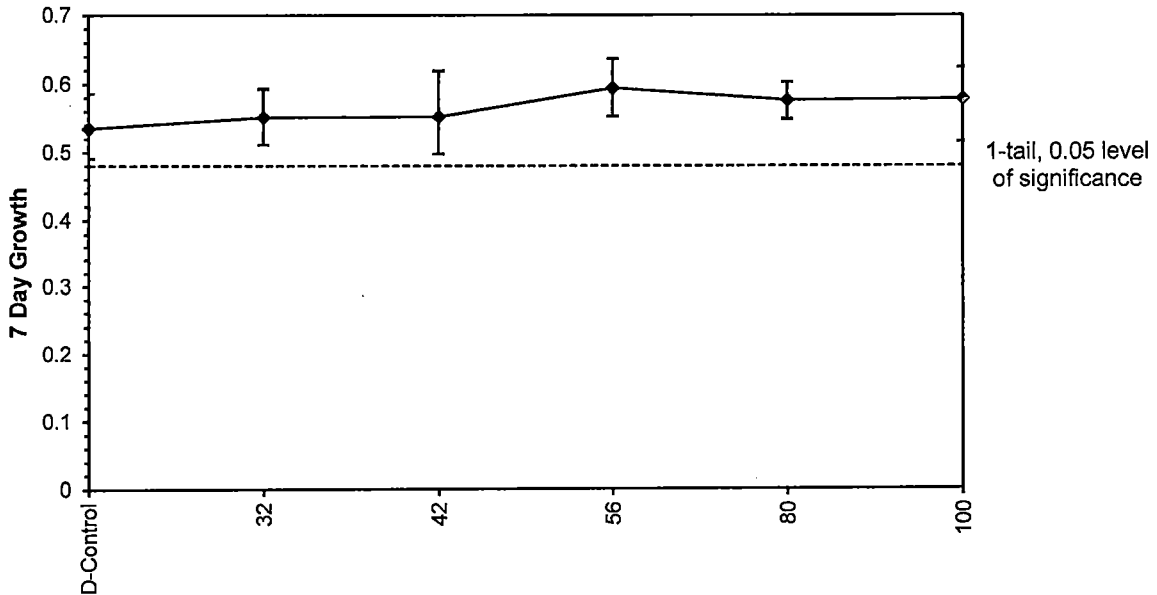
Conc-%	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed Critical	MSD
			Mean	Min	Max	CV%	N			
D-Control	0.5352	1.0000	0.5352	0.4912	0.5863	7.362	5			
32	0.5508	1.0291	0.5508	0.5113	0.5929	5.444	5	-0.661	2.360	0.0556
42	0.5518	1.0308	0.5518	0.4975	0.6188	9.642	5	-0.701	2.360	0.0556
56	0.5940	1.1098	0.5940	0.5525	0.6363	5.001	5	-2.496	2.360	0.0556
80	0.5763	1.0767	0.5763	0.5488	0.6029	4.145	5	-1.743	2.360	0.0556
100	0.5777	1.0794	0.5777	0.5150	0.6237	6.875	5	-1.805	2.360	0.0556

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9711	0.9	0.03229	-0.72134
Bartlett's Test indicates equal variances (p = 0.71)	2.96089	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.05556	0.1038	0.00239	0.00139	0.16724	5, 24

**Dose-Response Plot**



**CHRONIC TEST DATA SHEET**  
*Pimephales promelas*

Project: Walnut Ridge Beginning Date: 081516 Time: 1450 Test Species: *P. promelas*  
 Dilution H<sub>2</sub>O: 1:14973 Ending Date: 082216 Time: 1445 Age: < 24hrs

Test Type: ( \* )Static Renewal ( ) Flowthrough Toxicant/Effluent:

Conc.	Rep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Pan #
Control	1 *	8/0	8/0	8/0	8/0	8/0	8/0	8/0	1
	2	8/0	8/0	8/0	8/0	8/0	8/0	8/0	2
	3	8/0	8/0	8/0	8/0	8/0	8/0	8/0	3
	4	8/0	8/0	8/0	8/0	8/0	8/0	8/0	4
	5	8/0	8/0	8/0	8/0	8/0	8/0	8/0	5
32%	1	8/0	8/0	8/0	8/0	8/0	8/0	8/0	6
	2	8/0	8/0	8/0	8/0	8/0	8/0	8/0	7
	3	8/0	8/0	8/0	8/0	8/0	8/0	8/1	8
	4	8/0	8/0	8/0	8/0	8/0	8/0	8/0	9
	5	8/0	8/0	8/0	8/0	8/0	8/0	8/0	10
42%	1	8/0	8/0	8/0	8/0	8/0	8/0	8/0	11
	2	8/0	8/0	8/0	8/0	8/0	8/0	8/0	12
	3	8/0	8/0	8/0	8/0	8/0	8/0	8/0	13
	4	8/0	8/0	8/0	8/0	8/0	8/0	8/0	14
	5	8/0	8/0	8/0	8/0	8/0	8/0	8/0	15
56%	1	8/0	8/0	8/0	8/0	8/0	8/0	8/0	16
	2	8/0	8/0	8/0	8/0	8/0	8/1	7/0	17
	3	8/0	8/0	8/0	8/0	8/0	8/0	8/0	18
	4	8/0	8/0	8/0	8/0	8/0	8/0	8/0	19
	5	8/0	8/0	8/0	8/0	8/0	8/0	8/0	20
Date		081616	081716	081816	081916	082016	082116	082216	24 <sup>hrs</sup>
Initials		LABB	LABB	LABB	LABB	fr	f	CARR/M	

**CHRONIC TEST DATA SHEET**  
*Pimephales promelas*

Project: Walnut Ridge Beginning Date: 081516 Time: 1450 Test Species: P.promelas  
Dilution H<sub>2</sub>O: M#97 3 Ending Date: 082216 Time: 1445 Age: < 24hrs

Test Type: ( \* )Static Renewal ( ) Flowthrough Toxicant/Effluent

Conc.	Rep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Pan #
80%	1	8/6	8/6	8/6	8/1	7/0	7/0	7/0	✓ 22 21
	2	8/6	8/6	8/6	8/6	8/0	8/0	8/0	✓ 23 22
	3	8/6	8/6	8/6	8/6	8/0	8/0	8/0	✓ 24 23
	4	8/6	8/6	8/6	8/1	7/0	7/0	7/0	✓ 25 24
	5	8/6	8/6	8/6	8/6	8/0	8/0	8/6	✓ 26 25
100%	1	8/6	8/6	8/6	8/6	8/0	8/0	8/6	✓ 27 26
	2	8/6	8/6	8/6	8/6	8/0	8/0	8/6	✓ 28 27
	3	8/6	8/6	8/6	8/6	8/0	8/0	8/6	28
	4	8/6	8/6	8/6	8/6	8/0	8/0	8/6	29
	5	8/6	8/6	8/6	8/6	8/0	8/0	8/6	30
Date		081616	081716	081816	081916	082016	082116	082216	
Initials		LRB	LRB	LRB	LRB	f	f	LRB/MM	

22.8  
22.8  
22.6  
23.5



Final Water Chemistry for Chronic Tests  
Project: Walnut Ridge - *C. dubia*

Test Day:		1	2	3	4	5	6	7
Date:		081616	081716	081816	081916	082016	082116	082216
H <sub>2</sub> O Batch #:		MH973	MH973	MH973	MH973	MH973	MH973	MH973
Temp. (°C)	Control	23.3	23.0	23.1	23.8	23.2	23.8	24.5
	32%	23.7	23.3	23.3	23.4	23.2	23.3	24.4
	42%	23.7	23.4	23.0	23.0	23.1	23.4	24.3
	56%	23.8	23.4	23.0	23.3	23.2	23.8	24.1
	80%	23.8	23.4	23.2	23.0	23.2	23.3	24.0
	100%	23.9	23.9	23.2	23.1	23.2	23.4	24.0
pH	Control	8.29	8.49	8.17	8.38	8.41	8.41	8.24
	32%	8.40	8.58	8.29	8.56	8.64	8.60	8.49
	42%	8.41	8.63	8.34	8.58	8.66	8.69	8.55
	56%	8.46	8.65	8.35	8.65	8.74	8.71	8.63
	80%	8.46	8.76	8.36	8.73	8.76	8.81	8.66
	100%	8.50	8.79	8.37	8.73	8.79	8.84	8.72
DO (mg/L)	Control	8.8	8.8	8.9	9.0	8.8	9.1	8.8
	32%	8.5	8.8	8.6	8.8	8.8	8.9	8.8
	42%	8.7	8.8	8.6	8.9	8.9	9.1	8.8
	56%	8.5	8.8	8.6	9.0	8.8	9.1	8.8
	80%	8.6	8.9	8.6	9.1	8.7	9.0	8.8
	100%	8.6	8.9	8.5	9.1	8.8	9.2	8.8
Initials		JP	JP	JP	RLC	JP/aen	RLC	RLC



Final Water Chemistry for Chronic Tests  
Project: Walnut Ridge - *P. promelas*

Test Day:		1	2	3	4	5	6	7
Date:		08/16/16	08/17/16	08/18/16	08/19/16	08/20/16	08/21/16	08/22/16
H <sub>2</sub> O Batch #:		MH973	MH973	MH973	MH973	MH973	MH973	MH973
Temp. (°C)	Control	21.9	22.5	22.9	23.0	22.8	22.5	23.4
	32%	22.8	22.8	22.9	22.5	23.0	22.5	23.5
	42%	22.9	22.9	23.0	23.5	23.0	22.8	23.3
	56%	22.9	23.0	23.0	24.0	23.0	22.8	23.4
	80%	22.9	22.5	23.0	24.0	23.0	22.8	23.4
	100%	22.5	23.5	23.0	24.0	23.2	22.8	23.3
pH	Control	7.97	7.80	7.76	7.77	7.77	7.67	7.68
	32%	8.00	7.82	7.81	7.74	7.94	7.84	7.96
	42%	8.00	7.85	7.87	7.81	8.04	7.97	7.94
	56%	8.00	7.84	7.88	7.77	8.02	7.96	8.08
	80%	8.02	7.94	7.88	7.80	8.08	8.10	8.20
	100%	8.05	8.00	7.93	7.82	8.20	8.22	8.23
DO (mg/L)	Control	8.2	7.6	7.7	7.4	7.9	7.7	7.3
	32%	8.0	7.2	7.5	7.3	8.7 7.6 <sup>2</sup>	7.4	7.1
	42%	7.8	7.1	7.7	7.0	7.3	7.7	6.7
	56%	7.8	7.0	7.9	6.9	7.3 0.8 <sup>2</sup>	7.5	7.3
	80%	7.7	7.2	7.9	6.7	7.2	7.3	7.6
	100%	7.8	7.4	7.7	6.7	7.2	7.5	6.9
Initials		WBB/OP	WBB	WBB/AM	WBB	f/an	f	WBB/AM

# CHAIN OF CUSTODY RECORD

**Ecotoxicology Research Facility**  
 Arkansas State University  
 2645 Caddo Drive  
 State University, AR 72467  
 (870) 972-2570 Fax (870) 972-2577

Client Name <b>Walnut Ridge Wastewater Treatment</b>			Phone: (870) 886-2312				Analyses (List Below)				
Project # <i>3rd Qtr</i>			Fax:								
Sampler (sign) <i>[Signature]</i>			PO #:				Chronic C. dubia	Chronic P. promelas			
Remarks:			Contact: <b>Jonathan Kopp</b>								
Cont.#	Sample ID Number	Location	Sample Date	Sample Time	Sample Type		Matrix				
					Comp	Grab	Aqueous	Soil	Other		
<i>1</i>		<i>eff 001</i>	<i>8-14-16</i>	<i>9 AM</i>	<i>X</i>					<i>✓</i>	<i>/</i>
			<i>8-15-16</i>	<i>9 AM</i>							
Ice present at delivery:			<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No								
Temp:			<i>3.0°C</i>		<i>[Signature]</i> Initials						
1. Relinquished By (sign) <i>[Signature]</i>			Date <i>8-15-16</i>	Time <i>1130</i>	1. Received By (sign) <i>[Signature]</i>			Date <i>081516</i>	Time <i>1130</i>		
2. Relinquished By (sign)			Date	Time	2. Received By (sign)			Date	Time		



Ecotoxicology Research Facility

Ecotoxicology Research Facility

Arkansas State University

2645 Caddo Drive

State University, AR 72467

(870) 972-2570 Fax (870) 972-2577

# CHAIN OF CUSTODY RECORD



Client Name <b>Walnut Ridge Wastewater Treatment</b>			Phone: (870) 886-2312					Analyses (List Below)					
Project #			Fax:										
Sampler (sign) 			PO #:					Chronic C. dubia	Chronic P. promelas				
Remarks:			Contact: Jonathan Kopp										
Cont.#	Sample ID Number	Location	Sample Date	Sample Time	Sample Type		Matrix						
					Comp	Grab	Aqueous	Soil	Other				
	005		8-16 8-17	9am-9am	<input checked="" type="checkbox"/>						<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Ice present at delivery:		<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No											
Temp:		35°C  Initials											
1. Relinquished By (sign) 			Date	8-17	Time	12:11 pm	1. Received By (sign) 			Date	08/17/16	Time	12:10
2. Relinquished By (sign)			Date		Time		2. Received By (sign)			Date		Time	



Ecotoxicology Research Facility

### Ecotoxicology Research Facility

Arkansas State University

2645 Caddo Drive

State University, AR 72467

(870) 972-2570 Fax (870) 972-2577

# CHAIN OF CUSTODY RECORD



Client Name <b>Walnut Ridge Wastewater Treatment</b>			Phone: <b>(870) 886-2312</b>					Analyses (List Below)													
Project #			Fax:																		
Sampler (sign) 			PO #:					Chronic C. dubia	Chronic P. promelas												
Remarks:			Contact: <b>Jonathan Kopp</b>																		
Cont.#	Sample ID Number	Location		Sample Date	Sample Time	Sample Type		Matrix													
						Comp	Grab	Aqueous	Soil	Other											
1		eff	003	8-18	8-19	7am - 9am	<input checked="" type="checkbox"/>														
Ice present at delivery:			<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		Temp: 0.7 °C		PIC Initials														
1. Relinquished By (sign) 			Date 8-19-16		Time 12:40 pm		1. Received By (sign) 			Date 08/19/16		Time - 1240									
2. Relinquished By (sign)			Date		Time		2. Received By (sign)			Date		Time									



Ecotoxicology Research Facility

**SAMPLE CHECK IN**

Sample ID Number: WR 1

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

Date: 081516 Sampling Date: 081416-081516 Arrival Time: 11:30

Field Identification Number: EFFluent 001 Description: Composite

Shipped by: Federal Express  UPS  Hand delivered by: Jonathan Kopp

Drop-Off Location: ASU-ERF

Storage While Shipped: ICE/COOLER

Analysis Requested: Chronic C.dubia / Chronic p.promelas

Initial Water Chemistry Analysis:

Sample Received by: JK

Temperature (°C): 3.0

Ice Present upon delivery:  YES  NO

Date: 081516

Quality Assurance	Initial	Date	Yes	No
Chain of Custody	JK	081516	X	
Refrigerated at 4°C	JK	↓	X	
Field Record Received	JK		X	
Sample Label Affixed Properly	JK		X	
Project Leader Informed	JK		X	

Comments: \_\_\_\_\_

\_\_\_\_\_



Ecotoxicology Research Facility

**SAMPLE CHECK IN**

Sample ID Number: 002

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

Date: 0817/16 Sampling Date: ~~002~~ 8-16/8-17 Arrival Time: 1210

Field Identification Number: 002 Description: Composite - Aqueous

Shipped by: Federal Express \_\_\_\_\_ UPS \_\_\_\_\_ Hand delivered by: Jon Kopp

Drop-Off Location: ASU-ERF

Storage While Shipped: Ice Cooler

Analysis Requested: Chronic WET testing C. dubia/P. promelas

Initial Water Chemistry Analysis:

Sample Received by: Aen

Temperature (°C): 3.5 Ice Present upon delivery:  YES  NO

Date: 0817/16

Quality Assurance	Initial	Date	Yes	No
Chain of Custody	Aen	0817/16	X	
Refrigerated at 4°C	Aen	↓	X	
Field Record Received	Aen			X
Sample Label Affixed Properly	Aen		X	
Project Leader Informed	Aen		X	

Comments: \_\_\_\_\_



Ecotoxicology Research Facility

**SAMPLE CHECK IN**

Sample ID Number: 003

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

Date: 081916 Sampling Date: 0818-0819 Arrival Time: 1240

Field Identification Number: 003 Description: \_\_\_\_\_

Shipped by: Federal Express \_\_\_\_\_ UPS \_\_\_\_\_ Hand delivered by: Jon Kopp

Drop-Off Location: ASU-ERF

Storage While Shipped: ice cooler

Analysis Requested: chronic WET testing C. dubia/ P. promelas

Initial Water Chemistry Analysis:

Sample Received by: RIC

Temperature (°C): 0.7 Ice Present upon delivery: YES NO

Date: 081916

Quality Assurance	Initial	Date	Yes	No
Chain of Custody	RIC	081916	X	
Refrigerated at 4°C	RIC	↓	X	
Field Record Received	RIC			X
Sample Label Affixed Properly	RIC		X	
Project Leader Informed	RIC		X	

Comments: \_\_\_\_\_



Ecotoxicology Research Facility



P.O. Box 847  
State University, AR 72467  
Tel. 870-972-2570  
Fax 870-972-2577  
<http://ecotox.astate.edu/>

College of Sciences & Mathematics  
[www.astate.edu](http://www.astate.edu)

August 10, 2016

Jonathan Kopp  
Walnut Ridge Wastewater Treatment Plant  
216 Southwest 4<sup>th</sup> Street  
Walnut Ridge, AR 72476

Dear Jon,

Please find enclosed the results of the 7-day chronic tests using water collected from Walnut Ridge's plant facilities during the week of July 24, 2016. No lethal or sublethal effects were measured in *Pimephales promelas* or *Ceriodaphnia dubia* exposed to the critical flow concentration (100%) or other treated dilutions from this outfall.

All test conditions and acceptability criteria as suggested by our laboratory and the US EPA were met during these tests.

Please call if you have any questions regarding this particular test series or any other tests conducted in the past.

Sincerely,

Jennifer L. Bouldin, PhD  
Director Ecotoxicology Research Facility  
PO Box 847  
Arkansas State University  
State University, AR 72467



**Arkansas State University Ecotoxicology Research Facility**

**Laboratory Report**

Facility Director / Lab Contact: Jennifer Bouldin  
 Phone: (870) 972-2570

Client: Walnut Ridge Wastewater Treatment Plant  
 216 Southwest 4th Street  
 Walnut Ridge, AR 72476

Contact: Jon Kopp  
 870-866-2312

NPDES Permit #: AR0046566      AFIN#: 38-00040  
 Effluent Sampling Point/Type: 24hr Composite  
 Samples Collected:

Sample #	Sampling Times	Received	Arrival Temp
1	07/24/16 0900 hrs to 07/25/16 0900 hrs	07/25/16 1111 hrs	4.5 °C
2	07/26/16 0900 hrs to 07/27/16 0900 hrs	07/27/16 1155 hrs	2.0 °C
3	07/28/16 0900 hrs to 07/29/16 0900 hrs	07/29/16 1128 hrs	2.0 °C
4	07/31/16 0900 hrs to 08/01/16 0900 hrs	08/01/16 1225 hrs	0.3 °C

**Test Methods:**

7-Day Chronic Toxicity, Static renewal, Cladoceran, EPA 821/R-02/013, Section 13  
 7-Day Chronic Toxicity, Static renewal, Fathead minnow, EPA 821/R-02/013, Section 11

Organisms: *C. dubia* <24hrs, *P. promelas* <24hrs      Culture Source: ASU Ecotox

Dilutions: 0%, 32%, 42%, 56%, 80%, 100%      Critical Dilution: 100%


Statistical Method: Toxcalc 5.0.25

<i>C. dubia</i>			<i>P. promelas</i>		
whole effluent toxicity					
	lethality	sublethality		lethality	sublethality
DMR Code	22414 10	22414 P0	DMR Code	22414 10	22414 P0
Result	100%	100%	Result	100%	100%
	lethality	sublethality		lethality	sublethality
DMR Code	TGP3B	TLP3B	DMR Code	TGP6C	TLP6C
Result	0	0	Result	0	0
	NOEC lethality	NOEC sublethal		NOEC lethality	NOEC sublethal
DMR Code	TOP3B	TPP3B	DMR Code	TOP6C	TPP6C
Result	100%	100%	Result	100%	100%
	CV%			CV%	
DMR Code	TQP3B		DMR Code	TQP6C	
Result	33.9%		Result	10.0%	
	control survival	control mean reproduction		control survival	control mean weight
	100%	20.3		100%	0.4897
	critical dil. survival	critical mean reproduction		critical dil. survival	critical mean weight
	100%	25.8		100%	0.4400
				MSDp	
				0.1371	

Results Summary: No lethal or sublethal effects were measured in *C. dubia* or *P. promelas* to effluent dilutions.

QA/Reference Testing: Data attached

Reviewed By:

  
 Jennifer L. Bouldin, Director ASU Ecotoxicology Research Facility

Toxicity Test Performed: 7-day *Ceriodaphnia dubia* Survival and Reproduction  
 Effluent Sampling Point: Walnut Ridge WWT Plant  
 Date Test Started: 07/27/16 *C. dubia*  
 Time Test Started: 1310 *C. dubia*  
 Date Test Terminated: 08/03/16 *C. dubia*  
 Time Test Terminated: 1325 *C. dubia*  
 Laboratory Analyst: Cooper

Toxicity Test Performed: 7-day *Pimephales promelas* Survival and Growth  
 Effluent Sampling Point: Walnut Ridge WWT Plant  
 Date Test Started: 07/25/16 *P. promelas*  
 Time Test Started: 1615 *P. promelas*  
 Date Test Terminated: 08/01/16 *P. promelas*  
 Time Test Terminated: 1500 *P. promelas*  
 Laboratory Analyst: McCauley/Sweeney

## I. Test Methods

### A. Physical and Chemical Testing - APHA, Standard Methods for the Examination of Water and Wastewater; Vol. 21, 2005.

<u>Test</u>	<u>Method</u>
Alkalinity	2320B
Conductivity	2510B
Dissolved Oxygen (mg/L, DO)	4500-O-G
Hardness (mg/L CaCO <sub>3</sub> )	2340C
pH	4500-H <sup>+</sup> B
Temperature (°C)	2550B

### B. Toxicity Testing – EPA 821/R-02/013: Short Term Methods for Estimating the Chronic Toxicity of Effluents to Freshwater Organisms

<u>Test</u>	<u>Method</u>
Cladoceran Survival and Reproduction	Section 13
Fathead Minnow Survival and Growth	Section 11

## II. Test Organisms

- A. Name: *Ceriodaphnia dubia* (Cladoceran)  
 Source: Laboratory Culture  
 Age: <24 hours  
 Life Stage: Neonate

- B. Name: *Pimephales promelas* (Fathead minnow)  
Source: Laboratory Culture  
Age: <24 hours  
Life Stage: Larval

### III. External Factors

#### A. Incubator

Temperature (°C)

Average: 24.8

Range: 24.5-25.0

Light Cycle: 16 hours light/ 8 hours dark

Light Intensity: 100 footcandles

Control Water: Moderately Hard Synthetic Water (#MH 971/972)

#### B. *Ceriodaphnia dubia*

Test Chambers: 30 ml Solo cups

Volume per Chamber: 15-20 ml

Number of Organisms per Chamber: 1

Number of Replicates per Concentration: 10

Acclimation: Laboratory control water was added to cultures until >50% of the culture water consisted of control water.

Food: Cladocera were fed *Selenastrum* (#ABS 061316) and yeast/cereal/trout chow mix (#YCT 060716) one hour prior to test setup and once daily thereafter.

#### C. *Pimephales promelas*

Test Chambers: 250 ml storage dishes

Volume per Chamber: 200 ml

Number of Organisms per Chamber: 8

Number of Replicates per Concentration: 5

Acclimation: Laboratory control water was added to cultures until >50% of the culture water consisted of control water.

Food: Larval fish were fed 0.15ml of laboratory-cultured *Artemia* brine shrimp one hour prior to test setup and then 3X daily thereafter.

## IV. Quality Assurance

A. Standard Toxicant: Sodium Chloride

B. Organism: *Ceriodaphnia dubia*

Date and time of Reference Toxicant Test

Start: 07/05/16

Terminated: 07/12/16

Time of Reference Toxicant Test

Start: 1550

Terminated: 1505

Laboratory Analyst: McCauley

Dilution Water Used: Moderately Hard Synthetic Water #969/970

Results: Survival EC50 was slightly low but with 3SD and Reproduction was within control limit

Survival

LOEC: 1.82 g/L NaCl

EC50: 1.45 g/L NaCl

Reproduction

LOEC: 0.62 g/L NaCl

IC25: 0.58 g/L NaCl

C. Organism: *Pimephales promelas*

Date of Reference Toxicant Test

Start: 07/05/16

Terminated: 07/12/16

Time of Reference Toxicant Test

Start: 1600

Terminated: 1614

Laboratory Analyst: McCauley

Dilution Water Used: Moderately Hard Synthetic Water #969/970

Results: Survival LOEC was slightly above control limits but within 3SD and Growth was within control limits.

Survival

LOEC: 7.50 g/L NaCl

EC50: 6.13 g/L NaCl

Growth

LOEC: >7.50 g/L NaCl

IC25: 5.19 g/L NaCl

V. Physical and Chemical Data - See Attached

VI. Survival and Growth Data - See Attached

VII. Statistical Methods - See Attached

VIII. NPDES Permit Results - See Attached

**SUMMARY REPORTING FORM**  
**WET Testing**  
*Ceriodaphnia dubia* Survival and Reproduction

Permittee: Walnut Ridge WWT Plant

NPDES No.: AR0046566

		<u>Time</u>	<u>Date</u>		<u>Time</u>	<u>Date</u>
Composite 1:	Collected from	0900	7/26/16	to	0900	7/27/16
Composite 2:	Collected from	0900	7/28/16	to	0900	7/29/16
Composite 3:	Collected from	0900	7/31/16	to	0900	8/01/16

Test Initiated: 1310

Date: 07/27/16

Time Terminated: 1325

Date: 08/03/16

Dilution H<sub>2</sub>O: MH 972

**PERCENT SURVIVAL**

Percent Effluent

<u>Time of Reading</u>	<u>Control</u>	<u>32%</u>	<u>42%</u>	<u>56%</u>	<u>80%</u>	<u>100%</u>
24h	100	100	100	100	100	100
48h	100	100	100	100	100	100
7 day	100	100	100	100	100	100

**NUMBER OF YOUNG/FEMALE @ 7 DAYS**

Percent Effluent

<u>REP</u>	<u>0%</u>	<u>32%</u>	<u>42%</u>	<u>56%</u>	<u>80%</u>	<u>100%</u>
A	23	33	24	25	34	25
B	19	33	34	36	27	32
C	27	29	25	36	32	33
D	16	32	30	33	25	31
E	23	31	32	35	28	32
F	13	32	35	35	27	31
G	13	32	30	29	30	22
H	22	28	31	29	35	22
I	20	29	36	36	34	26
J	27	35	32	38	36	4
<b>Mean</b>	<b>20.3</b>	<b>31.4</b>	<b>30.9</b>	<b>33.2</b>	<b>30.8</b>	<b>25.8</b>
<b>CV%*</b>	<b>25.1</b>	<b>6.9</b>	<b>12.7</b>	<b>12.5</b>	<b>12.7</b>	<b>33.9</b>

\*Coefficient of Variation% = Standard Deviation x 100/Mean

### ***Ceriodaphnia dubia* Survival and Reproduction**

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 number of young produced per female by the critical dilution (100%) significantly different ( $p=0.05$ ) than the control's number of young per female?  
     Yes   X   No
  
3. If the NOEC for survival is less than the critical dilution, enter [1], otherwise enter [0] for parameter #TGP3B:   0
  
4. If the NOEC for reproduction is less than the critical dilution, enter [1], otherwise enter [0] for parameter #TLP3B:   0
  
5. Report the NOEC value for survival, Parameter #TOP3B:  
NOEC survival  100%  effluent
  
6. Report the NOEC value for reproduction, Parameter #TPP3B:  
NOEC reproduction  100%  effluent
  
7. Report the % coefficient of variation (largest of critical and control dilutions), Parameter #TQP3B:  
CV % reproduction  33.9%  (critical)

### **Whole Effluent Lethality Values for *Ceriodaphnia dubia***

1. Report the Whole Effluent Lethality values for the 30-Day average minimum, Parameter #22414:  
Daily Average Minimum NOEC:  100%
  
2. Report the Whole Effluent Lethality values for the 7-day minimum, Parameter #22414:  
7-Day Minimum NOEC:  100%

**WET Testing Summary Form**  
***Ceriodaphnia dubia* (Cladoceran)**  
**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant

Sample No. 1 Collected Ending Date: 7/27/16 Time: 0900

NPDES No.: AR0046566

Sample No. 2 Collected Ending Date: 7/29/16 Time: 0900

Contact: Jonathan Kopp

Sample No. 3 Collected Ending Date: 8/01/16 Time: 0900

Analyst: Cooper

Test Begin: Date: 7/27/16 Time: 1310 Test End: Date: 8/03/16 Time: 1325

Initial Water Chemistry for Chronic Tests								
Project: Walnut Ridge – <i>C. dubia</i>								
Test day		0	1	2	3	4	5	6
Date		7/27/2016	7/28/2016	7/29/2016	7/30/2016	7/31/2016	8/1/2016	8/2/2016
H <sub>2</sub> O #		MH972	MH972	MH972	MH972	MH972	MH972	MH972
Temp (°C)	Control	23.0	23.0	23.0	22.3	23.0	22.6	23.3
	32%	22.8	23.2	23.2	22.7	22.8	22.8	23.2
	42%	23.0	23.3	23.2	22.8	22.5	22.9	23.4
	56%	23.0	23.5	23.4	22.9	22.7	23.0	23.2
	80%	23.1	23.7	23.6	22.9	22.9	23.2	23.0
	100%	23.2	24.0	24.0	23.0	23.2	23.3	23.2
pH (Standard Units)	Control	8.07	8.14	8.09	8.13	8.19	8.18	8.12
	32%	8.25	8.28	8.20	8.28	8.33	8.15	8.21
	42%	8.28	8.30	8.28	8.29	8.34	8.18	8.21
	56%	8.31	8.32	8.25	8.31	8.38	8.16	8.23
	80%	8.29	8.33	8.26	8.33	8.40	8.14	8.23
	100%	8.28	8.33	8.25	8.32	8.37	8.11	8.23
DO (mg/L)	Control	8.6	8.7	8.3	8.7	8.7	8.7	8.2
	32%	8.7	8.7	8.5	8.7	8.6	8.6	8.3
	42%	8.5	8.5	8.5	8.7	8.5	8.5	8.4
	56%	8.4	8.5	8.5	8.7	8.5	8.6	8.4
	80%	8.4	8.4	8.5	8.6	8.4	8.6	8.3
	100%	8.5	8.4	8.5	8.7	8.3	8.7	8.4
Cond (µS/cm)	Control	317	316	318	314	314	313	325
	32%	319	323	326	322	318	313	325
	42%	321	327	329	325	321	311	326
	56%	323	330	334	329	328	311	324
	80%	329	338	344	335	340	311	326
	100%	336	348	355	341	346	310	326
Alk (mg/L)	Control	62		62			62	
	100%	180		186			164	
Hard (mg/L)	Control	90		90			90	
	100%	180		220			180	

**WET Testing Summary Form**  
***Ceriodaphnia dubia* (Cladoceran)**  
**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant

Sample No. 1 Collected Ending Date: 7/27/16 Time: 0900

NPDES No.: AR0046566

Sample No. 2 Collected Ending Date: 7/29/16 Time: 0900

Contact: Jonathan Kopp

Sample No. 3 Collected Ending Date: 8/01/16 Time: 0900

Analyst: Cooper

Test Begin: Date: 7/27/16 Time: 1310 Test End: Date: 8/03/16 Time: 1325

Final Water Chemistry for Chronic Tests								
Project: Walnut Ridge - <i>C. dubia</i>								
Test day		1	2	3	4	5	6	7
Date:		7/28/2016	7/29/2016	7/30/2016	7/31/2016	8/1/2016	8/2/2016	8/3/2016
H <sub>2</sub> O #		MH972	MH972	MH972	MH972	MH972	MH972	MH972
Temp (°C)	Control	24.0	24.0	23.2	23.0	23.4	23.5	24.0
	32%	23.8	24.0	23.2	23.0	23.0	23.3	24.1
	42%	23.9	24.0	23.1	23.0	23.2	23.1	24.3
	56%	24.0	23.9	23.3	23.1	23.2	23.2	24.0
	80%	24.0	24.0	23.0	23.0	22.7	23.1	24.8
	100%	24.0	24.0	23.2	23.0	23.3	23.1	24.4
pH (Standard Units)	Control	8.21	8.73	8.30	8.43	8.30	8.29	8.27
	32%	8.43	8.74	8.50	8.58	8.74	8.51	8.72
	42%	8.46	8.78	8.52	8.64	8.73	8.68	8.67
	56%	8.54	8.76	8.59	8.64	8.85	8.64	8.73
	80%	8.57	8.80	8.62	8.74	8.86	8.79	8.72
	100%	8.63	8.80	8.67	8.75	8.92	8.78	8.82
DO (mg/L)	Control	8.9	9.1	9.1	8.7	8.9	8.1	8.2
	32%	8.9	9.1	9.0	8.8	8.8	8.3	8.5
	42%	9.0	9.2	9.1	8.9	8.9	8.6	8.5
	56%	9.0	9.2	9.1	8.8	9.0	8.5	8.6
	80%	9.0	9.2	9.1	8.8	9.1	8.7	8.6
	100%	8.9	9.3	9.0	8.9	9.2	8.7	8.6



**Ceriodaphnia Survival and Reproduction Test-7 Day Survival**

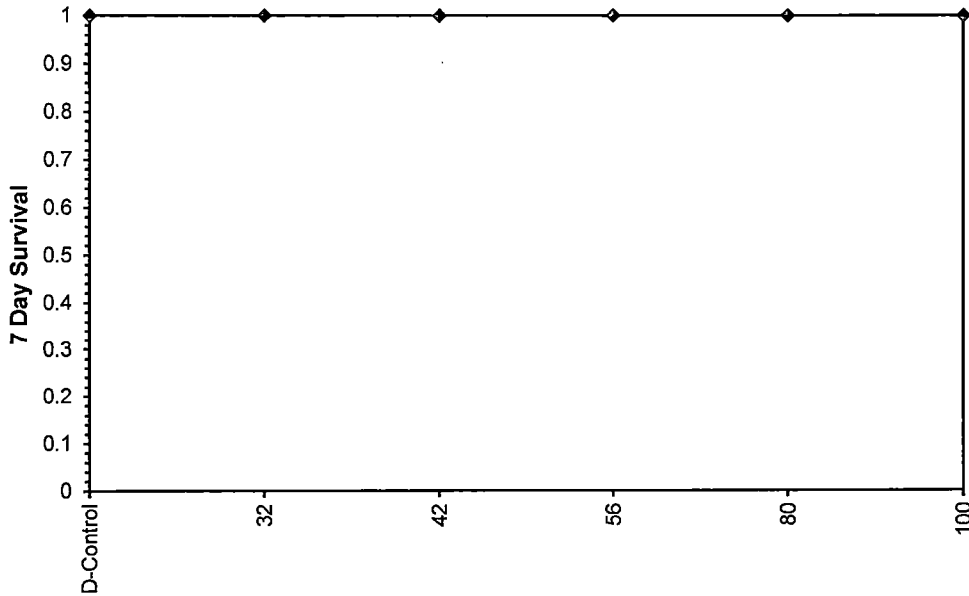
Start Date: 7/27/2016 13:10    Test ID: W. Ridge    Sample ID: NPDES Permit # AR0046566  
 End Date: 8/3/2016 13:25    Lab ID: ASU ERF    Sample Type: EFF1-POTW  
 Sample Date:    Protocol: EPAF 02-EPA Freshwater    Test Species: CD-Ceriodaphnia dubia  
 Comments:

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
32	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
42	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
56	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
80	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical
D-Control	1.0000	1.0000	0	10	10	10		
32	1.0000	1.0000	0	10	10	10	1.0000	0.0500
42	1.0000	1.0000	0	10	10	10	1.0000	0.0500
56	1.0000	1.0000	0	10	10	10	1.0000	0.0500
80	1.0000	1.0000	0	10	10	10	1.0000	0.0500
100	1.0000	1.0000	0	10	10	10	1.0000	0.0500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	100	>100		1

**Dose-Response Plot**



**Ceriodaphnia Survival and Reproduction Test-Reproduction**

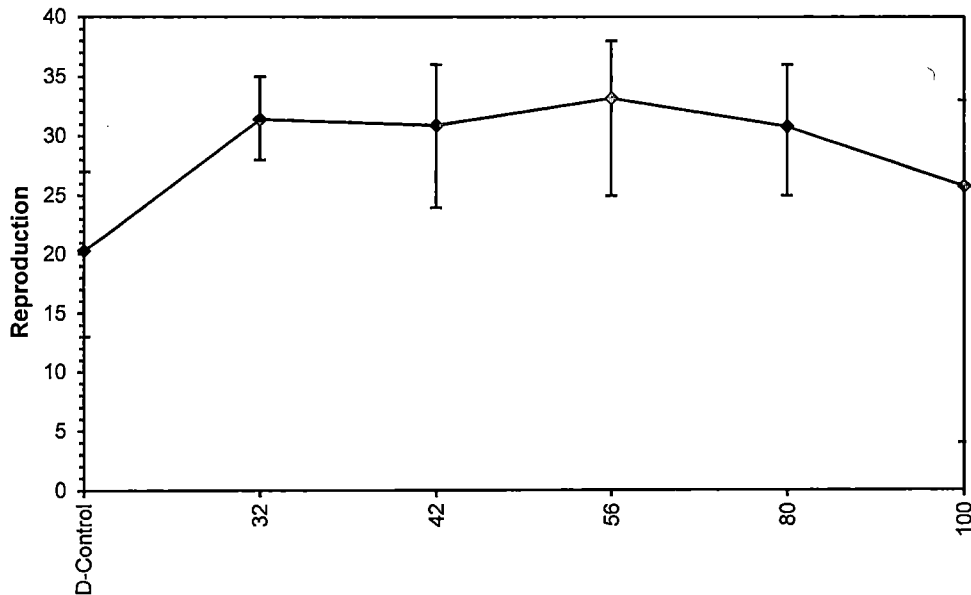
Start Date: 7/27/2016 13:10	Test ID: W. Ridge	Sample ID: NPDES Permit # AR0046566
End Date: 8/3/2016 13:25	Lab ID: ASU ERF	Sample Type: EFF1-POTW
Sample Date:	Protocol: EPAF 02-EPA Freshwater	Test Species: CD-Ceriodaphnia dubia
Comments:		

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	23.000	19.000	27.000	16.000	23.000	13.000	13.000	22.000	20.000	27.000
32	33.000	33.000	29.000	32.000	31.000	32.000	32.000	28.000	29.000	35.000
42	24.000	34.000	25.000	30.000	32.000	35.000	30.000	31.000	36.000	32.000
56	25.000	36.000	36.000	33.000	35.000	35.000	29.000	29.000	36.000	38.000
80	34.000	27.000	32.000	25.000	28.000	27.000	30.000	35.000	34.000	36.000
100	25.000	32.000	33.000	31.000	32.000	31.000	22.000	22.000	26.000	4.000

Conc-%	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%	N		
D-Control	20.300	1.0000	20.300	13.000	27.000	25.124	10		
32	31.400	1.5468	31.400	28.000	35.000	6.912	10	155.00	75.00
42	30.900	1.5222	30.900	24.000	36.000	12.714	10	151.00	75.00
56	33.200	1.6355	33.200	25.000	38.000	12.524	10	153.00	75.00
80	30.800	1.5172	30.800	25.000	36.000	12.695	10	151.00	75.00
100	25.800	1.2709	25.800	4.000	33.000	33.879	10	132.00	75.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.86259	1.035	-1.58752	5.45935
Bartlett's Test indicates unequal variances (p = 3.28E-03)	17.7462	15.0863		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1

**Dose-Response Plot**









**SUMMARY REPORTING FORM**  
**WET Testing**  
**Fathead Minnow Larvae (*Pimephales promelas*) Survival and Growth**

Permittee: Walnut Ridge WWT Plant

NPDES No.: AR0046566

		<u>Time</u>	<u>Date</u>		<u>Time</u>	<u>Date</u>
Composite 1:	Collected from	0900	07/24/16	to	0900	07/25/16
Composite 2:	Collected from	0900	07/26/16	to	0900	07/27/16
Composite 3:	Collected from	0900	07/28/16	to	0900	07/29/16

Test Initiated: 1615

Date: 07/25/16

Time Terminated: 1620

Date: 08/01/16

Dilution H<sub>2</sub>O: MH 971/972

**DATA TABLE FOR SURVIVAL**

Effluent Conc. %	% Survival in Replicate Chambers					Mean % Survival			CV%
	A	B	C	D	E	24h	48h	7 days	
Control	100	100	100	100	100	100	100	100	0.0
32	87.5	87.5	100	100	100	100	100	95	7.6
42	100	87.5	87.5	87.5	100	100	95	92.5	7.8
56	87.5	87.5	87.5	100	100	97.5	97.5	92.5	7.8
80	100	100	100	87.5	100	100	100	97.5	6.1
100	100	100	100	100	100	100	100	100	0.0

**DATA TABLE FOR GROWTH**

Effluent Conc %	Average Dry Weight in Replicate Chambers (mg)					Mean Dry Weight (mg)	CV%
	A	B	C	D	E		
Control	0.4912	0.5737	0.4650	0.4513	0.4675	0.4897	10.0
32	0.6400	0.4929	0.5825	0.4550	0.4975	0.5336	14.2
42	0.4525	0.5843	0.5386	0.4843	0.5113	0.5142	9.8
56	0.4600	0.4600	0.4800	0.4425	0.4237	0.4533	4.7
80	0.4400	0.4650	0.4575	0.4571	0.4225	0.4484	3.8
100	0.4262	0.4025	0.4413	0.4600	0.4700	0.4400	6.1

Coefficient of Variation = Standard Deviation x 100/Mean

## Fathead 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  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?

Yes  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  10.0%  (control)

### Whole Effluent Lethality Values

1. Report the Whole Effluent Lethality values for the 30-Day average minimum, Parameter #22414:

Daily Average Minimum NOEC:  100% 

2. Report the Whole Effluent Lethality values for the 7-day minimum, Parameter #22414:

7-Day Minimum NOEC:  100%

**WET Testing Summary Form**  
**Fathead Minnow Larvae (*Pimephales promelas*)**  
**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant      Sample No. 1 Collected Ending Date: 07/25/16    Time: 0900  
 NPDES No.: AR0046566      Sample No. 2 Collected Ending Date: 07/27/16    Time: 0900  
 Contact: Jon Kopp      Sample No. 3 Collected    Ending Date: 07/29/16    Time: 0900  
 Analyst: McCauley/Sweeney    Test Begin: Date: 07/25/16    Time: 1615    Test End: Date: 08/01/16    Time: 1620

Initial Water Chemistry for Chronic Tests								
Project: Walnut Ridge – <i>P. promelas</i>								
Test day		1	2	3	4	5	6	7
Date		7/25/2016	7/26/2016	7/27/2016	7/28/2016	7/29/2016	7/30/2016	7/31/2016
H <sub>2</sub> O #		MH971	MH972	MH972	MH972	MH972	MH972	MH972
Temp (°C)	Control	23.0	23.0	24.0	23.1	23.0	22.5	22.5
	32%	23.0	23.0	23.9	23.9	23.2	23.5	23.0
	42%	23.0	23.2	23.5	24.0	23.2	23.5	23.0
	56%	23.0	23.3	23.9	24.2	23.4	23.8	23.5
	80%	23.2	23.4	24.0	24.8	23.6	23.8	24.0
	100%	23.5	23.7	24.0	24.8	24.0	24.0	24.0
pH (Standard Units)	Control	8.19	8.18	8.07	8.14	8.09	8.13	8.19
	32%	8.32	8.35	8.25	8.28	8.20	8.28	8.33
	42%	8.35	8.39	8.28	8.30	8.23	8.29	8.34
	56%	8.36	8.43	8.31	8.32	8.25	8.31	8.38
	80%	8.37	8.44	8.29	8.33	8.26	8.33	8.40
	100%	8.38	8.45	8.28	8.33	8.25	8.32	8.37
DO (mg/L)	Control	8.9	8.8	8.6	8.7	8.3	8.7	8.7
	32%	8.7	8.6	8.7	8.7	8.5	8.7	8.6
	42%	8.6	8.6	8.5	8.5	8.5	8.7	8.5
	56%	8.5	8.5	8.4	8.5	8.5	8.7	8.5
	80%	8.5	8.4	8.4	8.4	8.5	8.6	8.4
	100%	8.5	8.2	8.5	8.4	8.5	8.7	8.3
Cond (µS/cm)	Control	319	314	317	316	318	314	314
	32%	324	319	319	323	326	322	318
	42%	326	321	321	327	329	325	321
	56%	330	325	323	330	334	329	328
	80%	332	333	329	338	344	335	340
	100%	338	338	336	348	355	341	346
Alk (mg/L)	Control	60		62		62		
	100%	170		180		186		
Hard (mg/L)	Control	90		90		90		
	100%	130		180		220		



**WET Testing Summary Form**  
**Fathead Minnow Larvae (*Pimephales promelas*)**  
**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant      Sample No. 1 Collected Ending Date: 07/25/16    Time: 0900  
 NPDES No.: AR0046566                              Sample No. 2 Collected Ending Date: 07/27/16    Time: 0900  
 Contact: Jon Kopp                                      Sample No. 3 Collected Ending Date: 07/29/16    Time: 0900  
 Analyst: McCauley/Sweeney    Test Begin: Date: 07/25/16    Time: 1615    Test End: Date: 08/01/16    Time: 1620

Final Water Chemistry for Chronic Tests								
Project: Walnut Ridge – <i>P. promelas</i>								
Test day		1	2	3	4	5	6	7
Date		7/26/2016	7/27/2016	7/28/2016	7/29/2016	7/30/2016	7/31/2016	8/1/2016
H <sub>2</sub> O #		MH971	MH972	MH972	MH972	MH972	MH972	MH972
Temp (°C)	Control	23.0	24.0	23.3	23.8	23.0	23.0	24.0
	32%	23.2	24.2	24.0	24.0	23.0	23.5	24.1
	42%	23.8	24.1	24.0	23.8	23.5	23.5	24.4
	56%	23.8	24.2	24.1	24.1	23.5	23.5	24.1
	80%	24.0	24.8	24.3	24.5	23.5	23.9	24.1
	100%	24.0	25.0	24.1	24.8	23.5	23.9	24.1
pH (Standard Units)	Control	7.95	7.69	7.55	7.65	7.79	7.72	7.80
	32%	8.17	7.80	7.80	7.85	8.05	8.08	8.22
	42%	8.28	7.92	7.94	8.04	8.15	8.19	8.40
	56%	8.37	8.02	7.96	8.13	8.24	8.25	8.60
	80%	8.47	8.13	8.02	8.30	8.29	8.33	8.61
	100%	8.51	8.22	8.17	8.30	8.40	8.41	8.66
DO (mg/L)	Control	8.9	6.6	6.5	7.1	7.9	7.3	7.6
	32%	8.0	6.4	6.5	6.8	7.7	7.2	7.7
	42%	7.9	6.0	6.7	6.9	7.9	7.5	8.2
	56%	7.9	6.1	6.7	7.2	7.9	7.6	8.6
	80%	8.0	6.3	6.2	7.6	7.9	7.5	8.8
	100%	8.0	6.3	6.4	7.2	7.8	7.5	8.8

**Larval Fish Growth and Survival Test-7 Day Survival**

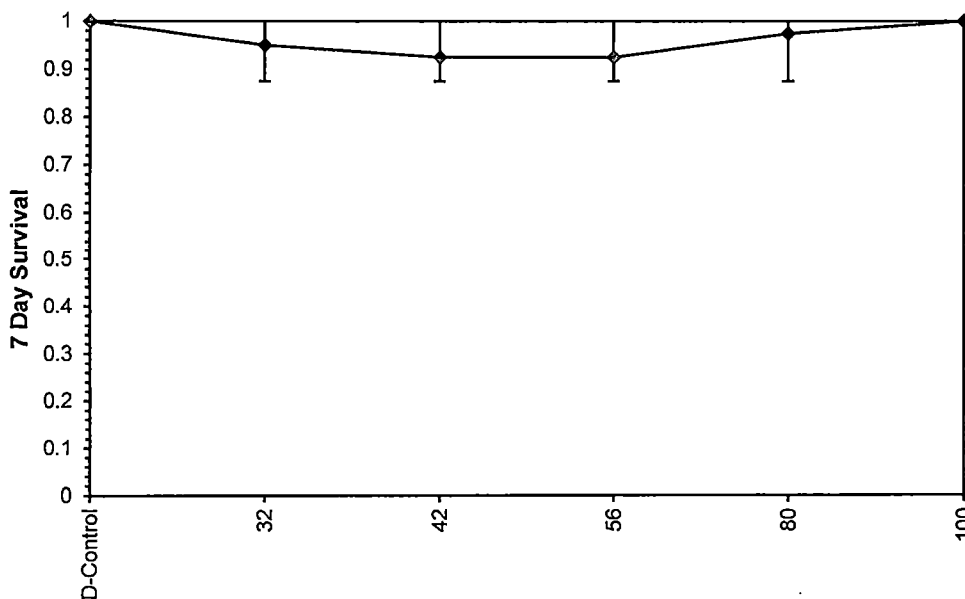
Start Date: 7/25/2015 16:15	Test ID: W. Ridge	Sample ID: AR0046566-NPDES Permit #
End Date: 8/1/2016 16:20	Lab ID: ASU ERF	Sample Type: EFF1-POTW
Sample Date:	Protocol: EPAF 02-EPA Freshwater	Test Species: PP-Pimephales promelas

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
32	0.8750	0.8750	1.0000	1.0000	1.0000
42	1.0000	0.8750	0.8750	0.8750	1.0000
56	0.8750	0.8750	0.8750	1.0000	1.0000
80	1.0000	1.0000	1.0000	0.8750	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root					N	Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%				
D-Control	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5			
32	0.9500	0.9500	1.3196	1.2094	1.3931	7.623	5	22.50	16.00	
42	0.9250	0.9250	1.2829	1.2094	1.3931	7.841	5	20.00	16.00	
56	0.9250	0.9250	1.2829	1.2094	1.3931	7.841	5	20.00	16.00	
80	0.9750	0.9750	1.3564	1.2094	1.3931	6.055	5	25.00	16.00	
100	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	16.00	

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92898	0.9	-0.15004	-0.7133
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1

**Dose-Response Plot**



**Larval Fish Growth and Survival Test-7 Day Growth**

Start Date: 7/25/2015 16:15	Test ID: W. Ridge	Sample ID: AR0046566-NPDES Permit #
End Date: 8/1/2016 16:20	Lab ID: ASU ERF	Sample Type: EFF1-POTW
Sample Date:	Protocol: EPAF 02-EPA Freshwater	Test Species: PP-Pimephales promelas

Comments:

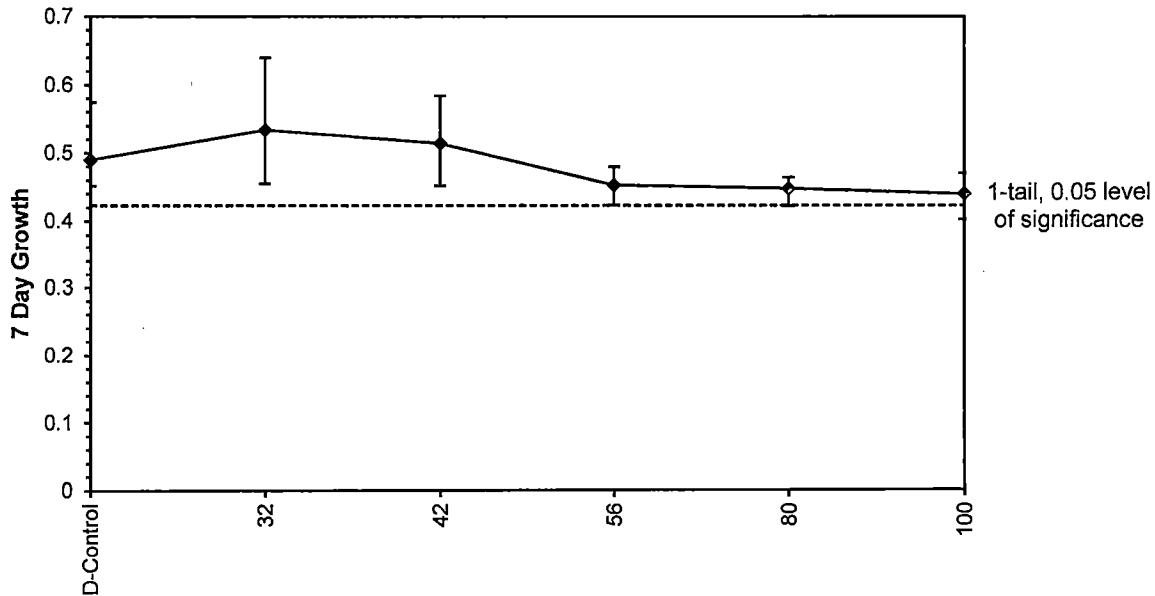
Conc-%	1	2	3	4	5
D-Control	0.4912	0.5737	0.4650	0.4513	0.4675
32	0.6400	0.4929	0.5825	0.4550	0.4975
42	0.4525	0.5843	0.5386	0.4843	0.5113
56	0.4600	0.4600	0.4800	0.4425	0.4237
80	0.4400	0.4650	0.4575	0.4571	0.4225
100	0.4262	0.4025	0.4413	0.4600	0.4700

Conc-%	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD
			Mean	Min	Max	CV%					
D-Control	0.4897	1.0000	0.4897	0.4513	0.5737	10.028	5				
32	0.5336	1.0895	0.5336	0.4550	0.6400	14.166	5	-1.540	2.360	0.0672	
42	0.5142	1.0499	0.5142	0.4525	0.5843	9.829	5	-0.858	2.360	0.0672	
56	0.4533	0.9255	0.4533	0.4237	0.4800	4.670	5	1.283	2.360	0.0672	
80	0.4484	0.9156	0.4484	0.4225	0.4650	3.824	5	1.452	2.360	0.0672	
100	0.4400	0.8984	0.4400	0.4025	0.4700	6.116	5	1.748	2.360	0.0672	

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96039	0.9	0.69548	0.80076
Bartlett's Test indicates equal variances (p = 0.05)	11.0088	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.06716	0.13712	0.00745	0.00202	0.01301	5, 24

**Dose-Response Plot**



**CHRONIC TEST DATA SHEET**  
*Pimephales promelas*

Project: Walnut Ridge Beginning Date: 072516 Time: 1615 Test Species: *P. promelas*  
 Dilution H<sub>2</sub>O: MH972 Ending Date: 080116 Time: 1620 Age: 24 hrs  
 Test Type: ( \* )Static Renewal ( ) Flowthrough Toxicant/ Effluent

Conc.	Rep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Pan #
Control	1	8/0	8/0	8/0	8/0	8/0	8/0	8/0	1
	2	8/0	8/0	8/0	8/0	8/0	8/0	8/0	2
	3	8/0	8/0	8/0	8/0	8/0	8/0	8/0	3
	4	8/0	8/0	8/0	8/0	8/0	8/0	8/0	4
	5	8/0	8/0	8/0	8/0	8/0	8/0	8/0	5
32%	1	8/0	8/0	8/1	7/0	8/0	7/0	7/0	6
	2	8/0	8/0	8/0	8/0	8/0	8/1	7/0	7
	3	8/0	8/0	8/0	8/0	8/0	8/0	8/0	8
	4	8/0	8/0	8/0	8/0	8/0	8/0	8/0	9
	5	8/0	8/0	8/0	8/0	8/0	8/0	8/0	10
42%	1	8/0	8/0	8/0	8/0	8/0	8/0	8/0	11
	2	8/0	8/1	7/0	7/0	7/0	<del>8/0</del> 7/0	7/0	12
	3	8/0	8/1	7/0	7/0	7/0	7/0	7/0	13
	4	8/0	8/0	8/0	8/1	8/0	7/0	7/0	14
	5	8/0	8/0	8/0	8/0	8/0	8/0	8/0	15
56%	1	8/0	8/0	8/0	8/1	7/0	7/0	7/0	16
	2	8/0	8/0	8/0	8/1	7/0	7/0	7/0	17
	3	8/1	7/0	7/0	7/0	7/0	7/0	7/0	18
	4	8/0	8/0	8/0	8/0	8/0	8/0	8/0	19
	5	8/0	8/0	8/0	8/0	8/0	8/0	8/0	20
Date		072616	072716	7/28/16	072916	073016	073116	080116	
Initials		J	J	J	AW	LAB	LAB	LAB	

**CHRONIC TEST DATA SHEET**  
*Pimephales promelas*

Project: Walnut Ridge Beginning Date: 072516 Time: 1615 Test Species: P.promelas

Dilution H<sub>2</sub>O: MH771 Ending Date: 080116 Time: 1620 Age: 24hrs  
MH972

Test Type: ( \* )Static Renewal ( ) Flowthrough Toxicant/Effluent: ( )

Conc.	Rep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Pan #
80%	1	8/0	8/0	8/0	8/0	8/0	8/0	8/0	21
	2	8/0	8/0	8/0	8/0	8/0	8/0	8/0	22
	3	8/0	8/0	8/0	8/0	8/0	8/0	8/0	23
	4	8/0	8/0	8/0	8/0	8/0	8/1	7/0	24
	5	8/0	8/0	8/0	8/0	8/0	8/0	8/0	25
100%	1	8/0	8/0	8/0	8/0	8/0	8/0	8/0	26
	2	8/0	8/0	8/0	8/0	8/0	8/0	8/0	27
	3	8/0	8/0	8/0	8/0	8/0	8/0	8/0	28
	4	8/0	8/0	8/0	8/0	8/0	8/0	8/0	29
	5	8/0	8/0	8/0	8/0	8/0	8/0	8/0	30
Date		072616	072716	072816	072916	073016	073116	080116	
Initials		f	f	P	all	LRB	LRB	LRB	

Initial Water Chemistry for Chronic Tests

Project: Walnut Ridge - *C. dubia*

Test Day:		0	1	2	3	4	5	6
Date:		072716	7/28/16	072916	073016	073116	080116	080216
H <sub>2</sub> O Batch #:		MH972	MH972	MH972	MH972	MH972	MH972	MH972
Temp. (°C)	Control	23.0	23.0	23.0	22.3	23.0	22.6	23.3
	32%	22.8	23.2	23.2	22.7	22.8	22.8	23.2
	42%	23.0	23.3	23.2	22.8	22.5	22.9	23.4
	56%	23.0	23.5	23.4	22.9	22.7	23.0	23.2
	80%	23.1	23.7	23.6	22.9	22.9	23.2	23.0
	100%	23.2	24.0	24.0	23.0	23.2	23.3	23.2
pH	Control	8.07	8.14	8.09	8.13	8.19	8.18	8.12
	32%	8.25	8.28	8.20	8.28	8.33	8.15	8.21
	42%	8.28	8.30	8.28	8.29	8.34	8.18	8.21
	56%	8.31	8.32	8.25	8.31	8.38	8.16	8.23
	80%	8.29	8.33	8.24	8.33	8.40	8.14	8.23
	100%	8.28	8.33	8.25	8.32	8.37	8.11	8.23
DO (mg/L)	Control	8.6	8.7	8.3	8.7	8.7	8.7	8.2
	32%	8.7	8.7	8.5	8.7	8.6	8.6	8.3
	42%	8.5	8.5	8.6	8.7	8.5	8.5	8.4
	56%	8.4	8.5	8.5	8.7	8.4 8.5	8.6	8.4
	80%	8.4	8.4	8.5	8.6	8.3 8.4	8.6	8.3
	100%	8.5	8.4	8.5	8.7	8.3	8.7	8.4
Cond. (µS/cm)	Control	317	316	318	314	314	313	325
	32%	319	323	326	322	318	313	325
	42%	321	327	329	325	321	311	326
	56%	323	330	334	329	328	311	324
	80%	329	338	344	335	340	311	326
	100%	336	348	355	341	346	310	326
Alk. (mg/L)	Control	62		62			62	
	100%	180		186			164	
Hard. (mg/L)	Control	90		90			90	
	100%	180		220			180	
Initials		RC/KK	JP/RIC	DM/RIC	JP/RIC	DM/RIC	RC	RC

Final Water Chemistry for Chronic Tests  
Project: Walnut Ridge - *C. dubia*

Test Day:		1	2	3	4	5	6	7
Date:		072816 <del>072616</del>	072916	073016	073116	080116	080216	080316
H <sub>2</sub> O Batch #:		MH972 <del>MH971</del>	MH972	MH972	MH972	MH972	MH972	MH972
Temp. (°C)	Control	23.5 <sup>24.0</sup>	24.0	23.2	23.0	23.4	23.5	24.0
	32%	23.4 <sup>23.8</sup>	24.0	23.2	23.0	23.0	23.3	24.1
	42%	23.8 <sup>23.9</sup>	24.0	23.1	23.0	23.2	23.1	24.3
	56%	23.8 <sup>24.0</sup>	23.9	23.3	23.1	23.2	23.2	24.0
	80%	23.7 <sup>24.0</sup>	24.0	23.0	23.0	22.7	23.1	24.8
	100%	23.8 <sup>24.0</sup>	24.0	23.2	23.0	23.3	23.1	24.4
pH	Control	8.21	8.73	8.30	8.43	8.30	8.29	8.27
	32%	8.43	8.74	8.50	8.58	8.74	8.51	8.72
	42%	8.46	8.78	8.52	8.64	8.73	8.68	8.67
	56%	8.54	8.76	8.59	8.64	8.85	8.64	8.73
	80%	8.57	8.80	8.62	8.74	8.86	8.79	8.72
	100%	8.63	8.80	8.67	8.75	8.92	8.78	8.82
DO (mg/L)	Control	8.9	9.1	9.1	8.7	8.9	8.1	8.2
	32%	8.9	9.1	9.0	8.8	8.8	8.3	8.5
	42%	9.0	9.2	9.1	8.9	8.9	8.6	8.5
	56%	9.0	9.2	9.1	8.8	9.0	8.5	8.6
	80%	9.0	9.2	9.1	8.8	9.0 <sup>am</sup>	8.7	8.6
	100%	8.9	9.3	9.0	8.9	9.2	8.7	8.6
Initials		ric/ps	ric	ric/ps	ric/ps	ric/	ric	ric

Initial Water Chemistry for Chronic Tests  
Project: Walnut Ridge - ~~C. dubia~~ / *P. promelas*

Test Day:		0	1	2	3	4	5	6
Date:		072516	072616	072716	7/28/16	072916	073016	073116
H <sub>2</sub> O Batch #:		MH971	MH972	MH972	MH972	MH972	MH972	MH972
Temp. (°C)	Control:	23.0	23.0	24.0	23.1	23.0	22.5	22.5
	32%:	23.0	23.0	23.9	23.9	23.2	23.5	23.0
	42%:	23.0	23.2	23.5	24.0	23.2	23.5	23.0
	56%:	23.0	23.3	23.9	24.2	23.4	23.8	23.5
	80%:	23.2	23.4	24.0	24.8	23.6	23.8	24.0
	100%:	23.5	23.7	24.0	24.8	24.0	24.0	24.0
pH	Control:	8.19	8.18	8.07	8.14	8.09	8.13	8.19
	32%:	8.32	8.35	8.25	8.28	8.20	8.28	8.33
	42%:	8.35	8.39	8.28	8.30	8.23	8.29	8.34
	56%:	8.36	8.43	8.31	8.32	8.25	8.31	8.38
	80%:	8.37	8.44	8.29	8.33	8.26	8.33	8.40
	100%:	8.38	8.45	8.28	8.33	8.25	8.32	8.37
DO (mg/L)	Control:	8.9	8.8	8.6	8.7	8.3	8.7	8.7
	32%:	8.7	8.6	8.7	8.7	8.5	8.7	8.6
	42%:	8.6	8.6	8.5	8.5	8.5	8.7	8.5
	56%:	8.5	8.5	8.4	8.5	8.5	8.7	8.5
	80%:	8.5	8.4	8.4	8.4	8.5	8.6	8.4
	100%:	8.5	8.2	8.5	8.4	8.5	8.7	8.3
Cond. (µS/cm)	Control:	319	314	317	316	318	314	314
	32%:	324	319	319	323	326	322	318
	42%:	326	321	321	327	329	325	321
	56%:	330	325	323	330	334	329	328
	80%:	332	333	329	338	344	335	340
	100%:	338	338	336	348	355	341	346
Alk. (mg/L)	Control:	60		62		62		
	100%:	170		180		186		
Hard. (mg/L)	Control:	90		90		90		
	100%:	130		180		280		
Initials		DS/LL	aev/rlc	RLC	DS	RLC/aev	DS/aev	aev



Final Water Chemistry for Chronic Tests  
Project: Walnut Ridge - *P. promelas*

Test Day:		1	2	3	4	5	6	7
Date:		072616	072716	072816	072916	073016	073116	080116
H <sub>2</sub> O Batch #:		MH972	MH972	MH972	MH972	MH972	MH972	MH972
Temp. (°C)	Control	23.0	24.0	23.3	23.8	23.0	23.0	24.0
	32%	23.2	24.2	24.0	24.0	23.0	23.5	24.1
	42%	23.8	24.1	24.0	23.8	23.5	23.5	24.4
	56%	23.8	24.2	24.1	24.1	23.5	23.5	24.1
	80%	24.0	24.9	24.3	24.5	23.5	23.9	24.1
	100%	24.0	25.0	24.1	24.8	23.5	23.9	24.1
pH	Control	7.95	7.69	7.55	7.65	7.79	7.72	7.80
	32%	8.17	7.80	7.80	7.85	8.05	8.08	8.22
	42%	8.28	7.92	7.94	8.04	8.15	8.19	8.40
	56%	8.37	8.02	7.96	8.13	8.24	8.25	8.60
	80%	8.47	8.13	8.02	8.30	8.29	8.33	8.61
	100%	8.51	8.22	8.17	8.3	8.40	8.41	8.66
DO (mg/L)	Control	8.9	6.6	6.5	7.1	7.9	7.3	7.6
	32%	8.0	6.4	6.5	6.8	7.7	7.2	7.7
	42%	7.9	6.0	6.7	6.9	7.9	7.5	8.2
	56%	7.9	6.1	6.7	7.2	7.9	7.6	8.6
	80%	8.0	6.3	6.2	7.6	7.9	7.5	8.8
	100%	8.0	6.3	6.4	7.2	7.8	7.5	8.8
Initials		ju	ju	2/JP	den	web/JP	web/JP	JP



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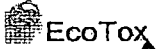
### Ecotoxicology Research Facility

Arkansas State University  
2645 Caddo Drive  
State University, AR 72467  
(870) 972-2570 Fax (870) 972-2577

# CHAIN OF CUSTODY RECORD



Client Name <b>Walnut Ridge Wastewater Treatment</b>			Phone: (870) 886-2312					Analyses (List Below)					
Project #			Fax:										
Sampler (sign) 			PO #:					Chronic C. dubia	Chronic P. promelas				
Remarks:			Contact: Jonathan Kopp										
Cont.#	Sample ID Number	Location	Sample Date	Sample Time	Sample Type		Matrix						
					Comp	Grab	Aqueous	Soil	Other				
1		eff 000	7-24-16	9AM	X					X	X		
			7-25-16	9AM									
Ice present at delivery:			X Yes ___ No										
Temp:			4.5°C		an Initials								
1. Relinquished By (sign) 			Date	7-25-16	Time	1. Received By (sign) 			Date	7/25/16	Time	11:11	
2. Relinquished By (sign)			Date		Time	2. Received By (sign)			Date		Time		



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### Ecotoxicology Research Facility

Arkansas State University  
2645 Caddo Drive  
State University, AR 72467  
(870) 972-2570 Fax (870) 972-2577

# CHAIN OF CUSTODY RECORD



Client Name		Phone: (870) 886-2312			Analyses (List Below)														
Walnut Ridge Wastewater Treatment		Fax:			Chronic C. dubia	Chronic P. promelas													
Project #		PO #:																	
Sampler (sign)		Remarks:																	
Contact: Jonathan Kopp																			
Cont.#	Sample ID Number	Location	Sample Date	Sample Time	Sample Type		Matrix												
					Comp	Grab	Aqueous	Soil	Other										
			7-26	7-27	9am	9am	<input checked="" type="checkbox"/>												
Ice present at delivery:		<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No																	
Temp:		2.0 °C		fle Initials															
1. Relinquished By (sign)		Date	Time		1. Received By (sign)			Date	Time										
		7-27-16	11:54am		Rebecca d. Lopez			072716	1155										
2. Relinquished By (sign)		Date	Time		2. Received By (sign)			Date	Time										



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Arkansas State University

2645 Caddo Drive

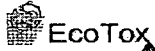
State University, AR 72467

(870) 972-2570 Fax (870) 972-2577

# CHAIN OF CUSTODY RECORD



Client Name <b>Walnut Ridge Wastewater Treatment</b>			Phone: (870) 886-2312					Analyses (List Below)					
Project #			Fax:										
Sampler (sign) 			PO #:					Chronic C. dubia	Chronic P. promelas				
Remarks:			Contact: Jonathan Kopp										
Cont.#	Sample ID Number	Location	Sample Date	Sample Time	Sample Type		Matrix						
					Comp	Grab	Aqueous	Soil	Other				
	EPC03		7-28	7-29	9am - 9am	<input checked="" type="checkbox"/>		X			X	X	
Ice present at delivery: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No													
Temp: 2.0 °C			aer Initials										
1. Relinquished By (sign) 			Date	Time	1. Received By (sign) 					Date	Time		
2. Relinquished By (sign)			Date	Time	2. Received By (sign)					Date	Time		
			7-29-16	11:30 am						07/29/2016	-11:28		



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Arkansas State University

2645 Caddo Drive

State University, AR 72467

(870) 972-2570 Fax (870) 972-2577

# CHAIN OF CUSTODY RECORD



Client Name		Phone: (870) 886-2312					Analyses (List Below)						
Walnut Ridge Wastewater Treatment		Fax:											
Project #		PO #:					Chronic C. dubia	Chronic P. promelas					
Sampler (sign)		Remarks:											
Contact: Jonathan Kopp													
Cont.#	Sample ID Number	Location	Sample Date	Sample Time	Sample Type		Matrix						
					Comp	Grab	Aqueous	Soil	Other				
X		est 001	7-31-16	9AM.									
			8-1-16	9AM.									
Ice present at delivery:		<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No											
Temp:		0.3 °C Alw/Initials											
1. Relinquished By (sign)		Date		Time		1. Received By (sign)		Date		Time			
		8-1-16		12:25				08/01/16		1225			
2. Relinquished By (sign)		Date		Time		2. Received By (sign)		Date		Time			



Ecotoxicology Research Facility

**SAMPLE CHECK IN**

Sample ID Number: WR 001

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

Date: 07 25 2014 Sampling Date: 0724- 0725 Arrival Time: 11:11

Field Identification Number: eff001 Description: Composite

Shipped by: Federal Express \_\_\_\_\_ UPS \_\_\_\_\_ Hand delivered by: Jon Kopp

Drop-Off Location: ASU-ERF

Storage While Shipped: cooler - ice

Analysis Requested: Chronic C. dubia Chronic P. promelas

Initial Water Chemistry Analysis:

Sample Received by: AEW

Temperature (°C): 4.5 Ice Present upon delivery:  YES  NO

Date: 0725 14

Quality Assurance	Initial	Date	Yes	No
Chain of Custody	<u>AEW</u>	<u>0725 14</u>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Refrigerated at 4°C	↓	↓	↓	
Field Record Received	↓	↓	↓	
Sample Label Affixed Properly	↓	↓	↓	
Project Leader Informed	↓	↓	↓	

Comments: \_\_\_\_\_

\_\_\_\_\_



Ecotoxicology Research Facility

**SAMPLE CHECK IN**Sample ID Number: WR 002

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

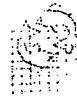
Date: 072716 Sampling Date: 072616-072717 Arrival Time: 1155Field Identification Number: EFF 002 Description: CompositeShipped by: Federal Express \_\_\_\_\_ UPS \_\_\_\_\_ Hand delivered by: Jow KoppDrop-Off Location: ASU-ERFStorage While Shipped: cooler - iceAnalysis Requested: Chronic C. dubia Chronic P. promelas

Initial Water Chemistry Analysis:

Sample Received by: RICTemperature (°C): 2.0 Ice Present upon delivery:  YES  NODate: 072716

Quality Assurance	Initial	Date	Yes	No
Chain of Custody	RIC	072716	X	
Refrigerated at 4°C	↓	↓	X	
Field Record Received				X
Sample Label Affixed Properly	↓	↓	X	
Project Leader Informed	↓	↓	X	

Comments: \_\_\_\_\_



EcoTox

Ecotoxicology Research Facility

## SAMPLE CHECK IN

Sample ID Number: W. Ridge # 3

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

Date: 072916 Sampling Date: 0728/0729 Arrival Time: 1128  
 Field Identification Number: EFF003 Description: Composite Aquatics

Shipped by: Federal Express \_\_\_\_\_ UPS \_\_\_\_\_ Hand delivered by: Jon Kopp

Drop-Off Location: \_\_\_\_\_ ASU-ERF \_\_\_\_\_

Storage While Shipped: \_\_\_\_\_

Analysis Requested: Chronic C. dubia Chronic P. promelas

Initial Water Chemistry Analysis: \_\_\_\_\_

Sample Received by: Aer/Ric

Temperature (°C): 2.0 Ice Present upon delivery:  YES  NO

Date: 0729/16

Quality Assurance	Initial	Date	Yes	No
Chain of Custody	<u>Aer</u>	<u>072916</u>	<input checked="" type="checkbox"/>	
Refrigerated at 4°C	<u>Aer</u>		<input checked="" type="checkbox"/>	
Field Record Received	<u>Aer</u>	↓		<input checked="" type="checkbox"/>
Sample Label Affixed Properly	<u>Aer</u>	↓	<input checked="" type="checkbox"/>	
Project Leader Informed	<u>Aer</u>	↓	<input checked="" type="checkbox"/>	

Comments: \_\_\_\_\_





Ecotoxicology Research Facility

## SAMPLE CHECK IN

Sample ID Number: W. Ridge #4

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

Date: 080116 Sampling Date: 073116/080116 Arrival Time: 1225  
 Field Identification Number: EFF 004 Description: Composite

Shipped by: Federal Express \_\_\_\_\_ UPS \_\_\_\_\_ Hand delivered by: Jon Kopp

Drop-Off Location: ASU-ERF

Storage While Shipped: \_\_\_\_\_

Analysis Requested: Chronic C. dubia

Initial Water Chemistry Analysis:

Sample Received by: AEN/RC

Temperature (°C): 0.3 Ice Present upon delivery:  YES  NO

Date: 080116

Quality Assurance	Initial	Date	Yes	No
Chain of Custody	RC	080116	X	
Refrigerated at 4°C	RC	↓	X	
Field Record Received	RC			X
Sample Label Affixed Properly	RC		X	
Project Leader Informed	RC		X	

Comments: \_\_\_\_\_



Ecotoxicology Research Facility



P.O. Box 847  
State University, AR 72467  
Tel. 870-972-2570  
Fax 870-972-2577  
<http://ecotox.astate.edu/>

College of Sciences & Mathematics  
[www.astate.edu](http://www.astate.edu)

June 23, 2016

Jonathan Kopp  
Walnut Ridge Wastewater Treatment Plant  
216 Southwest 4<sup>th</sup> Street  
Walnut Ridge, AR 72476

Dear Jon,

Please find enclosed the results of the 7-day chronic tests using water collected from Walnut Ridge's plant facilities during the week of June 12, 2016. Lethal effects were measured in *Pimephales promelas* and *Ceriodaphnia dubia* exposed to the critical flow concentration (100%) and other treated dilutions from this outfall. Additionally, sublethal effects were measured in both test organisms, thus the WET test will need to be repeated again next month.

All test conditions and acceptability criteria as suggested by our laboratory and the US EPA were met during these tests.

Please call if you have any questions regarding this particular test series or any other tests conducted in the past.

Sincerely,

Jennifer L. Bouldin, PhD  
Director Ecotoxicology Research Facility  
PO Box 847  
Arkansas State University  
State University, AR 72467

**Arkansas State University Ecotoxicology Research Facility**

**Laboratory Report**

Facility Director / Lab Contact: Jennifer Bouldin  
 Phone: (870) 972-2570

Client: Walnut Ridge Wastewater Treatment Plant  
 216 Southwest 4th Street  
 Walnut Ridge, AR 72476

Contact: Jon Kopp  
 870-866-2312

NPDES Permit #: AR0046566      AFIN#: 38-00040  
 Effluent Sampling Point/Type: 24hr Composite

**Samples Collected:**

Sample #	Sampling Times	Received	Arrival Temp
1	6/12/16 0900 hrs to 6/13/16 0900 hrs	6/13/16 1037 hrs	3.0 °C
2	6/14/16 0900 hrs to 6/15/16 0900 hrs	6/15/16 1145 hrs	3.0 °C
3	6/16/16 0900 hrs to 6/17/16 0900 hrs	6/17/16 1159 hrs	0.0 °C

**Test Methods:**

7-Day Chronic Toxicity, Static renewal, Cladoceran, EPA 821/R-02/013, Section 13  
 7-Day Chronic Toxicity, Static renewal, Fathead minnow, EPA 821/R-02/013, Section 11

Organisms: *C. dubia* <24hrs, *P. promelas* <24hrs      Culture Source: ASU Ecotox

Dilutions: 0%, 32%, 42%, 56%, 80%, 100%      Critical Dilution: 100%

Statistical Method: Toxcalc 5.0.25

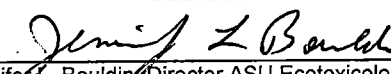
<i>C. dubia</i>			<i>P. promelas</i>		
whole effluent toxicity			whole effluent toxicity		
	lethality	sublethality		lethality	sublethality
DMR Code	22414 10	22414 P0	DMR Code	22414 10	22414 P0
Result	56%	<32%	Result	42%	42%
	lethality	sublethality		lethality	sublethality
DMR Code	TGP3B	TLP3B	DMR Code	TGP6C	TLP6C
Result	1	1	Result	1	1
	NOEC lethality	NOEC sublethal		NOEC lethality	NOEC sublethal
DMR Code	TOP3B	TPP3B	DMR Code	TOP6C	TPP6C
Result		<32%	Result	42%	42%
	CV%			CV%	
DMR Code	TQP3B		DMR Code	TQP6C	
Result	16.1%		Result	6.1%	
	control survival	control mean reproduction		control survival	control mean weight
	100%	29.1		97.5%	0.2706
	critical dil. survival	critical mean reproduction		critical dil. survival	critical mean weight
	0%	--		0%	--
				MSDp	
				0.2143	

**Results Summary:**

Effluent caused lethality to *C. dubia* at the 80 and 100% concentrations and sublethal results to all exposed dilutions. Significant differences in survival were also measured to *P. promelas* at the 56, 80 and 100% effluent dilutions. Sublethal effects were also measured in *P. promelas* at the 56% dilution.

QA/Reference Testing: Data attached

Reviewed By:

  
 Jennifer L. Bouldin, Director ASU Ecotoxicology Research Facility

Toxicity Test Performed: 7-day *Ceriodaphnia dubia* Survival and Reproduction  
 Effluent Sampling Point: Walnut Ridge WWT Plant  
 Date Test Started: 6/13/16 *C. dubia*  
 Time Test Started: 1445 *C. dubia*  
 Date Test Terminated: 6/20/16 *C. dubia*  
 Time Test Terminated: 1335 *C. dubia*  
 Laboratory Analyst: Cooper

Toxicity Test Performed: 7-day *Pimephales promelas* Survival and Growth  
 Effluent Sampling Point: Walnut Ridge WWT Plant  
 Date Test Started: 6/13/16 *P. promelas*  
 Time Test Started: 1520 *P. promelas*  
 Date Test Terminated: 6/20/16 *P. promelas*  
 Time Test Terminated: 1500 *P. promelas*  
 Laboratory Analyst: McCauley/Nicholson/Sweeney

I. Test Methods

A. Physical and Chemical Testing - APHA, Standard Methods for the Examination of Water and Wastewater; Vol. 21, 2005.

<u>Test</u>	<u>Method</u>
Alkalinity	2320B
Conductivity	2510B
Dissolved Oxygen (mg/L, DO)	4500-O-G
Hardness (mg/L CaCO <sub>3</sub> )	2340C
pH	4500-H <sup>+</sup> B
Temperature (°C)	2550B

B. Toxicity Testing – EPA 821/R-02/013: Short Term Methods for Estimating the Chronic Toxicity of Effluents to Freshwater Organisms

<u>Test</u>	<u>Method</u>
Cladoceran Survival and Reproduction	Section 13
Fathead Minnow Survival and Growth	Section 11

II. Test Organisms

- A. Name: *Ceriodaphnia dubia* (Cladoceran)
- Source: Laboratory Culture
- Age: <24 hours
- Life Stage: Neonate

- B. Name: *Pimephales promelas* (Fathead minnow)  
Source: Laboratory Culture  
Age: <24 hours  
Life Stage: Larval

### III. External Factors

#### A. Incubator

Temperature (°C)

Average: 25.2

Range: 25.0-25.5

Light Cycle: 16 hours light/ 8 hours dark

Light Intensity: 100 footcandles

Control Water: Moderately Hard Synthetic Water (#MH969)

#### B. *Ceriodaphnia dubia*

Test Chambers: 30 ml Solo cups

Volume per Chamber: 15-20 ml

Number of Organisms per Chamber: 1

Number of Replicates per Concentration: 10

Acclimation: Laboratory control water was added to cultures until >50% of the culture water consisted of control water.

Food: Cladocera were fed *Selenastrum* (#ABS 052016) and yeast/cereal/trout chow mix (#YCT 060716-1) one hour prior to test setup and once daily thereafter.

#### C. *Pimephales promelas*

Test Chambers: 250 ml storage dishes

Volume per Chamber: 200 ml

Number of Organisms per Chamber: 8

Number of Replicates per Concentration: 5

Acclimation: Laboratory control water was added to cultures until >50% of the culture water consisted of control water.

Food: Larval fish were fed 0.15ml of laboratory-cultured *Artemia* brine shrimp one hour prior to test setup and then 3X daily thereafter.

## IV. Quality Assurance

A. Standard Toxicant: Sodium Chloride

B. Organism: *Ceriodaphnia dubia*

Date and time of Reference Toxicant Test

Start: 06/06/16

Terminated: 06/14/16

Time of Reference Toxicant Test

Start: 1250

Terminated: 0930

Laboratory Analyst: McCauley

Dilution Water Used: Moderately Hard Synthetic Water #968

Results: Survival and Reproduction within control limits

Survival

LOEC: 2.60 g/L NaCl

EC50: 1.83 g/L NaCl

Reproduction

LOEC: 1.27 g/L NaCl

IC25: 0.83 g/L NaCl

C. Organism: *Pimephales promelas*

Date of Reference Toxicant Test

Start: 06/06/16

Terminated: 06/13/16

Time of Reference Toxicant Test

Start: 1220

Terminated: 1200

Laboratory Analyst: McCauley

Dilution Water Used: Moderately Hard Synthetic Water #968

Results: Survival and Growth were within control limits.

Survival

LOEC: 7.50 g/L NaCl

EC50: 6.15 g/L NaCl

Growth

LOEC: &gt;7.50 g/L NaCl

IC25: &gt;7.50 g/L NaCl

V. Physical and Chemical Data - See Attached

VI. Survival and Growth Data - See Attached

VII. Statistical Methods - See Attached

VIII. NPDES Permit Results - See Attached

**SUMMARY REPORTING FORM**  
**WET Testing**  
***Ceriodaphnia dubia* Survival and Reproduction**

Permittee: Walnut Ridge WWT Plant

NPDES No.: AR0046566

		<u>Time</u>	<u>Date</u>	to	<u>Time</u>	<u>Date</u>
Composite 1:	Collected from	0900	6/12/16		0900	6/13/16
Composite 2:	Collected from	0900	6/14/16		0900	6/15/16
Composite 3:	Collected from	0900	6/16/16		0900	6/17/16

Test Initiated: 1445

Date: 06/13/16

Time Terminated: 1335

Date: 06/20/16

Dilution H<sub>2</sub>O: MH 969

**PERCENT SURVIVAL**

Percent Effluent

<u>Time of Reading</u>	<u>Control</u>	<u>32%</u>	<u>42%</u>	<u>56%</u>	<u>80%</u>	<u>100%</u>
24h	100	100	100	100	100	100
48h	100	100	100	100	30	0
7 day	100	100	100	80	0	0

**NUMBER OF YOUNG/FEMALE @ 7 DAYS**

Percent Effluent

<u>REP</u>	<u>0%</u>	<u>32%</u>	<u>42%</u>	<u>56%</u>	<u>80%</u>	<u>100%</u>
A	30	22	14	2	X/0	X/0
B	38	22	13	7	X/0	X/0
C	29	14	16	1	X/0	X/0
D	29	31	3	6	X/0	X/0
E	36	25	2	X/0	X/0	X/0
F	24	23	3	4	X/0	X/0
G	25	23	13	X/4	X/0	X/0
H	29	23	10	5	X/0	X/0
I	25	25	19	0	X/0	X/0
J	26	23	19	7	X/0	X/0
<b>Mean</b>	<b>29.1</b>	<b>23.1</b>	<b>11.2</b>	<b>3.6</b>	<b>0.0</b>	<b>0.0</b>
<b>CV%*</b>	<b>16.1</b>	<b>18.0</b>	<b>58.0</b>	<b>75.5</b>	<b>0.0</b>	<b>0.0</b>

\*Coefficient of Variation% = Standard Deviation x 100/Mean

### ***Ceriodaphnia dubia* Survival and Reproduction**

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?  
 X  Yes        No
2. DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST AS APPROPRIATE:  
Is the mean number of young produced per female by the critical dilution (100%) significantly different ( $p=0.05$ ) than the control's number of young per female?  
 X  Yes        No
3. If the NOEC for survival is less than the critical dilution, enter [1], otherwise enter [0] for parameter #TGP3B:  1
4. If the NOEC for reproduction is less than the critical dilution, enter [1], otherwise enter [0] for parameter #TLP3B:  1
5. Report the NOEC value for survival, Parameter #TOP3B:  
NOEC survival  56  % effluent
6. Report the NOEC value for reproduction, Parameter #TPP3B:  
NOEC reproduction  <32  % effluent
7. Report the % coefficient of variation (largest of critical and control dilutions), Parameter #TQP3B:  
CV % reproduction  16.1%  (control)

### **Whole Effluent Lethality Values for *Ceriodaphnia dubia***

1. Report the Whole Effluent Lethality values for the 30-Day average minimum, Parameter #22414:  
Daily Average Minimum NOEC:  56%
2. Report the Whole Effluent Lethality values for the 7-day minimum, Parameter #22414:  
7-Day Minimum NOEC:  56%



**WET Testing Summary Form**  
***Ceriodaphnia dubia* (Cladoceran)**  
**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant  
 NPDES No.: AR0046566  
 Contact: Bruce Richart  
 Analyst: Cooper

Sample No. 1 Collected Ending Date: 6/13/16 Time: 0900  
 Sample No. 2 Collected Ending Date: 6/15/16 Time: 0900  
 Sample No. 3 Collected Ending Date: 6/17/16 Time: 0900  
 Test Begin: Date: 6/13/16 Time: 1445 Test End: Date: 6/20/16 Time: 1335

Initial Water Chemistry for Chronic Tests								
Project: Walnut Ridge – <i>C. dubia</i>								
Test day		0	1	2	3	4	5	6
Date		6/13/2016	6/14/2016	6/15/2016	6/16/2016	6/17/2016	6/18/2016	6/19/2016
H <sub>2</sub> O #		MH969	MH969	MH969	MH969	MH969	MH969	MH969
Temp (°C)	Control	22.5	23.0	24.0	22.6	23.0	23.4	23.0
	32%	22.3	23.2	24.0	22.5	23.0	22.6	22.5
	42%	22.1	23.4	24.2	22.8	23.2	22.8	22.3
	56%	22.0	23.5	24.4	23.0	23.5	22.9	22.4
	80%	21.8	23.8	24.6	23.0	-	-	-
	100%	21.7	24.0	24.9	-	-	-	-
pH (Standard Units)	Control	8.14	7.87	8.11	7.51	7.97	7.82	8.00
	32%	8.00	8.19	8.09	7.58	7.93	7.92	8.06
	42%	7.98	8.18	8.08	7.61	7.89	7.93	8.06
	56%	7.96	8.19	8.08	7.62	7.88	7.94	8.06
	80%	7.95	8.04	8.06	7.69	-	-	-
	100%	7.91	7.97	7.97	-	-	-	-
DO (mg/L)	Control	8.5	8.8	8.4	8.6	8.6	8.6	8.8
	32%	8.5	8.4	8.5	8.6	8.4	8.5	8.6
	42%	8.4	8.4	8.3	8.4	8.2	8.5	8.7
	56%	8.2	8.4	8.2	8.3	8.2	8.4	8.6
	80%	8.0	8.2	8.1	8.2	-	-	-
	100%	7.4	8.0	7.5	-	-	-	-
Cond (µS/cm)	Control	299	312	316	308	309	305	303
	32%	414	433	453	437	454	445	436
	42%	446	472	500	484	506	489	481
	56%	494	529	564	540	570	551	540
	80%	572	626	679	626	-	-	-
	100%	626	716	775	-	-	-	-
Alk (mg/L)	Control	62		62		62		
	100%	290		288		244		
Hard (mg/L)	Control	90		90		90		
	100%	210		200		220		

**WET Testing Summary Form**  
***Ceriodaphnia dubia* (Cladoceran)**

**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant

Sample No. 1 Collected Ending Date: 6/13/16 Time: 0900

NPDES No.: AR0046566

Sample No. 2 Collected Ending Date: 6/15/16 Time: 0900

Contact: Bruce Richart

Sample No. 3 Collected Ending Date: 6/17/16 Time: 0900

Analyst: Cooper

Test Begin: Date: 6/13/16 Time: 1445 Test End: Date: 6/20/16 Time: 1335

Final Water Chemistry for Chronic Tests								
Project: Walnut Ridge - <i>C. dubia</i>								
Test day		1	2	3	4	5	6	7
Date:		6/14/2016	6/15/2016	6/16/2016	6/17/2016	6/18/2016	6/19/2016	6/20/2016
H <sub>2</sub> O #		MH969	MH969	MH969	MH969	MH969	MH969	MH969
Temp (°C)	Control	23.3	24.3	23.0	23.2	23.0	22.8	23.8
	32%	23.4	24.2	23.3	23.4	22.4	22.9	24.1
	42%	23.4	24.3	23.3	23.7	23.1	22.6	23.9
	56%	23.8	24.5	23.4	23.6	23.2	22.4	24.1
	80%	23.4	24.6	23.3	-	-	-	-
	100%	23.9	24.4	-	-	-	-	-
pH (Standard Units)	Control	8.34	8.93	7.99	8.68	8.27	8.35	8.62
	32%	8.45	8.79	8.09	8.67	8.34	8.40	8.69
	42%	8.48	8.80	8.09	8.75	8.35	8.45	8.63
	56%	8.52	8.77	8.15	8.70	8.39	8.47	8.67
	80%	8.54	8.78	8.15	-	-	-	-
	100%	8.58	8.76	-	-	-	-	-
DO (mg/L)	Control	8.7	9.0	8.9	8.7	9.0	9.1	9.0
	32%	8.7	9.0	8.7	9.1	9.0	9.0	9.3
	42%	8.7	8.9	8.7	9.3	9.0	8.8	9.4
	56%	8.6	9.0	8.6	9.2	9.0	8.8	9.3
	80%	8.6	9.0	8.6	-	-	-	-
	100%	8.6	8.9	-	-	-	-	-

**SUMMARY REPORTING FORM**  
**WET Testing**  
**Fathead Minnow Larvae (*Pimephales promelas*) Survival and Growth**

Permittee: Walnut Ridge WWT Plant

NPDES No.: AR0046566

		<u>Time</u>	<u>Date</u>		<u>Time</u>	<u>Date</u>
Composite 1:	Collected from	0900	6/12/16	to	0900	6/13/16
Composite 2:	Collected from	0900	6/14/16	to	0900	6/15/16
Composite 3:	Collected from	0900	6/16/16	to	0900	6/17/16

Test Initiated: 1520

Date: 06/13/16

Time Terminated: 1500

Date: 06/20/16

Dilution H<sub>2</sub>O: MH 969

**DATA TABLE FOR SURVIVAL**

Effluent Conc. %	Survival in Replicate Chambers					Mean % Survival			CV%
	A	B	C	D	E	24h	48h	7 days	
Control	100	100	100	100	87.5	97.5	97.5	97.5	6.1
32	100	100	87.5	100	100	97.5	97.5	97.5	6.1
42	87.5	100	100	87.5	75	100	100	90	11.7
56	75	87.5	87.5	75	75	100	100	80	8.0
80	0	0	0	0	0	97.5	0	0	0.0
100	0	0	0	0	0	82.5	0	0	0.0

**DATA TABLE FOR GROWTH**

Effluent Conc %	Average Dry Weight in Replicate Chambers (mg)					Mean Dry Weight (mg)	CV%
	A	B	C	D	E		
Control	0.2337	0.2775	0.2713	0.2950	0.2757	0.2706	8.3
32	0.2763	0.2050	0.2571	0.3237	0.3250	0.2774	18.1
42	0.2443	0.1988	0.2862	0.3043	0.2533	0.2574	15.9
56	0.1883	0.1471	0.1471	0.2483	0.2250	0.1912	23.8
80	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-

Coefficient of Variation = Standard Deviation x 100/Mean

## Fathead 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     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?

Yes     No

3. If the NOEC for survival is less than the critical dilution, enter [1], otherwise enter [0] for parameter #TGP6C:   1  

4. If the NOEC for growth is less than the critical dilution, enter [1], otherwise enter [0] for parameter #TLP6C:   1  

5. Report the NOEC value for survival, Parameter #TOP6C:  
NOEC survival  42  % effluent

6. Report the NOEC value for growth, Parameter #TPP6C:  
NOEC growth  42  % effluent

7. Report the % coefficient of variation (largest of low flow and control dilutions), Parameter #TQP6C: CV % growth  8.3%  (control)

## Whole Effluent Lethality Values

1. Report the Whole Effluent Lethality values for the 30-Day average minimum, Parameter #22414:

Daily Average Minimum NOEC:  42% 

2. Report the Whole Effluent Lethality values for the 7-day minimum, Parameter #22414:

7-Day Minimum NOEC:  42%

**WET Testing Summary Form**  
**Fathead Minnow Larvae (*Pimephales promelas*)**

**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant

Sample No. 1 Collected Ending Date: 6/13/16 Time: 0900

NPDES No.: AR0046566

Sample No. 2 Collected Ending Date: 6/15/16 Time: 0900

Contact: Bruce Richart

Sample No. 3 Collected Ending Date: 6/17/16 Time: 0900

Analyst: Nicholson

Test Begin: Date: 6/13/16 Time: 1520 Test End: Date: 6/20/16 Time: 1500

Initial Water Chemistry for Chronic Tests								
Project: Walnut Ridge – <i>P. promelas</i>								
Test day		0	1	2	3	4	5	6
Date		6/13/2016	6/14/2016	6/15/2016	6/16/2016	6/17/2016	6/18/2016	6/19/2016
H <sub>2</sub> O #		MH969	MH969	MH969	MH969	MH969	MH969	MH969
Temp (°C)	Control	22.5	23.0	24.0	22.6	23.0	23.4	23.0
	32%	22.3	23.2	24.0	22.5	23.0	22.6	22.5
	42%	22.1	23.4	24.2	22.8	23.2	22.8	22.3
	56%	22.0	23.5	24.4	23.0	23.5	22.9	22.4
	80%	21.8	23.8	24.6	23.0	-	-	-
	100%	21.7	24.0	24.9	-	-	-	-
pH (Standard Units)	Control	8.14	7.87	8.11	7.51	7.97	7.82	8.00
	32%	8.00	8.19	8.09	7.58	7.93	7.92	8.06
	42%	7.98	8.18	8.08	7.61	7.89	7.93	8.06
	56%	7.96	8.19	8.08	7.62	7.88	7.94	8.06
	80%	7.95	8.04	8.06	7.69	-	-	-
	100%	7.91	7.97	7.97	-	-	-	-
DO (mg/L)	Control	8.5	8.8	8.4	8.6	8.6	8.6	8.8
	32%	8.5	8.4	8.5	8.6	8.4	8.5	8.6
	42%	8.4	8.4	8.3	8.4	8.2	8.5	8.7
	56%	8.2	8.4	8.2	8.3	8.2	8.4	8.6
	80%	8.0	8.2	8.1	8.2	-	-	-
	100%	7.4	8.0	7.5	-	-	-	-
Cond (µS/cm)	Control	299	312	316	308	309	305	303
	32%	414	433	453	437	454	445	436
	42%	446	472	500	484	506	489	481
	56%	494	529	564	540	570	551	540
	80%	572	626	679	626	-	-	-
	100%	626	716	775	-	-	-	-
Alk (mg/L)	Control	62		62		62		
	100%	290		288		244		
Hard (mg/L)	Control	90		90		90		
	100%	210		200		220		

**WET Testing Summary Form**  
**Fathead Minnow Larvae (*Pimephales promelas*)**  
**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant  
 NPDES No.: AR0046566  
 Contact: Bruce Richart  
 Analyst: Nicholson

Sample No. 1 Collected Ending Date: 6/13/16 Time: 0900  
 Sample No. 2 Collected Ending Date: 6/15/16 Time: 0900  
 Sample No. 3 Collected Ending Date: 6/17/16 Time: 0900  
 Test Begin: Date: 6/13/16 Time: 1520 Test End: Date: 6/20/16 Time: 1500

Final Water Chemistry for Chronic Tests								
Project: Walnut Ridge – <i>P. promelas</i>								
Test day		1	2	3	4	5	6	7
Date		6/14/2016	6/15/2016	6/16/2016	6/17/2016	6/18/2016	6/19/2016	6/20/2016
H <sub>2</sub> O #		MH969	MH969	MH969	MH969	MH969	MH969	MH969
Temp (°C)	Control	24.0	24.0	23.0	24.2	23.2	23.4	24.2
	32%	24.0	24.0	23.0	24.2	23.3	23.4	24.0
	42%	24.5	24.5	23.0	24.3	23.4	23.5	24.3
	56%	24.8	24.5	22.0	24.0	23.5	23.5	24.2
	80%	25.0	25.0	-	-	-	-	-
	100%	25.0	25.0	-	-	-	-	-
pH (Standard Units)	Control	7.81	7.85	7.24	7.51	7.56	7.38	7.85
	32%	8.12	8.12	7.58	7.77	7.60	7.41	7.35
	42%	8.19	8.22	7.64	7.82	7.60	7.47	7.41
	56%	8.22	8.29	7.66	7.89	7.60	7.51	7.46
	80%	8.25	8.36	-	-	-	-	-
	100%	8.26	8.38	-	-	-	-	-
DO (mg/L)	Control	7.9	8.3	7.5	6.8	7.0	5.9	7.7
	32%	7.6	7.0	8.4	6.8	6.4	4.4	4.1
	42%	7.3	6.7	8.5	6.0	5.5	4.0	4.3
	56%	7.3	6.7	8.6	6.2	5.0	3.9	3.9
	80%	7.3	6.3	-	-	-	-	-
	100%	7.0	6.0	-	-	-	-	-

**Ceriodaphnia Survival and Reproduction Test-7 Day Survival**

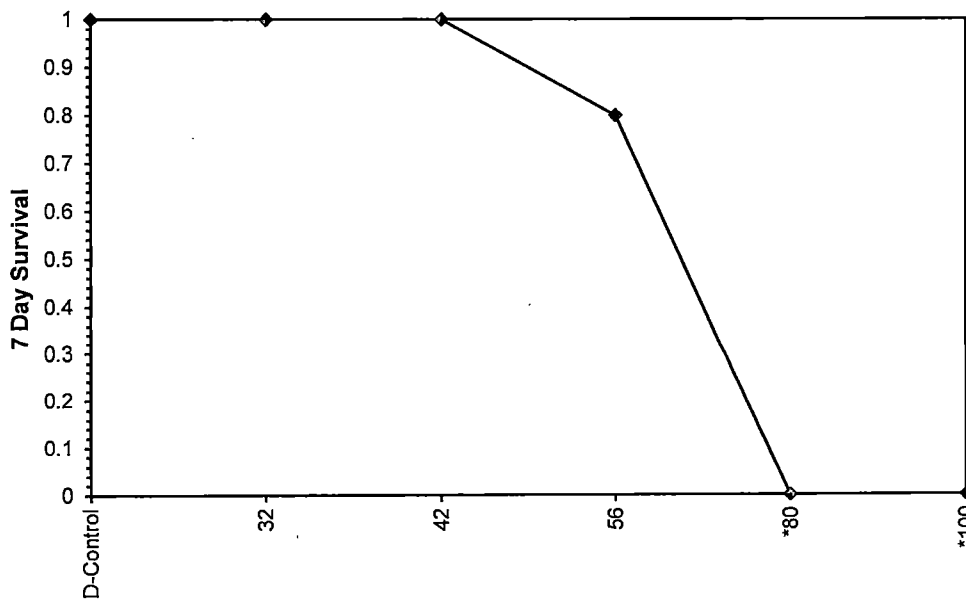
Start Date: 6/13/2016 14:45	Test ID: Walnut Rid	Sample ID: AR0046566-NPDES Permit #
End Date: 6/20/2016 13:35	Lab ID: ASU-ERF	Sample Type: EFF1-POTW
Sample Date:	Protocol: EPAF 02-EPA Freshwater	Test Species: CD-Ceriodaphnia dubia
Comments: June retest		

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
32	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
42	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
56	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000	0.0000	1.0000	1.0000	1.0000
80	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical
D-Control	1.0000	1.0000	0	10	10	10		
32	1.0000	1.0000	0	10	10	10	1.0000	0.0500
42	1.0000	1.0000	0	10	10	10	1.0000	0.0500
56	0.8000	0.8000	2	8	10	10	0.2368	0.0500
*80	0.0000	0.0000	10	0	10	10	0.0000	0.0500
*100	0.0000	0.0000	10	0	10	10	0.0000	0.0500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	56	80	66.9328	1.78571

**Dose-Response Plot**



**Ceriodaphnia Survival and Reproduction Test-Reproduction**

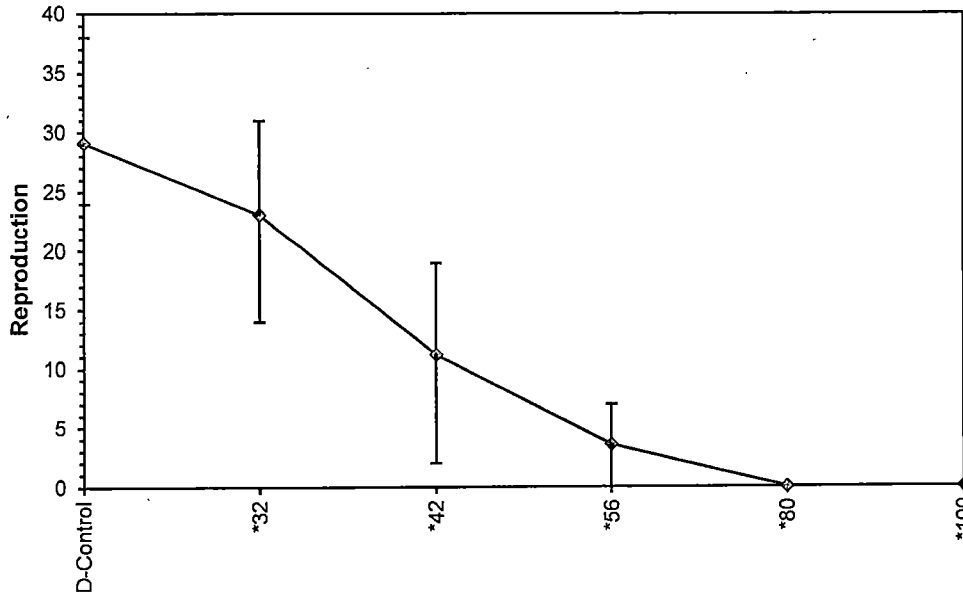
Start Date: 6/13/2016 14:45	Test ID: Walnut Rid	Sample ID: AR0046566-NPDES Permit #
End Date: 6/20/2016 13:35	Lab ID: ASU-ERF	Sample Type: EFF1-POTW
Sample Date:	Protocol: EPAF 02-EPA Freshwater	Test Species: CD-Ceriodaphnia dubia
Comments: June retest		

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	30.000	38.000	29.000	29.000	36.000	24.000	25.000	29.000	25.000	26.000
32	22.000	22.000	14.000	31.000	25.000	23.000	23.000	23.000	25.000	23.000
42	14.000	13.000	16.000	3.000	2.000	3.000	13.000	10.000	19.000	19.000
56	2.000	7.000	1.000	6.000	0.000	4.000	4.000	5.000	0.000	7.000
80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Conc-%	Mean	N-Mean	Transform: Untransformed				N	Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%			
D-Control	29.100	1.0000	29.100	24.000	38.000	16.073	10		
*32	23.100	0.7938	23.100	14.000	31.000	17.959	10	67.00	75.00
*42	11.200	0.3849	11.200	2.000	19.000	57.986	10	55.00	75.00
*56	3.600	0.1237	3.600	0.000	7.000	75.450	10	55.00	75.00
*80	0.000	0.0000	0.000	0.000	0.000	0.000	10	55.00	75.00
*100	0.000	0.0000	0.000	0.000	0.000	0.000	10	55.00	75.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.87632	1.035	-0.11463	1.51815
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<32	32		

**Dose-Response Plot**











**Larval Fish Growth and Survival Test-7 Day Survival**

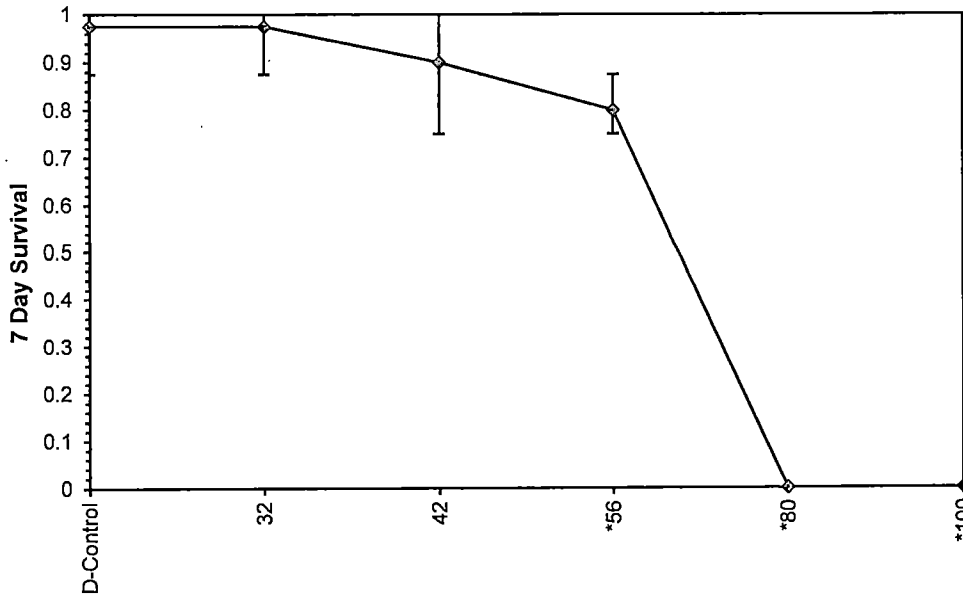
Start Date: 6/13/2016 15:20	Test ID: Walnut Rid	Sample ID: AR0046566-NPDES Permit #
End Date: 6/20/2016 15:00	Lab ID: ASU-ERF	Sample Type: EFF1-POTW
Sample Date:	Protocol: EPAF 02-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: June retest		

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	0.8750
32	1.0000	1.0000	0.8750	1.0000	1.0000
42	0.8750	1.0000	1.0000	0.8750	0.7500
56	0.7500	0.8750	0.8750	0.7500	0.7500
80	0.0000	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				N	Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%			
D-Control	0.9750	1.0000	1.3564	1.2094	1.3931	6.055	5		
32	0.9750	1.0000	1.3564	1.2094	1.3931	6.055	5	27.50	16.00
42	0.9000	0.9231	1.2504	1.0472	1.3931	11.683	5	22.00	16.00
*56	0.8000	0.8205	1.1121	1.0472	1.2094	7.990	5	16.00	16.00
*80	0.0000	0.0000	0.1777	0.1777	0.1777	0.000	5	15.00	16.00
*100	0.0000	0.0000	0.1777	0.1777	0.1777	0.000	5	15.00	16.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91535	0.9	-0.62481	1.20483
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	42	56	48.4974	2.38095

**Dose-Response Plot**



**Larval Fish Growth and Survival Test-7 Day Growth**

Start Date: 6/13/2016 15:20	Test ID: Walnut Rid	Sample ID: AR0046566-NPDES Permit #
End Date: 6/20/2016 15:00	Lab ID: ASU-ERF	Sample Type: EFF1-POTW
Sample Date:	Protocol: EPAF 02-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: June retest		

Conc-%	1	2	3	4	5
D-Control	0.2337	0.2775	0.2713	0.2950	0.2757
32	0.2763	0.2050	0.2571	0.3237	0.3250
42	0.2443	0.1988	0.2862	0.3043	0.2533
56	0.1883	0.1471	0.1471	0.2483	0.2250

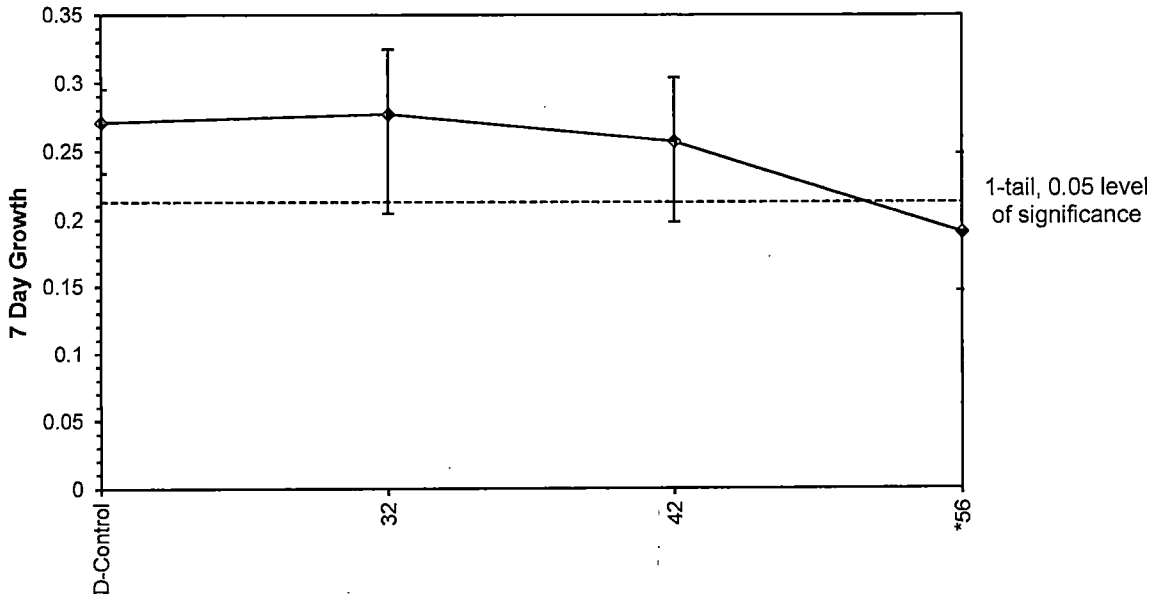
Conc-%	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed Critical	MSD
			Mean	Min	Max	CV%	N			
D-Control	0.2706	1.0000	0.2706	0.2337	0.2950	8.319	5			
32	0.2774	1.0251	0.2774	0.2050	0.3250	18.083	5	-0.261	2.230	0.0580
42	0.2574	0.9510	0.2574	0.1988	0.3043	15.852	5	0.510	2.230	0.0580
*56	0.1912	0.7064	0.1912	0.1471	0.2483	23.821	5	3.055	2.230	0.0580

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95845	0.868	-0.26004	-0.81285
Bartlett's Test indicates equal variances (p = 0.52)	2.23789	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	42	56	48.4974	2.38095	0.05799	0.21427	0.00781	0.00169	0.01637	3, 16

**Dose-Response Plot**



**CHRONIC TEST DATA SHEET**  
*Pimephales promelas*

Project: Walnut Ridge Beginning Date: 061316 Time: 1520 Test Species: P. promelas  
Dilution H<sub>2</sub>O: MH969 Ending Date: 062016 Time: 1500 Age: < 24hrs

Test Type: ( \* )Static Renewal ( ) Flowthrough Toxicant/Effluent

Conc.	Rep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Pan #
Control	1	8/0	8/0	8/0	8/0	8/0	8/0	8/0	1
	2	8/0	8/0	8/0	8/0	8/0	8/0	8/0	2
	3	8/0	8/0	8/0	8/0	8/0	8/0	8/0	3
	4	8/0	8/0	8/0	8/0	8/0	8/0	8/0	4
	5	8/1	7/0	8/0	7/0	7/0	7/0	7/0	5
32%	1	8/0	8/0	8/0	8/0	8/0	8/0	8/0	6
	2	8/0	8/0	8/0	8/0	8/0	8/0	8/0	7
	3	8/1	7/0	7/0	7/0	7/0	7/0	7/0	8
	4	8/0	8/0	8/0	8/0	8/0	8/0	8/0	9
	5	8/0	8/0	8/0	8/0	8/0	8/0	8/0	10
42%	1	8/0	8/0	8/0	8/0	8/1	7/0	7/0	11
	2	8/0	8/0	8/0	8/0	8/0	8/0	8/0	12
	3	8/0	8/0	8/0	8/0	8/0	8/0	8/0	13
	4	8/0	8/0	8/1	7/0	7/0	7/0	7/0	14
	5	8/0	8/0	8/0	8/2	6/0	6/0	6/0	15
56%	1	8/0	8/0	8/0	8/1	7/0	7/1	6/0	16
	2	8/0	8/0	8/0	8/0	8/0	8/1	7/0	17
	3	8/0	8/0	8/0	8/0	8/0	8/1	7/0	18
	4	8/0	8/0	8/1	7/1	6/0	6/0	6/0	19
	5	8/0	8/0	8/0	8/0	8/2	6/0	6/0	20
Date		061415	061516	061616	061716	061816	061916	062016	
Initials		OP	fu	WBP	JEN	REN	CBW	CAB	f

**CHRONIC TEST DATA SHEET**  
*Pimephales promelas*

Project: Walnut Ridge Beginning Date: 0613/16 Time: 1520 Test Species: P.promelas  
Dilution H<sub>2</sub>O: 1:14969 Ending Date: 0620/16 Time: 1500 Age: < 24hrs

Test Type: ( \* )Static Renewal ( ) Flowthrough Toxicant/Effluent: ( )

Conc.	Rep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Pan #
80%	1	8/1	7/7						
	2	8/0	8/8						
	3	8/0	8/8						
	4	8/0	8/8						
	5	8/0	8/8						
100%	1	8/0	8/8						
	2	8/1	7/7						
	3	8/0	8/8						
	4	8/3	5/5						
	5	8/3	5/5						
Date		0614/16	0615/16						
Initials		VLS JEN	JPR						

Initial Water Chemistry for Chronic Tests  
Project: Walnut Ridge - *C. dubia* / *P. promelas*

Test Day:		0	1	2	3	4	5	6
Date:		06/13/16	06/14/16	06/15/16	06/16/16	06/17/16	06/18/16	06/19/16
H <sub>2</sub> O Batch #:		MH969	MH969	MH969	MH969	MH969	MH969	MH969
Temp. (°C)	Control	22.5	23.0	24.0	22.6	23.0	23.4	23.0
	32%	22.3	23.2	24.0	22.5	23.0	22.6	22.5
	42%	22.1	23.4	24.2	22.8	23.2	22.8	22.3
	56%	22.0	23.5	24.4	23.0	23.5	22.9	22.4
	80%	21.8	23.8	24.6	23.0	—	—	—
	100%	21.7	24.0	24.9	—	—	—	—
pH	Control	8.14	7.87	8.11	7.51	7.97	7.82	8.00
	32%	8.00	8.19	8.09	7.58	7.93	7.92	8.06
	42%	7.98	8.18	8.08	7.61	7.89	7.93	8.06
	56%	7.96	8.19	8.08	7.62	7.88	7.94	8.06
	80%	7.95	8.04	8.06	7.69	—	—	—
	100%	7.91	7.97	7.97	—	—	—	—
DO (mg/L)	Control	8.5	8.82	8.4	8.6	8.6	8.6	8.8
	32%	8.5	8.41	8.5	8.4	8.4	8.5	8.6
	42%	8.4	8.40	8.3	8.4	8.2	8.5	8.7
	56%	8.2	8.35	8.2	8.3	8.2	8.4	8.6
	80%	8.0	8.17	8.1	8.2	—	—	—
	100%	7.4	7.95	7.5	—	—	—	—
Cond. (µS/cm)	Control	<del>298</del>	312	316	308	309	305	303
	32%	414	433	453	437	454	465	436
	42%	446	472	500	484	506	489	481
	56%	494	529	564	540	570	551	540
	80%	572	626	679	626	—	—	—
	100%	626	716	775	—	—	—	—
Alk. (mg/L)	Control	62		62		102		
	100%	290		288		244		
Hard. (mg/L)	Control	90		90		90		
	100%	210		200		220		
Initials		AW/SJC RLC	AD/RLC	AW RLC	AW RLC	AW RLC	AW RLC	RLC

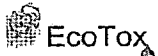




Final Water Chemistry for Chronic Tests  
Project: Walnut Ridge - *P. promelas*

Test Day:		1	2	3	4	5	6	7
Date:		06/15/16	06/15/16	06/16/16	06/17/16	06/18/16	06/19/16	06/20/16
H <sub>2</sub> O Batch #:		MH969	MH969	MH969	MH969	MH969	MH969	MH969
Temp. (°C)	Control	24.0	24.0	23.0	24.2	23.2	23.4	24.2
	32%	24.0	24.0	23.0	24.2	23.3	23.4	24.0
	42%	24.5	24.5	23.0	24.3	23.4	23.5	24.3
	56%	24.5	24.5	22.0	24.0	23.5	23.5	24.2
	80%	25.0	25.0	—	—	—	—	—
	100%	25.0	25.0	—	—	—	—	—
pH	Control	7.81	7.85	7.24	7.57 <sup>00</sup>	7.50	7.34	7.85
	32%	8.12	8.12	7.58	7.77	7.60	7.41	7.35
	42%	8.19	8.22	7.64	7.82	7.60	7.47	7.41
	56%	8.22	8.29	7.66	7.89	7.60	7.57	7.46
	80%	8.25	8.36	—	—	—	—	—
	100%	8.26	8.38	—	—	—	—	—
DO (mg/L)	Control	7.9	8.3	7.5	6.8	7.0	5.9	7.7
	32%	7.6	7.0	8.4	6.8	6.4	4.4	4.1
	42%	7.3	6.7	8.5	6.0	5.5	4.0	4.3
	56%	7.3	6.7	8.6	6.2	5.0	3.9	3.9
	80%	7.3	6.3	—	—	—	—	—
	100%	7.0	6.0	—	—	—	—	—
Initials		JLS/AEN	J	JS	AEN	AEN	AEN	LAB





Ecotoxicology Research Facility

### Ecotoxicology Research Facility

Arkansas State University

501 Iroquois Street

State University, AR 72467

(870) 972-2570 Fax (870) 972-2577

# CHAIN OF CUSTODY RECORD



Client Name <b>Walnut Ridge Wastewater Treatment</b>			Phone: <b>(870) 886-2312</b>				Analyses (List Below)											
Project #			Fax:															
Sampler (sign) 			PO #:				<table border="1"> <tr> <td rowspan="2">Chronic <i>C. dubia</i></td> <td rowspan="2">Chronic <i>P. promelas</i></td> <td></td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> <td></td> </tr> </table>				Chronic <i>C. dubia</i>	Chronic <i>P. promelas</i>						
Chronic <i>C. dubia</i>	Chronic <i>P. promelas</i>																	
Remarks:			Contact: <b>Jonathan Kopp</b>															
Cont.#	Sample ID Number	Location	Sample Date	Sample Time	Sample Type		Matrix											
					Comp	Grab	Aqueous	Soil	Other									
			6-14 - 6-15	9am	9am	<input checked="" type="checkbox"/>												
Ice present at delivery:		<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No																
Temp:		30°C		KL		Initials												
1. Relinquished By (sign) 			Date		Time		1. Received By (sign) 			Date		Time						
2. Relinquished By (sign)			Date		Time		2. Received By (sign)			Date		Time						
										06-15-16		11:45						



Ecotoxicology Research Facility

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Arkansas State University

2645 Caddo Drive

State University, AR 72467

(870) 972-2570 Fax (870) 972-2577

# CHAIN OF CUSTODY RECORD



Client Name <b>Walnut Ridge Wastewater Treatment</b>			Phone: <b>(870) 886-2312</b>				Analyses (List Below)									
Project #			Fax:													
Sampler (sign) <i>[Signature]</i>			PO #:				<table border="1"> <tr> <td rowspan="2">Chronic C. dubia</td> <td rowspan="2">Chronic P. promelas</td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> </tr> </table>				Chronic C. dubia	Chronic P. promelas				
Chronic C. dubia	Chronic P. promelas															
Remarks:			Contact: <b>Jonathan Kopp</b>													
Cont.#	Sample ID Number	Location	Sample Date	Sample Time	Sample Type		Matrix									
					Comp	Grab	Aqueous	Soil	Other							
			6-16/6-17	9am - 9am	<input checked="" type="checkbox"/>						<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>				
Ice present at delivery:			<input checked="" type="radio"/> Yes <input type="radio"/> No													
Temp:			<input checked="" type="radio"/> °C <i>[Signature]</i> Initials													
1. Relinquished By (sign) <i>[Signature]</i>			Date	Time	1. Received By (sign) <i>[Signature]</i>				Date	Time						
			6-17-16	11:59 am					6/17/16	11:58						
2. Relinquished By (sign)			Date	Time	2. Received By (sign)				Date	Time						



Ecotoxicology Research Facility

**SAMPLE CHECK IN**

Sample ID Number: WR # 1

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

Date: 061316 Sampling Date: 0612-0613 Arrival Time: 10:37 AM

Field Identification Number: EFF 001 Description: Composite Sample

Shipped by: Federal Express \_\_\_\_\_ UPS \_\_\_\_\_ Hand delivered by: Jon Kopp

Drop-Off Location: ASU-ERF

Storage While Shipped: Ice - Coolers

Analysis Requested: Chronic Cdubia Chronic P. promelas

Initial Water-Chemistry Analysis:

Sample Received by: JEN/JLS

Temperature (°C): 3°C Ice Present upon delivery: YES NO

Date: 061316

Quality Assurance	Initial	Date	Yes	No	
Chain of Custody	JEN	061316	X		
Refrigerated at 4°C	JEN	↓	X		
Field Record Received	JEN		X		
Sample Label Affixed Properly	JEN		X		
Project Leader Informed	JEN		✓	X	

Comments: \_\_\_\_\_



Ecotoxicology Research Facility

## SAMPLE CHECK IN

Sample ID Number: WR # 2

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

Date: 061516 Sampling Date: 0614 - 0615 Arrival Time: 1145

Field Identification Number: ERF 002 Description: Composite Sample

Shipped by: Federal Express \_\_\_\_\_ UPS \_\_\_\_\_ Hand delivered by: Jon Kopp

Drop-Off Location: ASU-ERF

Storage While Shipped: Ice - Cooler

Analysis Requested: Chronic C. dubia Chronic P. promelas

Initial Water Chemistry Analysis: \_\_\_\_\_

Sample Received by: KK

Temperature (°C): 3.0°C Ice Present upon delivery:  YES  NO

Date: 061516

Quality Assurance	Initial	Date	Yes	No
Chain of Custody	KK	061516	X	
Refrigerated at 4°C	KK	↓	X	
Field Record Received	KK		X	
Sample Label Affixed Properly	KK		X	
Project Leader Informed	KK		X	

Comments: \_\_\_\_\_



Ecotoxicology Research Facility

**SAMPLE CHECK IN**

Sample ID Number: WR #3

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

Date: 06/17/16 Sampling Date: 06/16 - 06/17 Arrival Time: 11:59am  
 Field Identification Number: EFF 003 Description: Composite

Shipped by: Federal Express \_\_\_\_\_ UPS \_\_\_\_\_ Hand delivered by: Jon Kopp

Drop-Off Location: ASU-ERF

Storage While Shipped: Ice - Coolers

Analysis Requested: Chronic C. dubia Chronic P. promelas

Initial Water Chemistry Analysis: \_\_\_\_\_

Sample Received by: JEN/RIC

Temperature (°C): 0°C Ice Present upon delivery:  YES  NO

Date: 06/17/16

Quality Assurance	Initial	Date	Yes	No
Chain of Custody	JEN	06/16/16	X	
Refrigerated at 4°C	JEN	↓	X	
Field Record Received	JEN		X	
Sample Label Affixed Properly	JEN		X	
Project Leader Informed	JEN		X	

Comments: \_\_\_\_\_





Ecotoxicology Research Facility



P.O. Box 847  
State University, AR 72467  
Tel. 870-972-2570  
Fax 870-972-2577  
<http://ecotox.astate.edu/>

College of Sciences & Mathematics  
[www.astate.edu](http://www.astate.edu)

June 2, 2016

Jonathan Kopp  
Walnut Ridge Wastewater Treatment Plant  
216 Southwest 4<sup>th</sup> Street  
Walnut Ridge, AR 72476

Dear Jon,

Please find enclosed the results of the 7-day chronic tests using water collected from Walnut Ridge's plant facilities during the week of May 16, 2016. No lethal effects were measured in *Pimephales promelas* or *Ceriodaphnia dubia* exposed to the critical flow concentration (100%) or other treated dilutions from this outfall. However, sublethal effects were measured in both test organisms, thus the WET test will need to be repeated monthly.

All test conditions and acceptability criteria as suggested by our laboratory and the US EPA were met during these tests.

Please call if you have any questions regarding this particular test series or any other tests conducted in the past.

Sincerely,

Jennifer L. Bouldin, PhD  
Director Ecotoxicology Research Facility  
PO Box 847  
Arkansas State University  
State University, AR 72467

**Arkansas State University Ecotoxicology Research Facility**

**Laboratory Report**

Facility Director / Lab Contact: Jennifer Bouldin  
 Phone: (870) 972-2570

Client: Walnut Ridge Wastewater Treatment Plant  
 216 Southwest 4th Street  
 Walnut Ridge, AR 72476

Contact: Jon Kopp  
 870-866-2312

NPDES Permit #: AR0046566      AFIN#: 38-00040  
 Effluent Sampling Point/Type: 24hr Composite

**Samples Collected:**

Sample #	Sampling Times	Received	Arrival Temp
1	5/15/16 0900 hrs to 5/16/16 0900 hrs	5/16/16 1116 hrs	.9 °C
2	5/17/16 0900 hrs to 5/18/16 0900 hrs	5/18/16 1135 hrs	1.0 °C
3	5/19/16 0900 hrs to 5/20/16 0900 hrs	5/20/16 1200 hrs	1.0 °C

**Test Methods:**

7-Day Chronic Toxicity, Static renewal, Cladoceran, EPA 821/R-02/013, Section 13  
 7-Day Chronic Toxicity, Static renewal, Fathead minnow, EPA 821/R-02/013, Section 11

Organisms: *C. dubia* <24hrs, *P. promelas* <24hrs      Culture Source: ASU Ecotox

Dilutions: 0%, 32%, 42%, 56%, 80%, 100%      Critical Dilution: 100%

Statistical Method: Toxcalc 5.0.25

<i>C. dubia</i>			<i>P. promelas</i>		
whole effluent toxicity			whole effluent toxicity		
	lethality	sublethality		lethality	sublethality
DMR Code	22414 10	22414 P0	DMR Code	22414 10	22414 P0
Result	100%	100%	Result	100%	100%
	lethality	sublethality		lethality	sublethality
DMR Code	TGP3B	TLP3B	DMR Code	TGP6C	TLP6C
Result	0	1	Result	0	1
	NOEC lethality	NOEC sublethal		NOEC lethality	NOEC sublethal
DMR Code	TOP3B	TPP3B	DMR Code	TOP6C	TPP6C
Result	100%	32%	Result	100%	56%
	CV%			CV%	
DMR Code	TQP3B		DMR Code	TQP6C	
Result	126.7%		Result	16.4%	
control survival	control mean reproduction		control survival	control mean weight	
100%	29.9		100%	0.9652	
critical dil. survival	critical mean reproduction		critical dil. survival	critical mean weight	
80%	2.9		75%	0.4784	
MSDp			MSDp		
0.2627			0.1150		

**Results Summary:**

QA/Reference Testing:

Data attached

Reviewed By:

  
 Jennifer L. Bouldin, Director ASU Ecotoxicology Research Facility

Toxicity Test Performed: 7-day *Ceriodaphnia dubia* Survival and Reproduction  
 Effluent Sampling Point: Walnut Ridge WWT Plant  
 Date Test Started: 5/16/16 *C. dubia*  
 Time Test Started: 1430 *C. dubia*  
 Date Test Terminated: 5/23/16 *C. dubia*  
 Time Test Terminated: 1345 *C. dubia*  
 Laboratory Analyst: Cooper

Toxicity Test Performed: 7-day *Pimephales promelas* Survival and Growth  
 Effluent Sampling Point: Walnut Ridge WWT Plant  
 Date Test Started: 5/16/16 *P. promelas*  
 Time Test Started: 1440 *P. promelas*  
 Date Test Terminated: 5/23/16 *P. promelas*  
 Time Test Terminated: 1520 *P. promelas*  
 Laboratory Analyst: McCauley/ Moland

## I. Test Methods

### A. Physical and Chemical Testing - APHA, Standard Methods for the Examination of Water and Wastewater; Vol. 21, 2005.

<u>Test</u>	<u>Method</u>
Alkalinity	2320B
Conductivity	2510B
Dissolved Oxygen (mg/L, DO)	4500-O-G
Hardness (mg/L CaCO <sub>3</sub> )	2340C
pH	4500-H <sup>+</sup> B
Temperature (°C)	2550B

### B. Toxicity Testing – EPA 821/R-02/013: Short Term Methods for Estimating the Chronic Toxicity of Effluents to Freshwater Organisms

<u>Test</u>	<u>Method</u>
Cladoceran Survival and Reproduction	Section 13
Fathead Minnow Survival and Growth	Section 11

## II. Test Organisms

- A. Name: *Ceriodaphnia dubia* (Cladoceran)  
 Source: Laboratory Culture  
 Age: <24 hours  
 Life Stage: Neonate

- B. Name: *Pimephales promelas* (Fathead minnow)  
Source: Laboratory Culture  
Age: <24 hours  
Life Stage: Larval

### III. External Factors

#### A. Incubator

Temperature (°C)

Average: 24.7

Range: 24.5-25.0

Light Cycle: 16 hours light/ 8 hours dark

Light Intensity: 100 footcandles

Control Water: Moderately Hard Synthetic Water (#967)

#### B. *Ceriodaphnia dubia*

Test Chambers: 30 ml Solo cups

Volume per Chamber: 15-20 ml

Number of Organisms per Chamber: 1

Number of Replicates per Concentration: 10

Acclimation: Laboratory control water was added to cultures until >50% of the culture water consisted of control water.

Food: Cladocera were fed *Selenastrum* (#ABS 050216) and yeast/cereal/trout chow mix (#YCT 030516-8) one hour prior to test setup and once daily thereafter.

#### C. *Pimephales promelas*

Test Chambers: 250 ml storage dishes

Volume per Chamber: 200 ml

Number of Organisms per Chamber: 8

Number of Replicates per Concentration: 5

Acclimation: Laboratory control water was added to cultures until >50% of the culture water consisted of control water.

Food: Larval fish were fed 0.15ml of laboratory-cultured *Artemia* brine shrimp one hour prior to test setup and then 3X daily thereafter.

## IV. Quality Assurance

## A. Standard Toxicant: Sodium Chloride

B. Organism: *Ceriodaphnia dubia*

Date and time of Reference Toxicant Test

Start: 04/04/16

Terminated: 04/11/16

Time of Reference Toxicant Test

Start: 1130

Terminated: 0950

Laboratory Analyst: Kilmer

Dilution Water Used: Moderately Hard Synthetic Water #963/964

Results: Survival and Reproduction within control limits

Survival

LOEC: 2.60 g/L NaCl

EC50: 2.21 g/L NaCl

Reproduction

LOEC: 1.82 g/L NaCl

IC25: 1.61 g/L NaCl

C. Organism: *Pimephales promelas*

Date of Reference Toxicant Test

Start: 04/04/16

Terminated: 04/11/16

Time of Reference Toxicant Test

Start: 1400

Terminated: 1355

Laboratory Analyst: Kilmer

Dilution Water Used: Moderately Hard Synthetic Water #963/964

Results: Survival and Growth within control limits.

Survival

LOEC: 4.22 g/L NaCl

EC50: 6.09 g/L NaCl

Growth

LOEC: &gt;7.50 g/L NaCl

IC25: 6.18 g/L NaCl

V. Physical and Chemical Data - See Attached

VI. Survival and Growth Data - See Attached

VII. Statistical Methods - See Attached

VIII. NPDES Permit Results - See Attached

**SUMMARY REPORTING FORM**  
**WET Testing**  
***Ceriodaphnia dubia* Survival and Reproduction**

Permittee: Walnut Ridge WWT Plant

NPDES No.: AR0046566

		<u>Time</u>	<u>Date</u>		<u>Time</u>	<u>Date</u>
Composite 1:	Collected from	0900	5/15/16	to	0900	5/16/16
Composite 2:	Collected from	0900	5/17/16	to	0900	5/18/16
Composite 3:	Collected from	0900	5/19/16	to	0900	5/20/16

Test Initiated: 1430

Date: 5/16/16

Time Terminated: 1345

Date: 5/23/16

Dilution H<sub>2</sub>O: MH 967

**PERCENT SURVIVAL**

Percent Effluent

<u>Time of Reading</u>	<u>Control</u>	<u>32%</u>	<u>42%</u>	<u>56%</u>	<u>80%</u>	<u>100%</u>
24h	100	100	100	100	100	100
48h	100	100	100	100	100	100
7 day	100	100	100	100	90	80

**NUMBER OF YOUNG/FEMALE @ 7 DAYS**

Percent Effluent

<u>REP</u>	<u>0%</u>	<u>32%</u>	<u>42%</u>	<u>56%</u>	<u>80%</u>	<u>100%</u>
A	32	16	30	7	6	7
B	35	20	31	26	21	0
C	35	28	31	22	4	0
D	34	28	13	15	3	x/0
E	31	32	21	19	3	x/0
F	34	33	20	15	1	2
G	36	37	17	26	8	0
H	32	20	21	9	7	0
I	14	19	11	22	x/0	5
J	16	12	10	18	7	9
<b>Mean</b>	<b>29.9</b>	<b>24.5</b>	<b>20.5</b>	<b>17.9</b>	<b>6.7</b>	<b>2.9</b>
<b>CV%*</b>	<b>26.8</b>	<b>33.6</b>	<b>39.2</b>	<b>36.3</b>	<b>87.8</b>	<b>126.7</b>

\*Coefficient of Variation% = Standard Deviation x 100/Mean

### ***Ceriodaphnia dubia* Survival and Reproduction**

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 number of young produced per female by the critical dilution (100%) significantly different ( $p=0.05$ ) than the control's number of young per female?  
  X   Yes      No
  
3. If the NOEC for survival is less than the critical dilution, enter [1], otherwise enter [0] for parameter #TGP3B:   0
  
4. If the NOEC for reproduction is less than the critical dilution, enter [1], otherwise enter [0] for parameter #TLP3B:   1
  
5. Report the NOEC value for survival, Parameter #TOP3B:  
NOEC survival  100  % effluent
  
6. Report the NOEC value for reproduction, Parameter #TPP3B:  
NOEC reproduction  32  % effluent
  
7. Report the % coefficient of variation (largest of critical and control dilutions), Parameter #TQP3B:  
CV % reproduction  126.7  % (critical)

### **Whole Effluent Lethality Values for *Ceriodaphnia dubia***

1. Report the Whole Effluent Lethality values for the 30-Day average minimum, Parameter #22414:  
Daily Average Minimum NOEC:  100%
  
2. Report the Whole Effluent Lethality values for the 7-day minimum, Parameter #22414:  
7-Day Minimum NOEC:  100%

**WET Testing Summary Form**  
***Ceriodaphnia dubia* (Cladoceran)**  
**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant

Sample No. 1 Collected Ending Date: 5/16/16 Time: 0900

NPDES No.: AR0046566

Sample No. 2 Collected Ending Date: 5/18/16 Time: 0900

Contact: Bruce Richart

Sample No. 3 Collected Ending Date: 5/20/16 Time: 0900

Analyst: Cooper

Test Begin: Date: 5/16/16 Time: 1430 Test End: Date: 5/23/16 Time: 1230

Initial Water Chemistry for Chronic Tests								
Project: Walnut Ridge – <i>C. dubia</i>								
Test day		0	1	2	3	4	5	6
Date		5/16/2016	5/17/2016	5/18/2016	5/19/2016	5/20/2016	5/21/2016	5/22/2016
H <sub>2</sub> O #		MH967	MH967	MH967	MH967	MH967	MH967	MH967
Temp (°C)	Control	22.0	22.8	21.8	21.2	22.1	22.0	22.0
	32%	22.2	22.6	22.2	21.7	22.2	22.2	22.0
	42%	22.4	22.4	22.1	21.6	22.5	22.0	22.1
	56%	22.7	22.7	22.4	21.8	22.7	22.2	22.0
	80%	22.9	22.9	22.5	22.0	22.8	22.7	22.5
	100%	23.0	23.0	22.8	22.2	23.0	22.9	22.8
pH (Standard Units)	Control	8.09	8.11	8.02	7.96	8.18	7.74	7.99
	32%	7.92	8.28	7.95	8.00	7.98	7.78	8.13
	42%	7.86	8.31	7.87	8.01	8.02	7.80	8.11
	56%	7.84	8.33	7.85	8.01	8.03	7.82	8.13
	80%	7.82	8.30	7.84	7.94	8.02	7.92	8.18
	100%	7.74	8.22	7.83	7.91	7.99	8.00	8.20
DO (mg/L)	Control	8.8	8.6	8.7	8.7	8.6	8.7	8.7
	32%	8.9	8.5	8.0	8.5	8.5	8.7	8.6
	42%	8.6	8.4	8.3	8.4	8.4	8.6	8.5
	56%	8.6	8.4	8.2	8.3	8.4	8.3	8.5
	80%	8.6	8.3	8.1	8.3	8.4	8.5	8.1
	100%	8.8	8.2	8.1	8.2	8.4	8.4	8.2
Cond (µS/cm)	Control	314	317	315	312	315	312	311
	32%	385	381	396	393	375	372	372
	42%	411	402	424	421	395	391	388
	56%	445	432	459	458	421	417	413
	80%	500	482	515	522	467	467	461
	100%	554	581	575	575	509	513	503
Alk (mg/L)	Control	58		58		58		
	100%	192		180		160		
Hard (mg/L)	Control	90		90		90		
	100%	165		230		180		



**WET Testing Summary Form**  
***Ceriodaphnia dubia* (Cladoceran)**  
**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant      Sample No. 1 Collected Ending Date: 5/16/16    Time: 0900  
 NPDES No.: AR0046566                              Sample No. 2 Collected Ending Date: 5/18/16    Time: 0900  
 Contact: Bruce Richart                                Sample No. 3 Collected Ending Date: 5/20/16    Time: 0900  
 Analyst: Cooper                                         Test Begin: Date: 5/16/16    Time: 1430    Test End: Date: 5/23/16 Time: 1230

Final Water Chemistry for Chronic Tests								
Project: Walnut Ridge - <i>C. dubia</i>								
Test day		1	2	3	4	5	6	7
Date:		5/17/2016	5/18/2016	5/19/2016	5/20/2016	5/21/2016	5/22/2016	5/23/2016
H <sub>2</sub> O #		MH967	MH967	MH967	MH967	MH967	MH967	MH967
Temp (°C)	Control	23.2	22.0	21.8	22.0	23.0	22.2	23.8
	32%	22.6	22.5	21.9	22.5	22.3	22.5	23.4
	42%	22.2	22.3	21.7	22.5	22.0	22.1	23.0
	56%	22.2	22.4	21.9	22.6	22.4	22.3	23.4
	80%	22.3	22.5	21.9	22.7	22.2	22.4	23.0
	100%	22.7	22.3	22.2	22.6	22.8	22.4	23.3
pH (Standard Units)	Control	8.52	8.64	8.22	8.82	8.50	8.57	8.47
	32%	8.54	8.64	8.36	8.81	8.56	8.57	8.58
	42%	8.56	8.68	8.39	8.82	8.53	8.62	8.56
	56%	8.59	8.65	8.45	8.81	8.61	8.57	8.63
	80%	8.58	8.66	8.46	8.82	8.53	8.63	8.56
	100%	8.61	8.65	8.51	8.75	8.57	8.60	8.60
DO (mg/L)	Control	9.0	9.1	9.1	9.0	8.6	8.5	8.9
	32%	9.0	9.1	8.8	9.1	8.8	8.7	9.1
	42%	8.9	9.3	8.8	9.2	9.0	8.8	9.2
	56%	9.0	9.1	8.8	9.3	9.1	8.8	9.1
	80%	8.9	9.2	8.7	9.3	9.0	8.8	9.0
	100%	8.8	9.1	8.7	9.1	8.9	8.8	8.8

## SUMMARY REPORTING FORM

### WET Testing

#### Fathead Minnow Larvae (*Pimephales promelas*) Survival and Growth

Permittee: Walnut Ridge WWT Plant

NPDES No.: AR0046566

		<u>Time</u>	<u>Date</u>		<u>Time</u>	<u>Date</u>
Composite 1:	Collected from	0900	5/15/16	to	0900	5/16/16
Composite 2:	Collected from	0900	5/17/16	to	0900	5/18/16
Composite 3:	Collected from	0900	5/19/16	to	0900	5/20/16

Test Initiated: 1430

Date: 5/16/16

Time Terminated: 1345

Date: 5/23/16

Dilution H<sub>2</sub>O: MH 967

#### DATA TABLE FOR SURVIVAL

Effluent Conc. %	% Survival in Replicate Chambers					Mean % Survival			CV%
	A	B	C	D	E	24h	48h	7days	
Control	100	100	100	100	100	100	100	100	0
32	100	100	100	100	100	100	100	100	0
42	87.5	100	100	100	100	100	100	97.5	6.1
56	100	87.5	100	100	100	100	100	97.5	6.1
80	100	75	87.5	87.5	100	100	100	90	11.7
100	100	50	75	75	75	100	100	75	20.3

Coefficient of Variation = Standard Deviation x 100/Mean

#### DATA TABLE FOR GROWTH

Effluent Conc %	Average Dry Weight in Replicate Chambers (mg)					Mean Dry Weight (mg)	CV%
	A	B	C	D	E		
Control	0.9700	0.9500	0.8550	0.9825	1.0687	0.9652	7.9
32	0.8925	1.0025	1.0225	0.9900	0.9013	0.9617	6.3
42	1.0043	0.9150	0.9825	0.8325	0.8738	0.9216	7.8
56	0.8988	0.9114	0.6987	0.9137	0.8550	0.8555	10.6
80	0.7350	0.8100	0.7229	0.6371	0.6850	0.7180	8.9
100	0.5888	0.5250	0.4317	0.4550	0.3917	0.4784	16.4

Coefficient of Variation = Standard Deviation x 100/Mean

## Fathead 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  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?

Yes \_\_\_\_ 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:   1  

5. Report the NOEC value for survival, Parameter #TOP6C:  
NOEC survival  100  % effluent

6. Report the NOEC value for growth, Parameter #TPP6C:  
NOEC growth  56  % effluent

7. Report the % coefficient of variation (largest of low flow and control dilutions), Parameter #TQP6C: CV % growth  16.4%  (critical)

## Whole Effluent Lethality Values

1. Report the Whole Effluent Lethality values for the 30-Day average minimum, Parameter #22414:

Daily Average Minimum NOEC:  100% 

2. Report the Whole Effluent Lethality values for the 7-day minimum, Parameter #22414:

7-Day Minimum NOEC:  100%

**WET Testing Summary Form**  
**Fathead Minnow Larvae (*Pimephales promelas*)**  
**Chemical Parameters Chart**

Permittee: Walnut Ridge Wastewater Plant

Sample No. 1 Collected Ending Date: 5/16/16 Time: 0900

NPDES No.: AR0046566

Sample No. 2 Collected Ending Date: 5/18/16 Time: 0900

Contact: Bruce Richart

Sample No. 3 Collected Ending Date: 5/20/16 Time: 0900

Analyst: Cooper

Test Begin: Date: 5/16/16 Time: 1430 Test End: Date: 5/23/16 Time: 1230

Initial Water Chemistry for Chronic Tests								
Project: Walnut Ridge - <i>P. promelas</i>								
Test day		1	2	3	4	5	6	7
Date		5/16/2016	5/17/2016	5/18/2016	5/19/2016	5/20/2016	5/21/2016	5/22/2016
H <sub>2</sub> O #		MH967	MH967	MH967	MH967	MH967	MH967	MH967
Temp (°C)	Control	22.0	22.8	21.8	21.2	22.1	22.0	22.0
	32%	22.2	22.6	22.2	21.7	22.2	22.2	22.0
	42%	22.4	22.4	22.1	21.6	22.5	22.0	22.1
	56%	22.7	22.7	22.4	21.8	22.7	22.2	22.0
	80%	22.9	22.9	22.5	22.0	22.8	22.7	22.5
	100%	23.0	23.0	22.8	22.2	23.0	22.9	22.8
pH (Standard Units)	Control	8.09	8.11	8.02	7.96	8.18	7.74	7.99
	32%	7.92	8.28	7.95	8.00	7.98	7.78	8.13
	42%	7.86	8.31	7.87	8.01	8.02	7.80	8.11
	56%	7.84	8.33	7.85	8.01	8.03	7.82	8.13
	80%	7.82	8.30	7.84	7.94	8.02	7.92	8.18
	100%	7.74	8.22	7.83	7.91	7.99	8.00	8.20
DO (mg/L)	Control	8.8	8.6	8.7	8.7	8.6	8.7	8.7
	32%	8.9	8.5	8.0	8.5	8.5	8.7	8.6
	42%	8.6	8.4	8.3	8.4	8.4	8.6	8.5
	56%	8.6	8.4	8.2	8.3	8.4	8.3	8.5
	80%	8.6	8.3	8.1	8.3	8.4	8.5	8.1
	100%	8.8	8.2	8.1	8.2	8.4	8.4	8.2
Cond (µS/cm)	Control	314	317	315	312	315	312	311
	32%	385	381	396	393	375	372	372
	42%	411	402	424	421	395	391	388
	56%	445	432	459	458	421	417	413
	80%	500	482	515	522	467	467	461
	100%	554	581	575	575	509	513	503
Alk (mg/L)	Control	58		58		58		
	100%	192		180		160		
Hard (mg/L)	Control	90		90		90		
	100%	165		230		180		

## WET Testing Summary Form

### Fathead Minnow Larvae (*Pimephales promelas*)

#### Chemical Parameters Chart

Permittee: Walnut Ridge Wastewater Plant  
 NPDES No.: AR0046566  
 Contact: Bruce Richart  
 Analyst: Cooper

Sample No. 1 Collected Ending Date: 5/16/16 Time: 0900  
 Sample No. 2 Collected Ending Date: 5/18/16 Time: 0900  
 Sample No. 3 Collected Ending Date: 5/20/16 Time: 0900  
 Test Begin: Date: 5/16/16 Time: 1430 Test End: Date: 5/23/16 Time: 1230

Final Water Chemistry for Chronic Tests								
Project: Walnut Ridge – <i>P. promelas</i>								
Test day		1	2	3	4	5	6	7
Date		5/17/2016	5/18/2016	5/19/2016	5/20/2016	5/21/2016	5/22/2016	5/23/2016
H <sub>2</sub> O #		MH967	MH967	MH967	MH967	MH967	MH967	MH967
Temp (°C)	Control	22.3	21.9	21.7	22.0	23.0	22.2	23.8
	32%	22.5	22.5	21.9	22.5	22.3	22.5	23.4
	42%	22.6	22.5	21.9	22.5	22.0	22.1	23.0
	56%	22.7	22.5	21.7	22.7	22.4	22.3	23.4
	80%	22.2	22.1	21.8	22.9	22.2	22.4	23.0
	100%	22.1	22.1	21.7	23.0	22.8	22.4	23.3
pH (Standard Units)	Control	7.64	7.29	7.08	7.66	8.50	8.57	8.47
	32%	8.02	7.70	7.56	7.88	8.56	8.57	8.58
	42%	8.10	7.80	7.65	7.94	8.53	8.62	8.56
	56%	8.17	7.91	7.76	7.97	8.61	8.57	8.63
	80%	8.25	8.06	7.88	8.02	8.53	8.63	8.56
	100%	8.32	8.14	8.00	8.08	8.57	8.60	8.60
DO (mg/L)	Control	7.8	6.2	5.5	6.6	8.6	8.5	8.9
	32%	7.7	6.5	5.6	6.2	8.8	8.7	9.1
	42%	7.5	6.6	5.4	6.0	9.0	8.8	9.2
	56%	7.3	6.5	5.3	5.8	9.1	8.8	9.1
	80%	7.2	5.7	5.3	5.2	9.0	8.8	9.0
	100%	7.3	6.1	5.3	4.8	8.9	8.8	8.8

**Ceriodaphnia Survival and Reproduction Test-7 Day Survival**

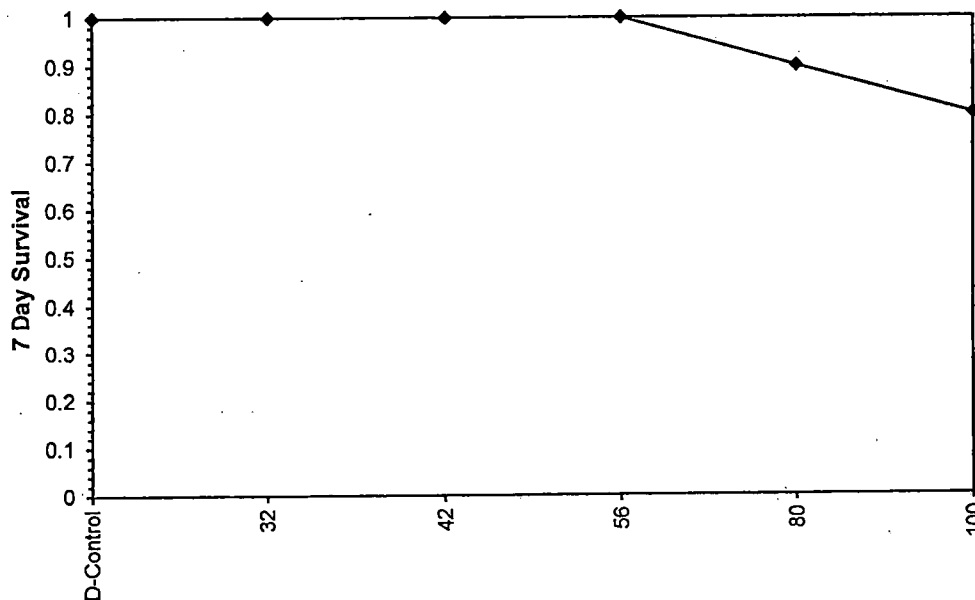
Start Date: 5/16/2016 14:30    Test ID: WR    Sample ID: AR0046566-NPDES Permit #  
 End Date: 5/23/2016 13:45    Lab ID: ASU-ERF    Sample Type: EFF1-POTW  
 Sample Date:    Protocol: EPAF 02-EPA Freshwater    Test Species: CD-Ceriodaphnia dubia  
 Comments: 2ndquarter WET

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
32	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
42	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
56	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
80	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000
100	1.0000	1.0000	1.0000	0.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical
D-Control	1.0000	1.0000	0	10	10	10		
32	1.0000	1.0000	0	10	10	10	1.0000	0.0500
42	1.0000	1.0000	0	10	10	10	1.0000	0.0500
56	1.0000	1.0000	0	10	10	10	1.0000	0.0500
80	0.9000	0.9000	1	9	10	10	0.5000	0.0500
100	0.8000	0.8000	2	8	10	10	0.2368	0.0500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	100	>100		1

**Dose-Response Plot**



**Ceriodaphnia Survival and Reproduction Test-Reproduction**

Start Date: 5/16/2016 14:30	Test ID: WR	Sample ID: AR0046566-NPDES Permit #
End Date: 5/23/2016 13:45	Lab ID: ASU-ERF	Sample Type: EFF1-POTW
Sample Date:	Protocol: EPAF 02-EPA Freshwater	Test Species: CD-Ceriodaphnia dubia
Comments: 2nd quarter WET		

Conc-%	1	2	3	4	5	6	7	8	9	10
D-Control	32.000	35.000	35.000	34.000	31.000	34.000	36.000	32.000	14.000	16.000
32	16.000	20.000	28.000	28.000	32.000	33.000	37.000	20.000	19.000	12.000
42	30.000	31.000	31.000	13.000	21.000	20.000	17.000	21.000	11.000	10.000
56	7.000	26.000	22.000	15.000	19.000	15.000	26.000	9.000	22.000	18.000
80	6.000	21.000	4.000	3.000	3.000	1.000	8.000	7.000	7.000	
100	7.000	0.000	0.000	2.000	0.000	0.000	5.000	9.000		

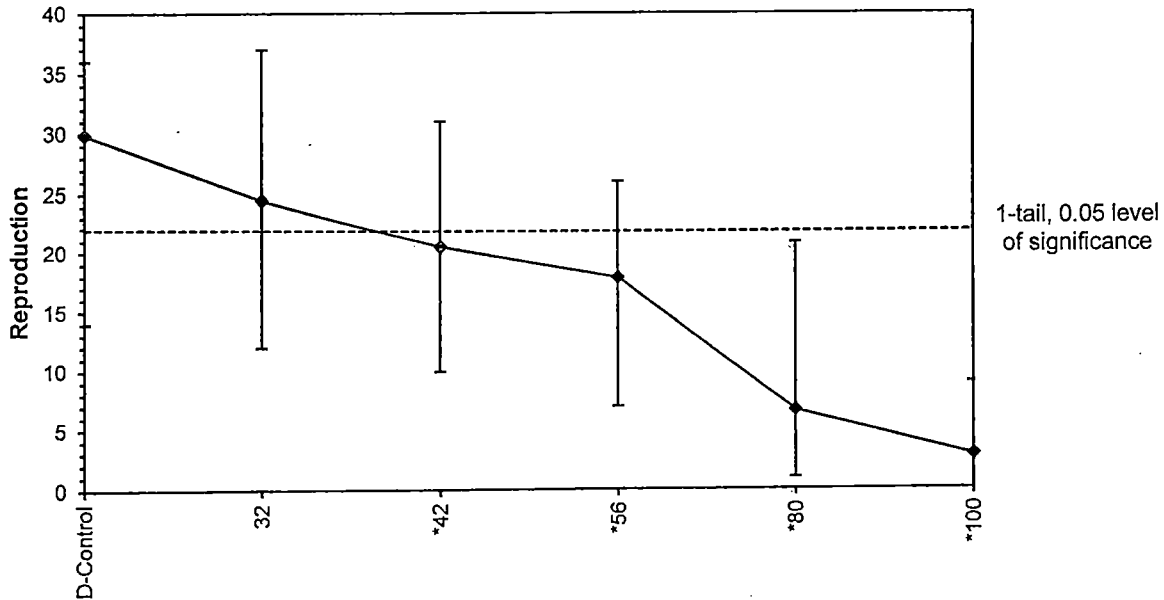
Conc-%	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD
			Mean	Min	Max	CV%					
D-Control	29.900	1.0000	29.900	14.000	36.000	26.823	10				
32	24.500	0.8194	24.500	12.000	37.000	33.562	10	1.719	2.402	7.544	
*42	20.500	0.6856	20.500	10.000	31.000	39.177	10	2.993	2.402	7.544	
*56	17.900	0.5987	17.900	7.000	26.000	36.344	10	3.821	2.402	7.544	
*80	6.667	0.2230	6.667	1.000	21.000	87.785	9	7.200	2.402	7.750	
*100	2.875	0.0962	2.875	0.000	9.000	126.696	8	8.112	2.402	8.001	

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.4905	1.035	-0.196	-0.20806
Bartlett's Test indicates equal variances (p = 0.35)	5.59007	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	-	32	42	36.6606	3.125	8.0012	0.2676	976.676	49.327	6.5E-11

**Dose-Response Plot**











**Larval Fish Growth and Survival Test-7 Day Survival**

Start Date: 5/16/2016 14:40    Test ID: WR    Sample ID: AR0046566-NPDES Permit #  
 End Date: 5/23/2016 15:20    Lab ID: ASU-ERF    Sample Type: EFF1-POTW  
 Sample Date:    Protocol: EPAF 02-EPA Freshwater    Test Species: PP-Pimephales promelas  
 Comments: 2nd quarter WET

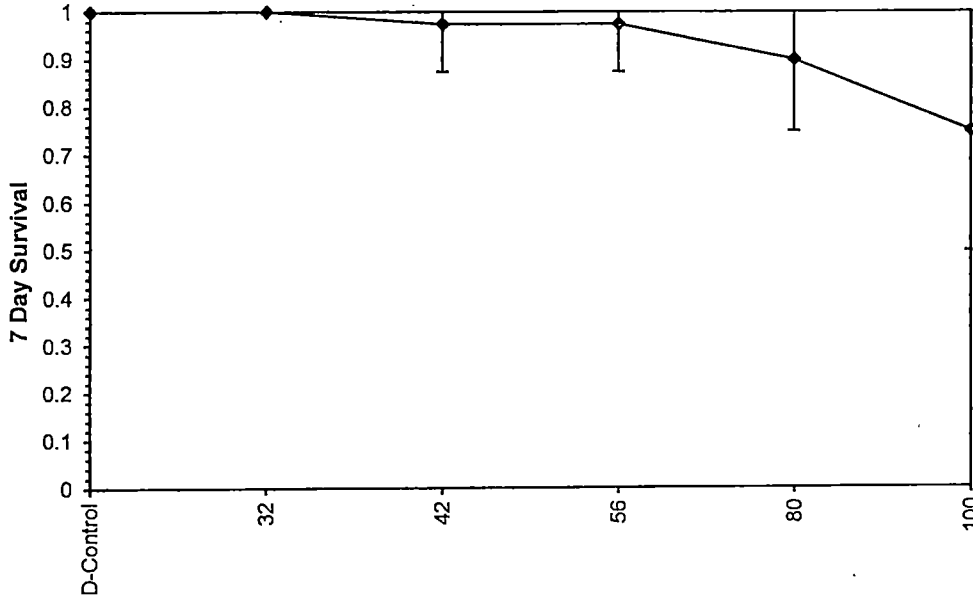
Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
32	1.0000	1.0000	1.0000	1.0000	1.0000
42	0.8750	1.0000	1.0000	1.0000	1.0000
56	1.0000	0.8750	1.0000	1.0000	1.0000
80	1.0000	0.7500	0.8750	0.8750	1.0000
100	1.0000	0.5000	0.7500	0.7500	0.7500

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				N	Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%			
D-Control	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5		
32	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	16.00
42	0.9750	0.9750	1.3564	1.2094	1.3931	6.055	5	25.00	16.00
56	0.9750	0.9750	1.3564	1.2094	1.3931	6.055	5	25.00	16.00
80	0.9000	0.9000	1.2504	1.0472	1.3931	11.683	5	20.00	16.00
100	0.7500	0.7500	1.0640	0.7854	1.3931	20.308	5	17.50	16.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.84327	0.9	0.16468	3.78793
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1

**Dose-Response Plot**



**Larval Fish Growth and Survival Test-7 Day Growth**

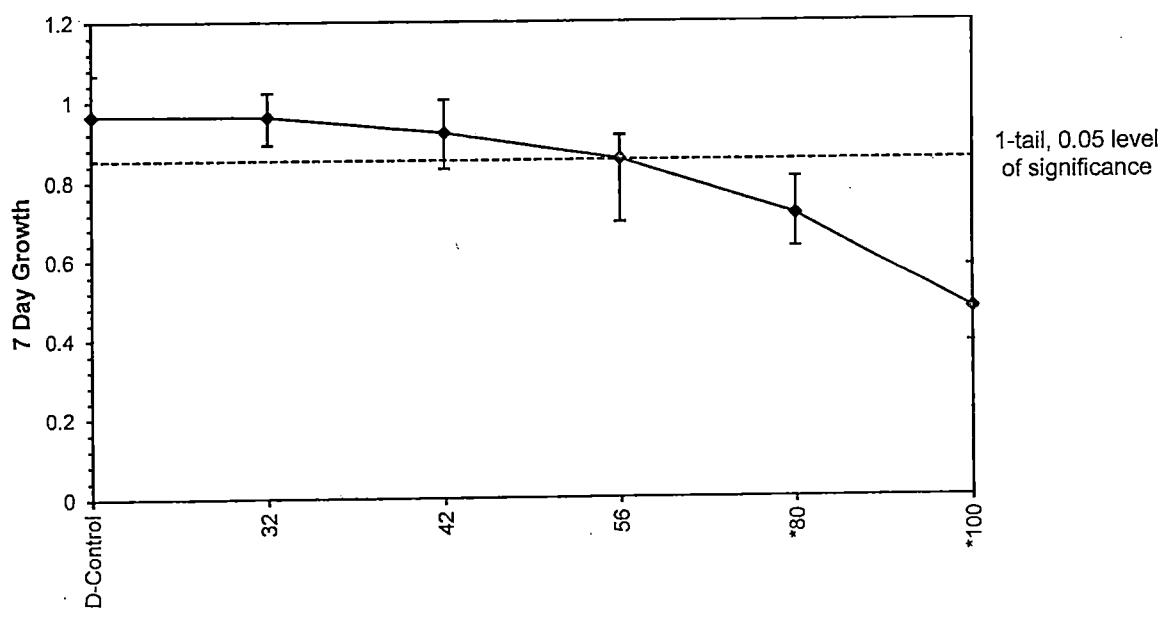
Start Date: 5/16/2016 14:40	Test ID: WR	Sample ID: AR0046566-NPDES Permit #
End Date: 5/23/2016 15:20	Lab ID: ASU-ERF	Sample Type: EFF1-POTW
Sample Date:	Protocol: EPAF 02-EPA Freshwater	Test Species: PP-Pimephales promelas
Comments: 2nd quarter WET		

Conc-%	1	2	3	4	5
D-Control	0.9700	0.9500	0.8550	0.9825	1.0687
32	0.8925	1.0025	1.0225	0.9900	0.9013
42	1.0043	0.9150	0.9825	0.8325	0.8738
56	0.8988	0.9114	0.6987	0.9137	0.8550
80	0.7350	0.8100	0.7229	0.6371	0.6850
100	0.5888	0.5250	0.4317	0.4550	0.3917

Conc-%	Mean	N-Mean	Transform: Untransformed					N	t-Stat	1-Tailed Critical	MSD
			Mean	Min	Max	CV%					
D-Control	0.9652	1.0000	0.9652	0.8550	1.0687	7.927	5				
32	0.9617	0.9964	0.9617	0.8925	1.0225	6.283	5	0.074	2.360	0.1110	
42	0.9216	0.9548	0.9216	0.8325	1.0043	7.828	5	0.928	2.360	0.1110	
56	0.8555	0.8863	0.8555	0.6987	0.9137	10.611	5	2.332	2.360	0.1110	
*80	0.7180	0.7438	0.7180	0.6371	0.8100	8.919	5	5.255	2.360	0.1110	
*100	0.4784	0.4956	0.4784	0.3917	0.5888	16.393	5	10.348	2.360	0.1110	

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution ( $p > 0.01$ )	0.97307	0.9	-0.35777	-0.49756						
Bartlett's Test indicates equal variances ( $p = 0.98$ )	0.78943	15.0863								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	56	80	66.9328	1.78571	0.11103	0.11503	0.1798	0.00553	6.2E-10	5, 24

**Dose-Response Plot**



**CHRONIC TEST DATA SHEET**

*Pimephales promelas*

Project: Walnut Ridge Beginning Date: 05/6/16 Time: 1440 Test Species: P. promelas

Walnut Ridge

Dilution H<sub>2</sub>O: 14967 Ending Date: 05/23/16 Time: 1520 Age: < 24hrs

Test Type: ( \* )Static Renewal ( ) Flowthrough Toxicant/ Effluent:

Conc.	Rep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Pan #
Control	1	9/0	8/0	8/0	8/0	8/0	8/0	8/0	1
	2	8/0	8/0	8/0	8/0	8/0	8/0	8/0	2
	3	8/0	8/0	8/0	8/0	8/0	8/0	8/0	3
	4	9/0	8/0	8/0	8/0	8/0	8/0	8/0	4
	5	10/0	8/0	8/0	8/0	8/0	8/0	8/0	5
32%	1	10/0	8/0	8/0	8/0	8/0	8/0	8/0	6
	2	10/0	8/0	8/0	8/0	8/0	8/0	8/0	7
	3	10/0	8/0	8/0	8/0	8/0	8/0	8/0	8
	4	10/0	8/0	8/0	8/0	8/0	8/0	8/0	9
	5	10/0	8/0	8/0	8/0	8/0	8/0	8/0	10
42%	1	9/0	8/0	8/0	8/0	8/1	7/0	9/0	11
	2	9/0	8/0	8/0	8/0	8/0	8/0	8/0	12
	3	9/0	8/0	8/0	8/0	8/0	8/0	8/0	13
	4	9/0	8/0	8/0	8/0	8/0	8/0	8/0	14
	5	10/0	8/0	8/0	8/0	8/0	8/0	8/0	15
56%	1	11/0	8/0	8/0	8/0	8/0	8/0	8/0	16
	2	10/0	8/0	8/0	8/1	7/0	7/0	9/0	17
	3	11/0	8/0	8/0	8/0	8/0	8/0	8/0	18
	4	9/0	8/0	8/0	8/0	8/0	8/0	8/0	19
	5	11/0	8/0	8/0	8/0	8/0	8/0	8/0	20
Date		0517/16	0519/16	0521/16	0520/16	0521/16	0522/16	0523/16	
Initials		DN	mm	DN	JM	mm	mm	JM	JM

**CHRONIC TEST DATA SHEET**

*Pimephales promelas*

Project: Walnut Ridge Beginning Date: 05/6/16 Time: 1440 Test Species: P.promelas

Dilution H<sub>2</sub>O: 1:14967 Ending Date: 05/23/16 Time: 1520 Age: < 24hrs

Test Type: (  ) Static Renewal (  ) Flowthrough Toxicant/Effluent

Conc.	Rep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Pan #
80%	1	8/0	8/0	8/0	8/0	8/0	8/0	8/0	21
	2	9/0	8/0	7/1	7/0	7/0	7/1	6/0	22
	3	8/0	8/0	8/0	8/1	7/0	7/0	7/0	23
	4	9/0	8/0	8/0	8/1	7/0	7/0	7/0	24
	5	9/0	8/0	8/0	8/0	8/0	8/0	8/0	25
100%	1	10/0	8/0	8/0	8/0	8/0	8/0	8/0	26
	2	10/0	8/0	5/3	4/0	4/0	4/0	4/0	27
	3	9/0	8/0	8/0	8/1	7/1	6/0	6/0	28
	4	11/0	8/0	8/0	8/1	6/0	6/0	6/0	29
	5	14/0	8/0	7/1	8/0	8/0	8/2	6/0	30
Date		05/17/16	05/18/16	05/19/16	05/20/16	05/21/16	05/22/16	05/23/16	
Initials		AW	MM	AW	JM	MM	MM	JM	JM

Initial Water Chemistry for Chronic Tests  
Project: Walnut Ridge - *C. dubia* / *P. promelas*

Test Day:		0	1	2	3	4	5	6
Date:		05/16/16	05/17/16	05/18/16	05/19/16	05/20/16	05/21/16	05/22/16
H <sub>2</sub> O Batch #:		MH967	MH967	MH967	MH967	MH967	MH967	MH967
Temp. (°C)	Control	22.0	22.8	21.8	21.2	22.1	22.0	22.0
	32%	22.2	22.6	22.2	21.7	22.2	22.2	22.0
	42%	22.4	22.4	22.1	21.6	22.5	22.0	22.1
	56%	22.7	22.7	22.4	21.8	22.7	22.2	22.0
	80%	22.9	22.9	22.5	22.0	22.8	22.7	22.5
	100%	23.0	23.0	22.8	22.2	23.0	22.9	22.8
pH	Control	8.09	8.11	8.02	7.96	8.18	7.74	7.99
	32%	7.92	8.28	7.95	8.00	7.98	7.78	8.13
	42%	7.86	8.31	7.87	8.01	8.02	7.80	8.11
	56%	7.84	8.33	7.85	8.01	8.03	7.82	8.13
	80%	7.82	8.30	7.84	7.94	8.02	7.92	8.18
	100%	7.74	8.22	7.83	7.91	7.99	8.00	8.20
DO (mg/L)	Control	8.8	8.6	8.7	8.7	8.6	8.7	8.7
	32%	8.9	8.5	8.0	8.5	8.5	8.7	8.6
	42%	8.6	8.4	8.3	8.4	8.4	8.6	8.5
	56%	8.6	8.4	8.2	8.3	8.4	8.3	8.5
	80%	8.6	8.3	8.1	8.2	8.4	8.5	8.1
	100%	8.8	8.2	8.1	8.2	8.4	8.4	8.2
Cond. (µS/cm)	Control	314	317	315	312	315	312	311
	32%	385	381	396	393	375	372	372
	42%	411	402	424	421	395	391	388
	56%	445	432	459	458	421	417	413
	80%	500	482	515	522	467	467	461
	100%	554	581	575	675	509	513	503
Alk. (mg/L)	Control	AN 9258		58		58		
	100%	AN 8192		180		160		
Hard (mg/L)	Control	AN 10590		90		90		
	100%	AN 91065	<del>230</del>	230		180		
Initials		AN/RIC	AN/RIC	AN/RIC	AN/RIC	AN/RIC	MH/RIC	CHPS/RIC

**Final Water Chemistry for Chronic Tests**  
Project: Walnut Ridge - *C. dubia*

Test Day:		1	2	3	4	5	6	7
Date:		051716	051816	051916	052016	052116	052216	052316
H <sub>2</sub> O Batch #:		MH967	MH967	MH967	MH967	MH967	MH967	MH967
Temp. (°C)	Control	23.2	22.0	21.8	22.0	23.0	22.2	23.8
	32%	22.6	22.5	21.9	22.5	22.3	22.5	23.4
	42%	22.2	22.3	21.7	22.5	22.0	22.1	23.0
	56%	22.2	22.4	21.9	22.6	22.4	22.3	23.4
	80%	22.3	22.5	21.9	22.7	22.2	22.4	23.0
	100%	22.7	22.3	22.2	22.6	22.8	22.4	23.3
pH	Control	8.52	8.64	8.22	8.82	8.50	8.57	8.47
	32%	8.54	8.64	8.36	8.81	8.56	8.57	8.58
	42%	8.56	8.68	8.39	8.82	8.53	8.62	8.56
	56%	8.59	8.65	8.45	8.81	8.61	8.57	8.63
	80%	8.58	8.66	8.46	8.82	8.53	8.63	8.56
	100%	8.61	8.65	8.51	8.75	8.57	8.60	8.60
DO (mg/L)	Control	9.0	9.1	9.1	9.0	8.6	8.5	8.9
	32%	9.0	9.1	8.8	9.1	8.8	8.7	9.1
	42%	8.9	9.3	8.8	9.2	9.0	8.8	9.2
	56%	9.0	9.1	8.8	9.3	9.1	8.8	9.1
	80%	8.9	9.2	8.7	9.3	9.0	8.8	9.0
	100%	8.8	9.1	8.7	9.1	8.9	8.8	8.8
Initials		RIC RIC	RIC	RIC	RIC	RIC/KK	RIC/KK	RIC



Final Water Chemistry for Chronic Tests  
Project: Walnut Ridge - *P. promelas*

Test Day:		1	2	3	4	5	6	7
Date:		05/17/16	05/18/16	05/19/16	05/20/16	05/21/16	05/22/16	05/23/16
H <sub>2</sub> O Batch #:		MH967	MH967	MH967	MH967	MH967	MH967	MH967
Temp. (°C)	Control	22.3	21.9	21.7	22.0	23.1	23.2	22.6
	32%	22.5	22.5	21.9	22.5	23.1	23.1	22.5
	42%	22.4	22.5	21.9	22.5	23.2	23.1	22.4
	56%	22.7	22.5	21.7	22.7	23.1	22.1	22.6
	80%	22.2	22.1	21.8	22.9	23.2	22.2	22.6
	100%	22.1	22.1	21.7	23.0	23.0	<del>23.0</del> 23.1 <sup>6</sup>	22.5
pH	Control	7.64	7.29	7.08	7.66	7.48	7.44	7.37
	32%	8.02	7.70	7.56	7.89	7.58	7.52	7.30
	42%	8.16	7.80	7.65	7.94	7.62	7.51	7.30
	56%	8.17	7.91	7.76	7.97	7.64	7.54	7.30
	80%	8.25	8.06	7.88	8.02	7.69	7.63	7.41
	100%	8.32	8.14	8.00	8.08	7.77	7.70	7.49
DO (mg/L)	Control	7.8	6.2	5.5	6.6	6.6	5.9	6.6
	32%	7.7	6.5	5.6	6.2	5.7	5.3	5.3
	42%	7.5	6.6	5.4	6.0	5.5	5.6	5.1
	56%	7.3	6.5	5.3	5.8	5.4	4.7	4.6
	80%	7.2	5.7	5.3	5.2	4.8	5.0	4.4
	100%	7.3	6.1	5.3	4.9	4.6	4.2	4.2
Initials:		JN	mm	JN	J	mm	mm	jan



EcoToxicology Research Facility

### Ecotoxicology Research Facility

Arkansas State University

501 Iroquois Street

State University, AR 72467

(870) 972-2570 Fax (870) 972-2577

# CHAIN OF CUSTODY RECORD



ARKANSAS STATE UNIVERSITY

Client Name <b>Walnut Ridge Wastewater Treatment</b>			Phone: (870) 886-2312					Analyses (List Below)				
Project #			Fax:									
Sampler (sign) 			PO #:					Chronic C. dubia	Chronic P. promelas			
Remarks:			Contact: <b>Jonathan Kopp</b>									
Cont.#	Sample ID Number	Location	Sample Date	Sample Time	Sample Type		Matrix					
					Comp	Grab	Aqueous	Soil	Other			
1		off #1001	5-15-16	9 AM	X						X	✓
			5-16-16	9 AM								
Ice present at delivery: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No			Temp: 19 °C					Initials: JK				
1. Relinquished By (sign) 			Date	Time	1. Received By (sign) 			Date	Time			
2. Relinquished By (sign)			Date	Time	2. Received By (sign)			Date	Time			





Ecotoxicology Research Facility

Ecotoxicology Research Facility

Arkansas State University

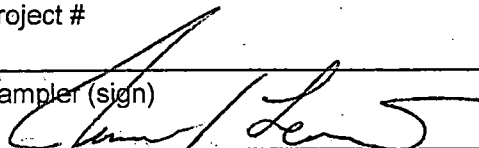
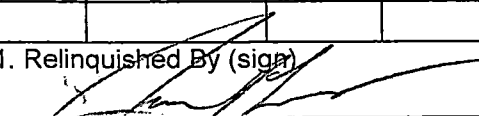
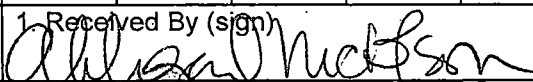
501 Iroquois

State University, AR 72467

(870) 972-2570 Fax (870) 972-2577

# CHAIN OF CUSTODY RECORD



Client Name <b>Walnut Ridge Wastewater Treatment</b>			Phone: <b>(870) 886-2312</b>				Analyses (List Below)						
Project #			Fax:										
Sampler (sign) 			PO #:				Chronic C. dubia	Chronic P. promelas					
Remarks: Contact: <b>Jonathan Kopp</b>													
Cont.#	Sample ID Number	Location	Sample Date	Sample Time	Sample Type		Matrix						
					Comp	Grab	Aqueous	Soil	Other				
			5-19	5-20	9am	9am	<input checked="" type="checkbox"/>						
Ice present at delivery:		<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No										
Temp:		1 °C	Initials										
1. Relinquished By (sign) 			Date	5-20-16	Time	12:00	1. Received By (sign) 			Date	05202016	Time	12:00
2. Relinquished By (sign)			Date		Time		2. Received By (sign)			Date		Time	



Ecotoxicology Research Facility

**SAMPLE CHECK IN**

Sample ID Number: WR001

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

Date: 05-16-16 Sampling Date: <sup>05-15-16 9AM</sup> 05-16-16 9AM Arrival Time: 11:16 am

Field Identification Number: EFF 001 Description: Comp Sample

Shipped by: Federal Express \_\_\_\_\_ UPS \_\_\_\_\_ Hand delivered by: Jonathon Kopp

Drop-Off Location: ASU-ERF

Storage While Shipped: on ice / CooTek

Analysis Requested: Chronic C. dubia Chronic P. promelas

Initial Water Chemistry Analysis:

Sample Received by: ZEN

Temperature (°C): 19°C Ice Present upon delivery:  YES  NO

Date: 05-16-16

Quality Assurance	Initial	Date	Yes	No
Chain of Custody	ZN	05-16-16	X	
Refrigerated at 4°C	ZN	05-16-16	X	
Field Record Received	ZN	05-16-16	X	
Sample Label Affixed Properly	ZN	05-16-16	X	
Project Leader Informed	ZN	05-16-16	X	

Comments: \_\_\_\_\_



Ecotoxicology Research Facility

**SAMPLE CHECK IN**

Sample ID Number: WR002

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

Date: 05-18-16 Sampling Date: 05-17-16 9am Arrival Time: 11:35am  
05-18-16 9am

Field Identification Number: EFF 002 Description: Comp Sample

Shipped by: Federal Express  UPS  Hand delivered by: Jonathon Kopp

Drop-Off Location: ASU-ERF

Storage While Shipped: on ice / cooler

Analysis Requested: Chronic C. dubia Chronic P. promelas

Initial Water Chemistry Analysis:

Sample Received by: JEN

Temperature (°C): 1°C Ice Present upon delivery:  YES  NO

Date: 05-18-16

Quality Assurance	Initial	Date	Yes	No
Chain of Custody	JN	05-18-16	X	
Refrigerated at 4°C	JN	05-18-16	X	
Field Record Received	JN	05-18-16	X	
Sample Label Affixed Properly	JN	05-18-16	X	
Project Leader Informed	JN	05-18-16	X	

Comments: \_\_\_\_\_

\_\_\_\_\_



Ecotoxicology Research Facility

**SAMPLE CHECK IN**

Sample ID Number: WR003

Fill out this information with each effluent or river water sample coming in for testing. Keep completed sheets with test data and file with the lab QA/QC officer.

Date: 05-20-16 Sampling Date: 05-19-16 9am Arrival Time: 12:00pm  
05-20-16 9am

Field Identification Number: EFF 003 Description: Comp Sample

Shipped by: Federal Express \_\_\_\_\_ UPS \_\_\_\_\_ Hand delivered by: Jonathon Kopp

Drop-Off Location: ASU-ERF

Storage While Shipped: on ice / cooler

Analysis Requested: Chronic C. dubia Chronic P. promelas

Initial Water Chemistry Analysis:

Sample Received by: JEN

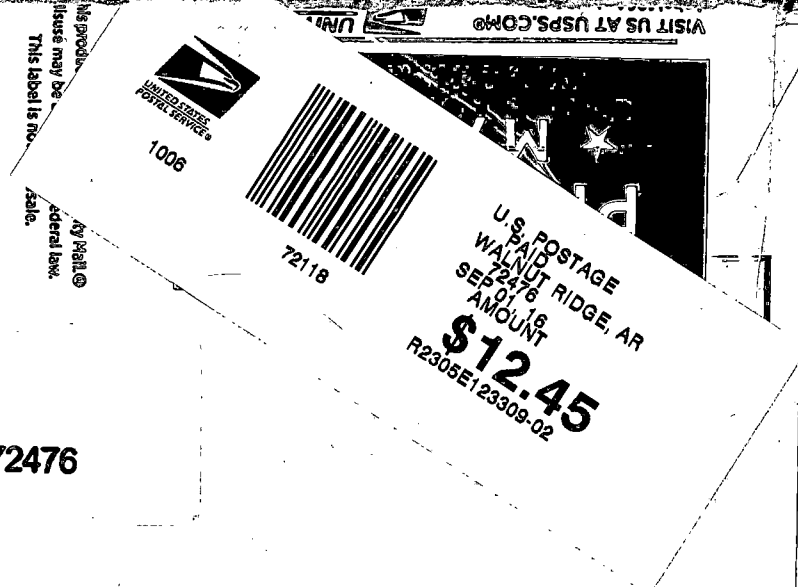
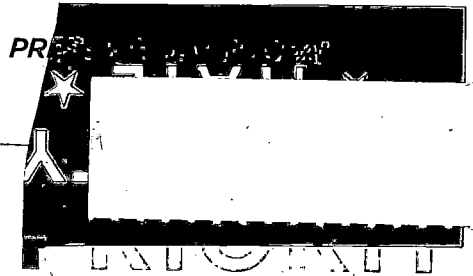
Temperature (°C): 1°C Ice Present upon delivery:  YES  NO

Date: 05-20-2016

Quality Assurance	Initial	Date	Yes	No
Chain of Custody	JN	05-20-16	X	
Refrigerated at 4°C	JN	05-20-16	X	
Field Record Received	JN	05-20-16	X	
Sample Label Affixed Properly	JN	05-20-16	X	
Project Leader Informed	JN	05-20-16	X	

Comments: \_\_\_\_\_

UNited STATES VISIT US AT USPS.COM



★ MAIL ★

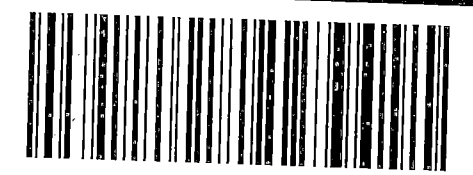
- DATE OF DELIVERY SPECIFIED\*
- USPS TRACKING™ INCLUDED\*
- INSURANCE INCLUDED\*
- PICKUP AVAILABLE

\* Domestic only

FROM:

City Water Works  
216 S. W. 4th St.  
Walnut Ridge, AR 72476

PLACE STICKER AT TOP OF ENVELOPE TO THE RIGHT OF THE RETURN ADDRESS, FOLD AT DOTTED LINE  
**CERTIFIED MAIL**



7015 0640 0004 4230 9481

TO:

ADEQ  
Enforcement Branch  
ATTN: Mary Barnett  
5301 Northshore Drive  
North Little Rock AR 72118-5317

RETURN RECEIPT  
REQUESTED

WHEN USED INTERNATIONALLY,  
A CUSTOMS DECLARATION  
LABEL MAY BE REQUIRED.

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