Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Report Date: 7/26/12
Ohio EPA Permit No.: 2PH00007*JD
Alloway Lab #: L12-16182-01

Report of Chronic Biomonitoring

Samples Collected on:

7/9/12
7/11/12
7/13/12

Pimephales promelas
Ceriodaphnia dubia

Allen County Sanitary Engineer
America-Bath WWTP
3226 North Cole Street
Lima, OH 45805

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Alloway Lab #: L12-16182-01

Date: 7/26/12

Melanie Marshall
Allen County Sanitary Engineer
3226 North Cole Street
Lima, OH 45805

Dear Ms. Marshall:

Enclosed is the report of the biomonitoring on the sample listed above. The objective of the chronic biomonitoring was to determine the effects, both the acute endpoint (mortality) and chronic endpoints (mortality and growth, or reproduction), from the test water. Fathead minnow larvae (*Pimephales promelas*) and neonate water fleas (*Ceriodaphnia dubia*) were the test organisms.

Acute toxicity was determined by locating the median lethal concentration (LC50) and the median effective concentration (EC50). Growth was determined from the dry weight of surviving *Pimephales promelas* larvae. Reproduction was monitored daily by counting the number of young produced by each female *Ceriodaphnia dubia*.

Dissolved oxygen, pH, and temperature were recorded daily at each renewal and at the end of each 24-hour exposure period. Results may be found in Appendix C "Parameter Data Sheets". Samples were analyzed for alkalinity, hardness and conductivity. These results may also be found in Appendix C.

Alloway (Lima, Ohio) performs biomonitoring testing in accordance with procedures established by the Environmental Protection Agency. The Standard Operating Procedures for the testing is on file with the Ohio EPA.

Please call if you have any questions or need additional information.

Paul J. Crerar
Biology Lab Manager

Richard F. Rupp
Senior Biologist

Alloway
Your Resource for Defensible Data

Outfall: Effluent 001
Report Date: 7/26/12
Ohio EPA Permit No.: 2PH00007*JD

1. Facility Name	Allen County Sanitary Engineer America-Bath WWTP
2. Facility Address	3226 North Cole Street Lima, OH 45805
3. Ohio EPA Permit No.	2PH00007*JD
4. NPDES No.	OH0023841
5. Facility Contact	Melanie Marshall
6. Telephone No.	419-225-8084
7. Consulting Laboratory	Alloway
8. Lab Contact	Paul Crerar
9. Lab Telephone No.	419-223-1362
10. Receiving water(s) of discharge	Pike Run
11. Outfall(s) tested	Effluent 001

12. Is your current Standard Operating Procedure (SOP) Manual on file with Ohio EPA? Yes
If yes, indicate date in which SOP was submitted to the EPA: 1/21/2005

I certify under penalty of law that I have personally examined and am familiar with the information submitted in this document and based on my inquiry of those individuals immediately responsible for obtaining information, I believe the submitted information is true, accurate and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment.

Signature _____ Date _____

Name (typed or printed) _____ Title _____

Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00007*JD
Date of Test: 7/10-17/12

Summary of Chronic Toxicity Test Results
Pimephales promelas

	4500 Form Value	MDL	4500 Comments Required
Effluent/Outfall:			
Chronic Toxicity, *Pimephales promelas* - TUc	AA	1.0	Growth
Acute Toxicity, *Pimephales promelas* - TUa	AA	0.2	
Upstream:			
96 Hr. Acute Toxicity, *Pimephales promelas* - % Affected	AA	2.5	
7 day Chronic Toxicity, *Pimephales promelas* - % Affected	AA	2.5	

AA = below detectable

Conclusion/Comments:

1101 N. Cole Street • Lima, Ohio 45805
419.223.1362 • Fax 419.227.3792
800.436.1243

508 Bissman Ct. • Mansfield, Ohio 44903
419.525.1644 • Fax 419.524.5575
800.635.3222

1776 Marion-Waldo Rd. • Marion, Ohio 43302
740.389.5991 • Fax 740.389.1481
800.873.2835

www.alloway.com

Alloway
Your Resource for Defensible Data

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00007*JD
Date of Test: 7/10-16/12

Summary of Chronic Toxicity Tedt Results
Ceriodaphnia dubia

	4500 Form Value	MDL	4500 Comments Required
Effluent/Outfall:			
Chronic Toxicity, *Ceriodaphnia dubia* - TUc	AA	1.0	Reproduction
Acute Toxicity, *Ceriodaphnia dubia* - TUa	AA	0.2	
Upstream:			
48 Hr. Acute Toxicity, *Ceriodaphnia dubia* - % Affected	AA	10.0	
7 day Chronic Toxicity, *Ceriodaphnia dubia* - % Affected	10	10.0	

AA = below detectable

Conclusion/Comments:

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00007*JD
Date of Test: 7/10-17/12

Chronic Toxicity Test Results

Results of a 7-Day Pimephales promelas Survival and Growth Test

Test Solutions	Cumulative Percent Mortality* (Cumulative Percent Adversely Affected)* Test Day							Mean Dry Weight*	
	1	2	3	4	5	6	7	Mean	SD
Primary Control Upstream	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.745	0.090
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.917	0.106
20%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.799	0.066
40%	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	0.751	0.076
60%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	2.5% (2.5%)	0.744	0.048
80%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.779	0.058
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.913	0.078

LC50 Values (%):		>100	>100	>100	>100	>100	>100	>100	**Calculated TUc for Survival** <1.0
95% Confidence Limits	LL								
	UL								
EC50 Values (%):		>100	>100	>100	>100	>100	>100	>100	**Calculated TUc for Growth (100/IC25)** <1.0
95% Confidence Limits	LL								
	UL								

7-Day NOEC for Mortality (%)	100	7-Day NOEC for Growth (%)	100	**Method(s) used to determine Values:**
7-Day LOEC for Mortality (%)	>100	7-Day LOEC for Growth (%)	>100	
Chronic Value for Mortality (%)	>100	Chronic Value for Growth (%)	>100	
IC25 (%)	>100	IC50 (%)	>100	IC Pin, Dunnett's

*indicates significant differences from the primary control with an * ($p<0.05$)

Chronic Value = square root of (NOEC x LOEC)

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Ohio EPA Permit No.: 2PH00007*JD
Date of Test: 7/10-16/12

Chronic Toxicity Test Results

Results of a 7-Day Ceriodaphnia dubia Survival and Reproduction

Test Solutions	Cumulative Percent Mortality* (Cumulative Percent Adversely Affected)* Test Day							Number of Young Produced
	1	2	3	4	5	6	7	Mean
Primary Control Upstream	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	--- (---)	23.6
Secondary Control Lab	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	11.6
20%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	22.1
40%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	25.1
60%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	23.4
80%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	--- (---)	24.0
100%	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	10.0% (10.0%)	--- (---)	23.9
LC50 Values (%):	>100	>100	>100	>100	>100	>100	---	**Calculated TUc Value for Survival**
95% Confidence Limits LL								
95% Confidence Limits UL								<1.0
EC50 Values (%):	>100	>100	>100	>100	>100	>100	---	**Calculated TUc Value for Reproduction**
95% Confidence Limits LL								
95% Confidence Limits UL								<1.0

7-Day NOEC for Mortality (%)	100		7-Day NOEC for Reprod (%)	100	**Method(s) used to determine Values:**
7-Day LOEC for Mortality (%)	>100		7-Day LOEC for Reprod (%)	>100	
Chronic Value for Mortality (%)	>100		Chronic Value for Reprod (%)	>100	
IC25 (%)	>100		IC50 (%)	>100	IC Pin, Dunnett's

*indicates significant differences from the primary control with an * ($p<0.05$)

Chronic Value = square root of (NOEC x LOEC)

Customer: Allen County Sanitary Engineer
Facility: America-Bath WWTP
Outfall: Effluent 001
Report Date: 7/26/12
Ohio EPA Permit No.: 2PH00007*JD

Sampling Summary for Chronic Toxicity Tests

Sample Location & Description		Sample	Sample Collection Beginning	Sample Collection Ending
Final Effluent:				
Outfall No.:	Effluent 001	1st	7/8/12 @ 09:00	7/9/12 @ 09:00
Type (Grab/Comp):	Comp	2nd	7/10/12 @ 09:00	7/11/12 @ 08:50
Volume Collected:	5 Gal.	3rd	7/12/12 @ 09:00	7/13/12 @ 09:00
Upstream Station:				
Waterbody:	Pike Run			
Station No.:	801	1st		7/9/12 @ 10:30
Type (Grab/Comp):	Grab	2nd		7/11/12 @ 09:10
Volume Collected:	5 Gal.	3rd		7/13/12 @ 09:40

Samples collected by: Alloway

OEPA Permit No.: 2PH00007*JD Allen Co. (American-Bath WWTP)

PIMEPHALES PROMELAS CHRONIC TOXICITY TEST CONDITIONS

Summary of Toxicity Test Conditions (Ohio EPA 1998; EPA-821R-02-013, Oct. 2002)	
1. Test Species and Age:	*Pimephales promelas* < 24 hours old
2. Test Type and Duration:	Static renewal; 7 days
3. Test Dates:	7/10-17/12
4. Test Temperature (°C):	25°C ± 1°C
5. Light Quality:	Ambient laboratory illumination
6. Photoperiod:	16 h light, 8 h dark
7. Feeding Regime:	Feed 0.1 g (0.1 to 0.2 mL) newly hatched brine shrimp nauplii three times daily (M-F) at 4-h intervals. On weekends 0.1g of nauplii are fed at the beginning of the workday, after renewal, and at the end of the workday. Sufficient larvae are added to provide an excess. Fish are not fed during the final 12-h of the test.
8. Size of Test Vessel:	500 mL plastic cup
9. Volume and Depth of Test Solutions:	250 mL, 6.7 cm deep
10. No. of Test Organisms per Test Vessel:	10 per test vessel
11. No. of Test Vessels per Test Solution:	4 per solution tested
12. Total No. of Test Organisms per Test Solution:	40 per solution tested
13. Test Concentrations (as percent by volume effluent):	100, 80, 60, 40 and 20% effluent (001)
14. Renewal of Test Solutions:	Daily - day 0, 1 - 7/9; day 2, 3 - 7/11; day 4, 5, 6 - 7/13
15. Dilution Control Water:	Upstream grab from the receiving stream
16. Secondary Control Water:	Lab rearing unit water
17. Aeration During Test:	None
18. Endpoints Measured:	Mortality, and atypical behavior or appearance; and growth (dry weight).
19. If secondary control water used as diluent due to toxicity in primary control water, indicate number of consecutive tests conducted with alternative diluent:	

OEPA Permit No.: 2PH00007*JD Allen Co. (American-Bath WWTP)

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST CONDITIONS

Summary of Toxicity Test Conditions (Ohio EPA 1998; EPA-821R-02-013, Oct. 2002)	
1. Test Species and Age:	*Ceriodaphnia dubia* 20-22 hours
2. Test Type and Duration:	Static renewal; 6 to 8 days (6 days)
3. Test Dates:	7/10-16/12
4. Test Temperature (^{o}C):	25°C ± 1°C
5. Light Quality:	Ambient laboratory illumination
6. Photoperiod:	16 h light, 8 h dark
7. Feeding Regime:	Fed 0.1 mL YCT and 0.1 mL (3.5 million cells) *Selenastrum capricornutum.*
8. Size of Test Vessel:	1 fl. oz. (30 mL) clear plastic cup
9. Volume and Depth of Test Solutions:	15 mL, 20 mm deep
10. No. of Test Organisms per Test Vessel:	1 per test vessel
11. No. of Test Vessels per Test Solution:	10 per solution tested
12. Total No. of Test Organisms per Test Solution:	10 per solution tested
13. Test Concentrations (as percent by volume effluent):	100, 80, 60, 40 and 20% effluent (001)
14. Renewal of Test Solutions:	Daily - day 0, 1 - 7/9; day 2, 3 - 7/11; day 4, 5, 6 - 7/13
15. Dilution Control Water:	Upstream grab from the receiving stream
16. Secondary Control Water:	Lab rearing unit water
17. Aeration During Test:	None
18. Endpoints Measured:	Mortality, and atypical behavior or appearance; and reproduction (number of young produced).
19. If secondary control water used as diluent due to toxicity in primary control water, indicate number of consecutive tests conducted with alternative diluent:	

Appendix A

Field Data

Alloway
Your Resource for Defensible Data

1101 North Cole Street, Lima, OH 45805
Phone 419-223-1362 Fax 419-227-3792

02

Chain of Custody Record

This is a legal document that authorizes Alloway to perform testing on samples submitted under this agreement.

Project: L12-16182

Report To: Name: Company: Address:	Melanie Marshall Allen County Sanitary 3226 North Cole St. Lima, OH 45805	Invoice To (If Different): Name: Company: Address: PO#:	Melanie Marshall Allen County Sanitary 3226 North Cole St. Lima, OH 45805
Phone #:	419-996-4689	Fax #:	
E-mail:	mmarshall@allencountyohio.com		

Notes/Comments

Project Name: Chronic Bio Assay - Day 1

Sampler: (Print) Scott Pfeiffer/Chad Carroll (Signature) Scott Pfeiffer/Chad Carroll

Matrix Codes:
ww – wastewater, s – solid, sg – sludge
gw – groundwater, w – water, o – other
dw – drinking water, oil – oil

	Customer Sample ID / Sample Location	Sample Date	Sample Time	Comp.	Grab	Matrix	Number of Containers	Analysis Required	Alloway Lims # (For Lab Use Only)
1	Effluent	7/8/12 7/9/12	0900 0900	X		WW	1	Bio Assay	16182-01
2									
3	Upstream	7/9/12	1030		X	W	1	Bio Assay	
4									
5									
6									
7									
8									

	Relinquished by:	Received by:	Date	Time
1	Scott Pfeiffer			
2				
3				

Method of Sample Delivery: UPS / FedEx ☐ Other _____ Client Delivery ☐ Alloway Pick-up ☐

Received for Laboratory by: (Signature) [signature] 7-9-12 10:51

Sample Receiving (For Lab Use Only)
Ice Present? Y ☐ N ☐
Proper Preservation? Y ☐ N ☐
Container Temperature: _____

Priority (For Client Use)
Note: Rush Charges May Apply
24 Hrs ☐
48 Hrs ☐
3 Working Days ☐
Routine (5-10 Working days) ☐

White – Lab Copy Yellow – Client Copy

ACSE Biomonitoring Field Data Sheet

Client: BATH **Field Technicians:** SP

Date: 7/9/12 CMC

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	1015	23.8	969	10.75	6.76
Upstream	1030	23.2	899	9.28	7.12

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

- ☐ Cloudy
- ☐ Heavy Rain
- ☐ Light Rain
- ☐ Clear Skies
- ☒ Sunny
- ☐ Snow
- ☐ _____

Stream Conditions

- ☐ Rapid and Complete Mixing
- ☒ Mixing is not Rapid and Complete

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____

Alloway
Your Resource for Defensible Data

Chain of Custody Record

This is a legal document that authorizes Alloway to perform testing on samples submitted under this agreement.

○ 1101 North Cole Street, Lima, OH 45805
Phone 419-223-1362 Fax 419-227-3792

○ 1776 Marion-Waldo Road, Marion, OH 43302
Phone 740-389-5991 Fax 740-389-1481

○ 508 Bissman Court, Mansfield, OH 44903
Phone 419-525-1644 Fax 419-524-5575

Report To: Name: Company: Address:	Melanie Marshall Allen County Sanitary 3226 North Cole St. Lima, OH 45805	Invoice To (If Different): Name: Company: Address: PO#:	Melanie Marshall Allen County Sanitary 3226 North Cole St. Lima, OH 45805
Phone #:	419-996-4689	Fax #:	
E-mail:	mmarshall@allencountyohio.com		

Notes/Comments

Project Name: Bioassay Day 2

Sampler: (Print) Mark Zwiesel / Chad Carrow (Signature) [illegible] / Chad Carrow

Matrix Codes:
ww – wastewater, s – solid, sg – sludge
gw – groundwater, w – water, o – other
dw – drinking water, oil – oil

	Customer Sample ID / Sample Location	Sample Date	Sample Time	Comp.	Grab	Matrix	Number of Containers	Analysis Required	Alloway Lims # (For Lab Use Only)
1	Effluent	7-10-12 – 7-11-12	09:00 – 08:50	X			1	Toxicity	
2	Upstream	7-11-12	9:10		X		1	"	
3									
4									
5									
6									
7									
8									

	Relinquished by:	Received by:	Date	Time
1	[signature]			
2				
3				

Method of Sample Delivery:
UPS / FedEx ☐ Client Delivery ☐
Other _____ Alloway Pick-up ☐

Received for Laboratory by: (Signature) [signature] 7/11/12 1015

Sample Receiving (For Lab Use Only)
Ice Present? Y ☐ N ☐
Proper Preservation? Y ☐ N ☐
Container Temperature: _____

Priority (For Client Use)
Note: Rush Charges May Apply
24 Hrs ☐
48 Hrs ☐
3 Working Days ☐
Routine (5-10 Working days) ☐

White – Lab Copy Yellow – Client Copy

ACSE Biomonitoring Field Data Sheet

Client: Bath **Field Technicians:** CMC

Date: 7-11-12 MZ

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)	
9:4 Effluent	8:50	23.3	1004	10.65	7.50	21.9
Upstream	8:57	22.0	956	~~5.64~~ 2.98 mg	7.64	21.6

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

- ☐ Cloudy
- ☐ Heavy Rain
- ☐ Light Rain
- ☐ Clear Skies
- ☐ Sunny
- ☐ Snow
- ☐ _____

Stream Conditions

- ☐ Rapid and Complete Mixing
- ☐ Mixing is not Rapid and Complete

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____

9:45 Eff 9:56
9:47 UP 9:55 2.98 mg/L

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Chain of Custody Record

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○ 1776 Marion-Waldo Road, Marion, OH 43302
Phone 740-389-5991 Fax 740-389-1481

○ 508 Bissman Court, Mansfield, OH 44903
Phone 419-525-1644 Fax 419-524-5575

Report To:	Melanie Marshall	Invoice To (If Different):	Melanie Marshall
Name:	Allen County Sanitary	Name:	Allen County Sanitary
Company:	3226 North Cole St.	Company:	3226 North Cole St.
Address:	Lima, OH 45805	Address:	Lima, OH 45805
		PO#:	
Phone #:	419-996-4689	Fax #:	
E-mail:	mmarshall@allencountyohio.com		

Notes/Comments

Project Name: Chronic Bio Assay. Day 3

Sampler: (Print) Scott Pfriffer / Bruce Emerson (Signature) Scott Pfriffer / [illegible]

Matrix Codes:
ww – wastewater, s – solid, sg – sludge
gw – groundwater, w – water, o – other
dw – drinking water, oil – oil

	Customer Sample ID / Sample Location	Sample Date	Sample Time	Comp.	Grab	Matrix	Number of Containers	Analysis Required	Alloway Lims # (For Lab Use Only)
1	Upstream	7/13/12	0940		X	W	1	Bio Assay	
2									
3	Effluent	7/12/12 7/13/12	0900 0900	X		WW	1	Bio Assay	
4									
5									
6									
7									
8									

	Relinquished by:	Received by:	Date	Time
1	Scott Pfriffer			
2				
3				

Method of Sample Delivery:
UPS / FedEx ☐ Client Delivery ☐
Other ____ Alloway Pick-up ☐

Received for Laboratory by: (Signature) Paul Emerson — Date: 7/13/12 — Time: 1040

Sample Receiving (For Lab Use Only)
Ice Present? Y ☐ N ☐
Proper Preservation? Y ☐ N ☐
Container Temperature: _____

Priority (For Client Use)
Note: Rush Charges May Apply
24 Hrs ☐
48 Hrs ☐
3 Working Days ☐
Routine (5-10 Working days) ☐

White – Lab Copy Yellow – Client Copy

ACSE Biomonitoring Field Data Sheet

Client: Bath **Field Technicians:** SP

Date: 7/13/12 JE

Grab Parameters for Collection Site Determinations

	Parameter Time	Temperature (°C)	Conductivity (umhos/cm)	Dissolved Oxygen (mg/L)	pH (s.u.)
Effluent	0915	23.1	983	8.36	7.33
Upstream	0940	~~23~~ SP 23.0	961	7.21	7.56

Diagram of Collection Sites

~ Width of stream=
~ Depth of stream=

Weather Conditions

- ☐ Cloudy
- ☐ Heavy Rain
- ☐ Light Rain
- ☐ Clear Skies
- ☒ Sunny
- ☐ Snow
- ☐ _____

Stream Conditions

- ☐ Rapid and Complete Mixing
- ☐ Mixing is not Rapid and Complete

Other Conditions

- ☐ Outfall Flooded Due to Heavy Rain
- ☐ _____

Appendix B

Raw Data Sheets

CONDITION OF ORGANISMS* IN REPLICATE TEST VESSELS
7-DAY *PIMEPHALES PROMELAS* CHRONIC TOXICITY TEST

Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Contact:
Effluent Serial No.: 001 **NPDES Permit No.:** 2PH00007*

Person(s) Conducting Test: PJC RFR
Beginning: Date 7/10/12 **Time** 1335
Ending: Date 7/17/12 **Time** 1335
Age of *Pimephales promelas*: <24H #1
Dilution Water Used: upstream

Day	Lab Control					Upstream Control					20% Effluent					40% Effluent				
	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total
start	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
1	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
2	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
3	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	9G 1D	10G	10G	39G 1D
4	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	9G 1D	10G	10G	39G 1D
5	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	9G 1D	10G	10G	39G 1D
6	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	9G 1D	10G	10G	39G 1D
7	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	9G 1D	10G	10G	39G 1D

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; A = Atypical Behavior or Appearance; LA = Lab Accident (not used in analyses of data).

CONDITION OF ORGANISMS* IN REPLICATE TEST VESSELS
7-DAY *PIMEPHALES PROMELAS* CHRONIC TOXICITY TEST

Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Contact:
Effluent Serial No.: 001 **NPDES Permit No.:** 2PA00007*

Person(s) Conducting Test: RJC RFR
Beginning: Date 7/10/12 **Time** 1335
Ending: Date 7/17/12 **Time**
Age of *Pimephales promelas*: <24H #11
Dilution Water Used: Upstream

Day	60% Effluent					80% Effluent					100% Effluent					~~Far-field Mixing Zone~~				
	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total	1	2	3	4	Total
start	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G
1	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G					
2	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G					
3	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G					
4	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G					
5	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G					
6	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G					
7	9G 1D	10G	10G	10G	39G 1D	10G	10G	10G	10G	40G	10G	10G	10G	10G	40G					

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; A = Atypical Behavior or Appearance; LA = Lab Accident (not used in analyses of data).

WEIGHT OF FISH IN REPLICATE TEST VESSELS
7-DAY *PIMEPHALES PROMELAS* CHRONIC TOXICITY TEST

Discharge: Allen Co. (American - Bath WWTP) Date of Test: 7/10-17/12
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007* *Pimephales promelas* Age at Start: <24 H #11
Drying Temperature (C°): 60 Drying Time (h): 24 Date Weighed: 7-19-12 Analyst: KEK

Balance Calibration Check: 1 gram weight = 0.99999 grams Date: 7-19-12 Analyst: KEK

PJC ✓ 7/20/12

Test Solution	Replicate	A Number of Fish Weighed	B Dry Weight (g) of Boat	C Dry Weight (g) Boat + Fish	D No. of Original Fish	E = C-B Total Dry Weight of Fish (mg)	F = E/D Avg. Dry Weight of Fish (mg)
Fish at Start of Test		10	1.13020	1.13098	10	0.780	0.078
Lab Control	1	10	1.08960	1.09853	10	8.93	0.893
	2	10	1.13012	1.13791	10	7.79	0.779
	3	10	1.08264	1.09240	10	9.76	0.976
	4	10	1.10393	1.11414	10	10.21	1.021
Upstream Control	1	10	1.11059	1.11692	10	6.33	0.633
	2	10	1.13076	1.13853	10	7.77	0.777
	3	10	1.12677	1.13523	10	8.46	0.846
	4	10	1.09231	1.09953	10	7.22	0.722
20% Effluent	1	10	1.13202	1.13979	10	7.77	0.777
	2	10	1.09757	1.10551	10	7.94	0.794
	3	10	1.09167	1.10058	10	8.91	0.891
	4	10	1.12430	1.13164	10	7.34	0.734
40% Effluent	1	10	1.12984	1.13747	10	7.63	0.763
	2	9	1.12377	1.13057	10	6.80	0.680
	3	10	1.08322	1.09174	10	8.52	0.852
	4	10	1.08781	1.09488	10	7.07	0.707

WEIGHT OF FISH IN REPLICATE TEST VESSELS
7-DAY *PIMEPHALES PROMELAS* CHRONIC TOXICITY TEST

Discharge: Allen Co. (American-Bath WWTP) Date of Test: 7/10-17/12
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007* *Pimephales promelas* Age at Start: <24H #11
Drying Temperature (C°): 60 Drying Time (h): 24 Date Weighed: 7/19/12 Analyst: KEK

BSC ✓ 7/20/12

Test Solution	Replicate	A Number of Fish Weighed	B Dry Weight (g) of Boat	C Dry Weight (g) Boat + Fish	D No. of Original Fish	E = C-B Total Dry Weight of Fish (mg)	F = E/D Avg. Dry Weight of Fish (mg)
60% Effluent	1	9	1.12879	1.13564	10	6.85	0.685
	2	10	1.09477	1.10270	10	7.93	0.793
	3	10	1.13199	1.13970	10	7.71	0.771
	4	10	1.09079	1.09805	10	7.26	0.726
80% Effluent	1	10	1.10015	1.10760	10	7.45	0.745
	2	10	1.09074	1.09921	10	8.47	0.847
	3	10	1.10152	1.10870	10	7.18	0.718
	4	10	1.12537	1.13343	10	8.06	0.806
100% Effluent	1	10	1.12107	1.12942	10	8.35	0.835
	2	10	1.08394	1.09268	10	8.74	0.874
	3	10	1.12318	1.13247	10	9.29	0.929
	4	10	1.09958	1.10972	10	10.14	1.014
~~Far-field Mixing Zone~~	1				10		
	2				10		
	3				10		
	4				10		

EPA ICP 6/94 XZR 7/26/12 AW

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	20	40	60	80	100
Response 1	.633	.777	.763	.685	.745	.835
Response 2	.777	.794	.680	.793	.847	.874
Response 3	.846	.891	.852	.771	.718	.929
Response 4	.722	.734	.707	.726	.806	1.014

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: American-Bath WWTP 001
Test Start Date: 7/10/12 Test Ending Date: 7/17/12
Test Species: P. promelas
Test Duration: 7 days
DATA FILE: Amb0712p.icp

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	4	0.000	0.745	0.090	0.788
2	4	20.000	0.799	0.066	0.788
3	4	40.000	0.751	0.076	0.788
4	4	60.000	0.744	0.048	0.788
5	4	80.000	0.779	0.058	0.788
6	4	100.000	0.913	0.078	0.788

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 75% of the control response mean.

TOXSTAT 3.5

Title: American-Bath WWTP 001 P. promelas 7/10-17/12 Growth mg RJA 7/26/12 BCV
File: AMBA0712.PPW Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Upst. Control	0.7445	0.7445		
2	20 %	0.7990	0.7990	-1.0897	
3	40 %	0.7505	0.7505	-0.1200	
4	60 %	0.7437	0.7437	0.0150	
5	80 %	0.7790	0.7790	-0.6898	
6	100 %	0.9130	0.9130	-3.3692	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: American-Bath WWTP 001 P. promelas 7/10-17/12 Growth mg
File: AMBA0712.PPW Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Upst. Control	4			
2	20 %	4	0.1205	16.2	-0.0545
3	40 %	4	0.1205	16.2	-0.0060
4	60 %	4	0.1205	16.2	0.0008
5	80 %	4	0.1205	16.2	-0.0345
6	100 %	4	0.1205	16.2	-0.1685

TOXSTAT 3.5

Title: American-Bath WWTP 001 P. promelas 7/10-17/12 Growth mg
File: AMBA0712.PPW Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Upst. Control	4	0.6330	0.8460	0.7445
2	20 %	4	0.7340	0.8910	0.7990
3	40 %	4	0.6800	0.8520	0.7505
4	60 %	4	0.6850	0.7930	0.7437
5	80 %	4	0.7180	0.8470	0.7790
6	100 %	4	0.8350	1.0140	0.9130

Title: American-Bath WWTP 001 P. promelas 7/10-17/12 Growth mg
File: AMBA0712.PPW Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Upst. Control	0.0081	0.0900	0.0450	12.0879
2	20 %	0.0044	0.0663	0.0332	8.3013
3	40 %	0.0058	0.0760	0.0380	10.1245
4	60 %	0.0023	0.0481	0.0240	6.4644
5	80 %	0.0034	0.0584	0.0292	7.4962
6	100 %	0.0060	0.0776	0.0388	8.4987

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: RFR, PJC
Contact:
Beginning Date: 7/10/12 Time: 1310
Ending Date: 7/16/12 Time: 1310
Age of *Ceriodaphnia dubia*: 20-22H ECT2
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 / G	0 / G	0 / G	2 / G	0 / G	About 0 / G	12 / G	/	14	3
2	/	/	/	1 /	0 /	About 0 /	10 /	/	11	~~2~~ 3
3	/	/	/	1 /	About 0 /	0 /	9 /	/	10	3
4	/	/	/	About 0 /	0 /	1 /	9 /	/	10	3
5	/	/	/	2 /	0 /	1 /	9 /	/	12	3
6	/	/	/	1 /	0 /	About 0 /	11 /	/	12	3
7	/	/	/	About 0 /	About 0 /	0 /	10 /	/	10	3
8	/	/	/	1 /	About 0 /	0 /	8 /	/	9	3
9	/	/	/	2 /	0 /	About 0 /	11 /	/	13	3
10	/	/	/	1 /	0 /	About 0 /	14 /	/	15	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	–		
									SUM	116	30
									MEAN	11.6	3.0
									S.D.	2.0	–

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; A = Atypical Behavior or Appearance; LA = Lab Accident (not used in analyses of data).

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: RFR, PJC
Contact:
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:10
Age of *Ceriodaphnia dubia*: 20-22h
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0 G	0 G	0 G	4 G	5 G	0 G	15 G			24	3
2				5	8	0	14 G			27	3
3				4	7	0	12 G			23	3
4				4	0	6	9 D		D	19	3
5				4	6	0	12 G			22	3
6				2	5	0	14			21	3
7				4	10	0	15			29	3
8				4	8	0	12			24	3
9				4	6	0	12			22	3
10				3	8	0	14			25	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	~~10G~~ 9G 1D	–			
									SUM	236	30
									MEAN	23.6	3.0
									S.D.	2.9	–

RFR

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; A = Atypical Behavior or Appearance; LA = Lab Accident (not used in analyses of data).

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: AFR, PJC
Contact:
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:15
Age of *Ceriodaphnia dubia*: 20-22H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 / G	0 / G	0 / G	4 / G	9 / G	0 / G	16 / G	/	29	3
2	/	/	/	4 /	10 /	1 /	12 /	/	27	3
3	/	/	/	0 /	6 /	0 /	14 /	/	20	2
4	/	/	/	0 /	7 /	12 /	0 /	/	19	2
5	/	/	/	4 /	8 /	0 /	5 /	/	17	3
6	/	/	/	4 /	4 /	0 /	1 /	/	9	3
7	/	/	/	4 /	12 /	0 /	13 /	/	29	3
8	/	/	/	4 /	8 /	~~+13~~ /	0 /	/	25	3
9	/	/	/	5 /	11 /	0 /	10 /	/	26	3
10	/	/	/	4 /	11 /	0 /	15 /	/	30	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	–	SUM 221	28
									MEAN 22.1	2.8
									S.D. 8.3	–

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; A = Atypical Behavior or Appearance; LA = Lab Accident (not used in analyses of data).

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 40% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: RFA, PJC
Contact:
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:10
Age of *Ceriodaphnia dubia*: 20-22H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 / G	0 / G	0 / G	4 / G	10 / G	0 / G	14 / G	/	28	3
2	/	/	/	4 /	7 /	0 /	12 /	/	23	3
3	/	/	/	3 /	6 /	0 /	13 /	/	22	3
4	/	/	/	1 /	9 /	0 /	10 /	/	20	3
5	/	/	/	4 /	10 /	0 /	16 /	/	30	3
6	/	/	/	3 /	8 /	0 /	15 /	/	26	3
7	/	/	/	4 /	11 /	12 /	0 /	/	27	3
8	/	/	/	4 /	9 /	0 /	10 /	/	23	3
9	/	/	/	5 /	9 /	0 /	8 /	/	22	3
10	/	/	/	4 /	11 /	0 /	15 /	/	30	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	–		
									SUM	251	30
									MEAN	25.1	3.0
									S.D.	3.6	–

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; A = Atypical Behavior or Appearance; LA = Lab Accident (not used in analyses of data).

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 60% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: AFR, PJC
Contact:
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:10
Age of *Ceriodaphnia dubia*: 20-22H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 / G	0 / G	0 / G	4 / G	7 / G	0 / G	16 / G	/	27	3
2	/	/	/	2 /	6 /	0 /	13 /	/	21	3
3	/	/	/	3 /	6 /	0 /	16 /	/	25	3
4	/	/	/	3 /	6 /	0 /	12 /	/	21	3
5	/	/	/	4 /	9 /	1 /	12 /	/	26	3
6	/	/	/	4 /	7 /	0 /	14 /	/	25	3
7	/	/	/	4 /	10 /	0 /	14 /	/	28	3
8	/	/	/	About 0 /	0 /	0 /	11 /	/	11	2
9	/	/	/	4 /	8 /	0 /	10 /	/	22	3
10	/	/	/	4 /	0 /	8 /	16 /	/	28	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	–		
									SUM	234 / 29
									MEAN	23.4 / 2.9
									S.D.	5.1 / –

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; A = Atypical Behavior or Appearance; LA = Lab Accident (not used in analyses of data).

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 80% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH60007*

Person(s) Conducting Test: RFR, PJC
Contact:
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:10
Age of *Ceriodaphnia dubia*: 20-22H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7	TOTAL PER REP No. Young	No. Broods
1	0 / G	0 / G	0 / G	3 / G	6 / G	0 / G	13 / G	/	22	3
2	/	/	/	0 /	7 /	0 /	12 /	/	19	2
3	/	/	/	2 /	9 /	0 /	13 /	/	24	3
4	/	/	/	1 /	8 /	0 /	11 /	/	20	3
5	/	/	/	4 /	10 /	0 /	14 /	/	28	3
6	/	/	/	4 /	9 /	0 /	14 /	/	27	3
7	/	/	/	3 /	11 /	0 /	16 /	/	30	3
8	/	/	/	4 /	7 /	0 /	12 /	/	23	3
9	/	/	/	3 /	8 /	0 /	10 /	/	21	3
10	/	/	/	4 /	8 /	0 /	14 /	/	26	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	10G	—	SUM 240	29
									MEAN 24.0	2.9
									S.D. 3.7	—

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; A = Atypical Behavior or Appearance; LA = Lab Accident (not used in analyses of data).

CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001
NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: RFR, PSC
Contact:
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:10
Age of *Ceriodaphnia dubia*: 20-22H
Dilution Water Used: Upstream

NO. YOUNG PRODUCED / CONDITION OF ADULT*

Rep.	DAY of TEST 0	1	2	3	4	5	6	7		TOTAL PER REP No. Young	No. Broods
1	0/G	0/G	0/G	3/G	6/G	0/G	11/G	/		20	3
2	/	/	/	5/	0/	9/	12/G	/		26	3
3	/	/	/	2/	0/	7/	16/D	/	D	25	3
4	/	/	/	1/	5/	0/	10/G	/		16	3
5	/	/	/	4/	7/	0/	12/	/		23	3
6	/	/	/	4/	7/	0/	14/	/		25	3
7	/	/	/	4/	12/	0/	12/	/		28	3
8	/	/	/	3/	11/	0/	12/	/		26	3
9	/	/	/	4/	9/	0/	11/	/		24	3
10	/	/	/	5/	9/	0/	12/	/		26	3
Total Adult Cond.	10G	10G	10G	10G	10G	10G	9G LR ~~10G~~ 10	–	SUM	239	30
									MEAN	23.9	3.0
									S.D.	3.5	–

* Condition of Adult: G = Good Condition; D = Dead; IM = Immotile; LE = Loss of Equilibrium; A = Atypical Behavior or Appearance; LA = Lab Accident (not used in analyses of data).

TOXSTAT 3.5

Title: American-Bath WWTP C. dubia 7/10-17/12 Reproduction RTA 7/26/12
File: AMBA0712.CDR Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Upst. Control	23.6000	23.6000		
2	20 %	22.1000	22.1000	0.6901	
3	40 %	25.1000	25.1000	-0.6901	
4	60 %	23.4000	23.4000	0.0920	
5	80 %	24.0000	24.0000	-0.1840	
6	100 %	23.9000	23.9000	-0.1380	

Dunnett critical value = 2.3100 (1 Tailed, alpha = 0.05, df [used] = 5,40)
(Actual df = 5,54)

Title: American-Bath WWTP C. dubia 7/10-17/12 Reproduction
File: AMBA0712.CDR Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Upst. Control	10			
2	20 %	10	5.0208	21.3	1.5000
3	40 %	10	5.0208	21.3	-1.5000
4	60 %	10	5.0208	21.3	0.2000
5	80 %	10	5.0208	21.3	-0.4000
6	100 %	10	5.0208	21.3	-0.3000

EPA ICP 6/54

AEN 7/26/12 RLW

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	20	40	60	80	100
Response 1	24	29	28	27	22	20
Response 2	27	27	23	21	19	26
Response 3	23	20	22	25	24	25
Response 4	19	19	20	21	20	16
Response 5	22	17	30	26	28	23
Response 6	21	9	26	25	27	25
Response 7	29	29	27	28	30	28
Response 8	24	25	23	11	23	26
Response 9	22	26	22	22	21	24
Response 10	25	30	30	28	26	26

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: American-Bath WWTP 001
Test Start Date: 7/10/12 Test Ending Date: 7/16/12
Test Species: C. dubia
Test Duration: 6 days
DATA FILE: Amb0712c.icp

Conc. ID	Number Replicates	Concentration %	Response Means	Std. Dev.	Pooled Response Means
1	10	0.000	23.600	2.914	23.933
2	10	20.000	23.100	6.724	23.933
3	10	40.000	25.100	3.573	23.933
4	10	60.000	23.400	5.103	23.767
5	10	80.000	24.000	3.651	23.767
6	10	100.000	23.900	3.510	23.767

*** No Linear Interpolation Estimate can be calculated from the input data since none of the (possibly pooled) group response means were less than 75% of the control response mean.

TOXSTAT 3.5

Title: American-Bath WWTP C. dubia 7/10-17/12 Reproduction RFR 7/26/12
File: AMBA0712.CDR Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Upst. Control	10	19.0000	29.0000	23.6000
2	20 %	10	7.0000	30.0000	22.1000
3	40 %	10	20.0000	30.0000	25.1000
4	60 %	10	11.0000	28.0000	23.4000
5	80 %	10	19.0000	30.0000	24.0000
6	100 %	10	16.0000	28.0000	23.9000

Title: American-Bath WWTP C. dubia 7/10-17/12 Reproduction
File: AMBA0712.CDR Transform: NO TRANSFORMATION

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Upst. Control	8.4889	2.9136	0.9214	12.3456
2	20 %	68.7667	8.2926	2.6223	37.5229
3	40 %	12.7667	3.5730	1.1299	14.2352
4	60 %	26.0444	5.1034	1.6138	21.8093
5	80 %	13.3333	3.6515	1.1547	15.2145
6	100 %	12.3222	3.5103	1.1101	14.6875

CERIODAPHNIA BROOD STOCK INDIVIDUAL CULTURE INFORMATION FORM FOR *C. DUBIA* BROODS USED IN CHRONIC TESTS

Discharger/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Type of Test: Chronic Date of Test: 7/10-17/12
Effluent Serial No.: 001 Permit No.: 2PH00007*

Investigator: _____ Culture No.: ECT #2
Culture initiation date: _____ Culture ending date: _____
Origin of parental organisms: _____
Age at start of culture: _____
Culture water renewal frequency: minimum 3 times per week Culture water: MHSF
Culture temperature: 25°C Fed daily: 0.1 mL YCT & 0.1 mL *Selenastrum capricornutum*

Number of brood organisms at start of culture: _____
Percent survival of parental organisms after 7 days: _____
Percent of non-reproducing adults after 7 days: _____
Average young production after 7 days of brood adults for test: _____

	BROOD ADULT NO.	BROOD SIZE	HATCHING TIME WINDOW 1500 to 1700	TOTAL; YOUNG PROD. (7 DAYS)	USED IN REP. NO.
1)	1	8	7/9/12		1
2)	2	9			2
3)	3	10			3
4)	4	11			4
5)	5	11			5
6)	6	12			6
7)	7	9			7
8)	8	9			8
9)	9	10			9
10)	10	10			10

COMMENTS: _____

Appendix C

Parameter Data

PHYSICAL-CHEMICAL DATA OF SAMPLES WHEN RECEIVED BY LAB
For Chronic Toxicity Testing

Industry/Toxicant: Allen Co. (American - Bath WWTP) Type of Test: Chronic
Address: Lima, OH Date of Test: 7/10-7/12
Effluent Serial No.: 001 Permit No.: 2PH00007*

1st Sample Collection

Date Samples Collected: 7/9/12 Date Samples Read: 7/9/12 Analyst: KEK

	100% Effluent	Upstream
Temperature (°C)	20.3	23.0
Dissolved Oxygen (mg/L)	7.6	6.0
pH (s.u.)	7.60	7.57
Conductivity (µmhos)	1004	975
Chlorine, total (mg/L)	<0.02	
Visual Description	light tan Stain	Green ~~Stain~~ KK

2nd Sample Collection

Date Samples Collected: 7/11/12 Date Samples Read: 7/11/12 Analyst: PJC

	100% Effluent	Upstream
Temperature (°C)	20.7	21.9
Dissolved Oxygen (mg/L)	8.0	3.9
pH (s.u.)	7.53	7.46
Conductivity (µmhos)	1023	954
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light tan Stain	Green

3rd Sample Collection

Date Samples Collected: 7/13/12 Date Samples Read: 7/13/12 Analyst: PJC

	100% Effluent	Upstream
Temperature (°C)	23.6	23.8
Dissolved Oxygen (mg/L)	8.0	5.1
pH (s.u.)	7.37	7.32
Conductivity (µmhos)	1002	999
Chlorine, total (mg/L)	<0.02	<0.02
Visual Description	light Stain	light green

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: Lab Control
Industry/Toxicant: Allen Co. (American - Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 7/10/12 Time: 1335
Ending Date: 7/17/12 Time: 1335
Test Organism: *Pimephales promelas* Age: <24H #11
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.3	25.0 to 25.5	25.4	24.9 to 25.2	25.3	25.2 to 25.5	25.3	25.2 to 25.6	25.4	25.3 to 25.5	25.2	25.1 to 25.3	25.2 to 25.6
Dissolved Oxygen (mg/L)	8.6	8.9	6.2	8.9	6.3	8.5	6.6	8.0	6.8	8.0	5.8	8.6	6.0	7.2
pH (s.u.)	7.18	7.11	7.30	7.28	7.47	7.15	7.34	7.28	7.36	7.05	7.30	7.17	7.39	7.30
Conductivity (µmho/cm)	382	383	397	371	398	372	388	377	386	377	389	378	403	391
Hardness (mg/L $CaCO_3$)	120			120				103						
Alkalinity (mg/L $CaCO_3$)	86			86				68						

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: Upstream Control
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 7/10/12 Time: 1335
Ending Date: 7/17/12 Time: 1335
Test Organism: *Pimephales promelas* Age: <24H #11
Dilution Water Used: upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.3	25.0 to 25.5	25.4	24.9 to 25.2	25.3	25.2 to 25.5	25.1	25.2 to 25.4	25.4	25.3 to 25.5	25.3	25.1 to 25.3	25.2 to 25.4
Dissolved Oxygen (mg/L)	7.2	8.6	5.8	8.6	4.2	8.5	6.4	7.5	6.7	7.9	5.3	8.6	5.5	7.1
pH (s.u.)	7.46	7.46	7.39	7.47	7.43	7.59	7.47	7.10	7.32	7.35	7.44	7.42	7.45	7.50
Conductivity (µmho/cm)	974	973	1001	953	999	953	980	1002	990	1002	1010	1003	1035	1024
Hardness (mg/L $CaCO_3$)	325			325				325						
Alkalinity (mg/L $CaCO_3$)	171			171				154						

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent
Industry/Toxicant: Allen Co. (American - Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PA00007*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 7/10/12 Time: 1335
Ending Date: 7/17/12 Time: 1335
Test Organism: *Pimephales promelas* Age: <24hr #16
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.3	25.0 to 25.5	25.4	24.9 to 25.2	25.3	25.2 to 25.5	25.4	25.2 to 25.6	25.4	25.3 to 25.5	25.4	25.1 to 25.3	25.2 to 25.6
Dissolved Oxygen (mg/L)	7.6	8.4	5.2	8.4	6.4	8.3	6.5	7.8	6.7	8.0	5.0	8.2	5.9	6.9
pH (s.u.)	7.61	7.65	7.48	7.53	7.60	7.61	7.49	7.51	7.50	7.50	7.45	7.51	7.53	7.52
Conductivity (µmho/cm)	980	981	998	967	1024	968	1008	1003	1003	1004	1026	1004	1051	1046
Hardness (mg/L $CaCO_3$)														
Alkalinity (mg/L $CaCO_3$)														

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: 40% Effluent
Industry/Toxicant: Allen Co. (American - Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 7/10/12 Time: 1335
Ending Date: 7/17/12 Time: 1335
Test Organism: *Pimephales promelas* Age: <24H #11
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.3	25.0 to 25.3	25.3	24.9 to 25.2	25.2	25.2 to 25.5	25.5	25.2 to 25.6	25.4	25.3 to 25.5	25.3	25.1 to 25.3	25.2 to 25.6
Dissolved Oxygen (mg/L)	7.6	7.8	5.6	8.1	6.8	8.4	6.4	7.8	6.6	7.9	5.2	8.8	5.6	7.0
pH (s.u.)	7.63	7.53	7.52	7.57	7.62	7.59	7.52	7.55	7.54 ~~5.5~~ AFR	7.57	7.48	7.47	7.56	7.55
Conductivity (µmho/cm)	986	988	1019	982	1028	982	1029	1003	1022	1004	1053	1005	1059	1056
Hardness (mg/L $CaCO_3$)														
Alkalinity (mg/L $CaCO_3$)														

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: 60% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: PJC RFR
Beginning Date: 7/10/12 Time: 1335
Ending Date: 7/17/12 Time: 1335
Test Organism: *Pimephales promelas* Age: <24h #10
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.3	25.0 to 25.5	25.3	24.9 to 25.2	25.2	25.2 to 25.5	25.4	25.2 to 25.6	25.3	25.3 to 25.5	25.3	25.1 to 25.3	25.2 to 25.6
Dissolved Oxygen (mg/L)	7.9	8.2	5.9	8.2	5.4	8.7	6.4	7.9	6.6	8.1	5.3	8.5	6.0	7.4
pH (s.u.)	7.68	7.57	7.55	7.62	7.66	7.61	7.52	7.55	7.54	7.59	7.52	7.40	7.59	7.58
Conductivity (µmho/cm)	991	992	1023	995	1024	994	1035	1004	1018	996	1033	1006	1046	1048
Hardness (mg/L $CaCO_3$)														
Alkalinity (mg/L $CaCO_3$)														

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: 80% Effluent
Industry/Toxicant: Allen Co. (American - Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: PJC RFR
Beginning Date: 7/10/12 Time: 1335
Ending Date: 7/17/12 Time: 1335
Test Organism: *Pimephales promelas* Age: <24hr #11
Dilution Water Used: upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.3	25.0 to 25.5	25.4	24.9 to 25.2	25.3	25.2 to 25.5	25.4	25.2 to 25.4	25.2	25.3 to 25.5	25.3	25.1 to 25.3	25.2 to 25.6
Dissolved Oxygen (mg/L)	8.1	8.5	6.2	8.4	6.3	8.3	6.4	7.9	6.5	8.2	5.8	8.5	6.2	7.4
pH (s.u.)	7.70	7.61	7.57	7.68	7.68	7.65	7.49	7.60	7.53	7.63	7.54	7.51	7.60	7.58
Conductivity (µmho/cm)	996	997	1022	1000	1028	1008	1035	1004	1034	1005	1029	1005	1042	1043
Hardness (mg/L $CaCO_3$)														
Alkalinity (mg/L $CaCO_3$)														

PHYSICAL-CHEMICAL DATA
PIMEPHALES PROMELAS 7-DAY CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: PJC, RFR
Beginning Date: 7/10/12 Time: 1335
Ending Date: 7/17/12 Time: 1335
Test Organism: *Pimephales promelas* Age: <24H #11
Dilution Water Used: upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.3	25.0 to 25.5	25.3	24.9 to 25.7	25.3	25.2 to 25.5	25.4	25.2 to 25.6	25.4	25.3 to 25.5	25.3	25.1 to 25.3	25.2 to 25.6
Dissolved Oxygen (mg/L)	8.1	8.6	6.1	8.5	6.5	8.0	6.3	7.9	6.5	8.3	5.7	8.3	5.4	7.3
pH (s.u.)	7.80	7.70	7.57	7.72	7.69	7.74	7.50	7.58	7.54	7.66	7.54	7.57	7.57	7.57
Conductivity (µmho/cm)	1002	1002	1030	1023	1052	1022	1070	1005	1065	1004	1042	1005	1067	1063
Hardness (mg/L $CaCO_3$)	325			359				257						
Alkalinity (mg/L $CaCO_3$)	171			154				154						

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Lab Control
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: AFA, MJC
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:10
Test Organism: *Ceriodaphnia dubia*: Age: 24-22H ECT 2
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	~~Old~~
Temperature (°C)	25.3	25.3	24.9 to 25.2	25.3	24.8 to 25.0	25.3	24.8 to 25.0	25.3	25.0 to 25.2	25.4	25.0 to 25.1	25.2	24.9 to 25.1	–
Dissolved Oxygen (mg/L)	8.4	8.6	7.9	8.9	8.8	8.9	7.5	8.2	7.5	8.2	7.7	8.7	7.8	
pH (s.u.)	7.24	7.25	7.32	7.21	7.52	7.27	7.40	7.30	7.38	7.15	7.07	7.11	7.41	
Conductivity (µmho/cm)	327	326	331	325	335	326	337	326	335	325	336	323	335	
Hardness (mg/L $CaCO_3$)	103			103				103						
Alkalinity (mg/L $CaCO_3$)	103			86				103						

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: Upstream Control
Industry/Toxicant: Allen Co. (American - Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: AFR, ASC
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:10
Test Organism: *Ceriodaphnia dubia*: Age: 20-22H
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		~~7~~
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	~~Old~~
Temperature (°C)	25.4	25.3	24.9 to 25.2	25.4	24.8 to 25.0	25.3	24.8 to 25.0	25.1	25.0 to 25.2	25.4	25.0 to 25.1	25.3	24.9 to 25.1	–
Dissolved Oxygen (mg/L)	>.2	8.4	7.6	8.6	8.1	8.5	7.8	7.5	7.6	7.9	7.9	8.8	7.6	
pH (s.u.)	7.46	7.46	7.67	7.47	7.74	7.59	7.69	7.40	7.51	7.35	7.39	7.42	7.58	
Conductivity (µmho/cm)	974	973	958	953	981	953	958	1000	955	1002	994	1003	997	
Hardness (mg/L $CaCO_3$)	325			325				325						
Alkalinity (mg/L $CaCO_3$)	171			171				154						

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 20% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: RFR, PJC
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:10
Test Organism: *Ceriodaphnia dubia*: Age: 20-22 H
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		~~7~~
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	~~Old~~
Temperature (°C)	25.4	25.3	24.9 to 25.2	25.4	24.8 to 25.0	25.3	24.8 to 25.0	25.4	25.0 to 25.2	25.4	25.0 to 25.1	25.4	24.9 to 25.1	–
Dissolved Oxygen (mg/L)	7.6	8.4	7.7	8.4	7.5	8.3	7.9	7.8	7.6	8.0	7.9	8.2	7.7	
pH (s.u.)	7.61	7.65	7.71	7.53	7.77	7.61	7.64	7.51	7.65	7.50	7.67	7.51	7.64	
Conductivity (µmho/cm)	980	981	990	967	1002	968	998	1003	994	1004	1029	1004	1031	
Hardness (mg/L $CaCO_3$)														
Alkalinity (mg/L $CaCO_3$)														

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 40% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: AFR, PJC
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:10
Test Organism: *Ceriodaphnia dubia*: Age: 20-22H
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.3	24.9 to 25.2	25.3	24.8 to 25.0	25.2	~~24.8~~ ~~24.9~~ NA to 25.0	25.5	25.0 to 25.2	25.4	25.0 to 25.1	25.3	24.9 to 25.1	–
Dissolved Oxygen (mg/L)	7.6	7.8	7.7	8.1	7.6	8.4	7.9	7.8	7.6	7.9	7.8	8.8	7.7	
pH (s.u.)	7.63	7.53	7.72	7.57	7.79	7.59	7.64	7.55	7.67	7.57	7.74	7.47	7.69	
Conductivity (µmho/cm)	986	988	996	987	1007	982	1010	1003	1004	1004	1028	1005	1041	
Hardness (mg/L $CaCO_3$)														
Alkalinity (mg/L $CaCO_3$)														

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 60% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PA00007*

Person(s) Conducting Test: RFR, PJC
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:10
Test Organism: *Ceriodaphnia dubia*: Age: 20-22H
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		7
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
Temperature (°C)	25.4	25.3	24.9 to 25.2	25.3	24.8 to 25.0	25.2	24.8 to 25.0	25.4	25.0 to 25.2	25.3	25.0 to 25.1	25.3	24.9 to 25.1	—
Dissolved Oxygen (mg/L)	7.9	8.2	7.8	8.2	8.2	8.7	7.9	7.9	7.6	8.1	7.8	8.5	7.6	
pH (s.u.)	7.68	7.57	7.72	7.62	7.79	7.61	7.65	7.58	7.67	7.59	7.75	7.48	7.72	
Conductivity (µmho/cm)	991	992	1003	995	1007	994	1025	1004	1014	996	1031	1006	1035	
Hardness (mg/L $CaCO_3$)														
Alkalinity (mg/L $CaCO_3$)														

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 80% Effluent
Industry/Toxicant: Allen Co. (American-Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PH00007*

Person(s) Conducting Test: AFR, PJC
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:10
Test Organism: *Ceriodaphnia dubia*: Age: 20-22H
Dilution Water Used: Upstream

Day	0	1		2		3		4		5		6		~~7~~
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	~~Old~~
Temperature (°C)	25.4	25.3	24.9 to 25.2	25.4	24.8 to 25.0	25.3	24.8 to 25.0	25.4	25.0 to 25.2	25.2	25.0 to 25.1	25.3	24.9 to 25.1	-
Dissolved Oxygen (mg/L)	8.1	8.5	7.9	8.4	8.2	8.3	7.9	7.9	7.7	8.2	7.8	8.5	7.7	
pH (s.u.)	7.70	7.67	7.73	7.66	7.80	7.65	7.65	7.60	7.67	7.63	7.78	7.51	7.74	
Conductivity (µmho/cm)	996	997	1007	1008	1014	1008	1035	1004	1034	1005	1031	1005	1035	
Hardness (mg/L $CaCO_3$)														
Alkalinity (mg/L $CaCO_3$)														

PHYSICAL-CHEMICAL DATA
CERIODAPHNIA DUBIA CHRONIC TOXICITY TEST

Solution Tested: 100% Effluent
Industry/Toxicant: Allen Co. (American - Bath WWTP)
Address: Lima, OH
Effluent Serial No.: 001 NPDES Permit No.: 2PA00007*

Person(s) Conducting Test: AFR, PJC
Beginning Date: 7/10/12 Time: 13:10
Ending Date: 7/16/12 Time: 13:10
Test Organism: *Ceriodaphnia dubia*: Age: 20-22hr
Dilution Water Used: Upstream

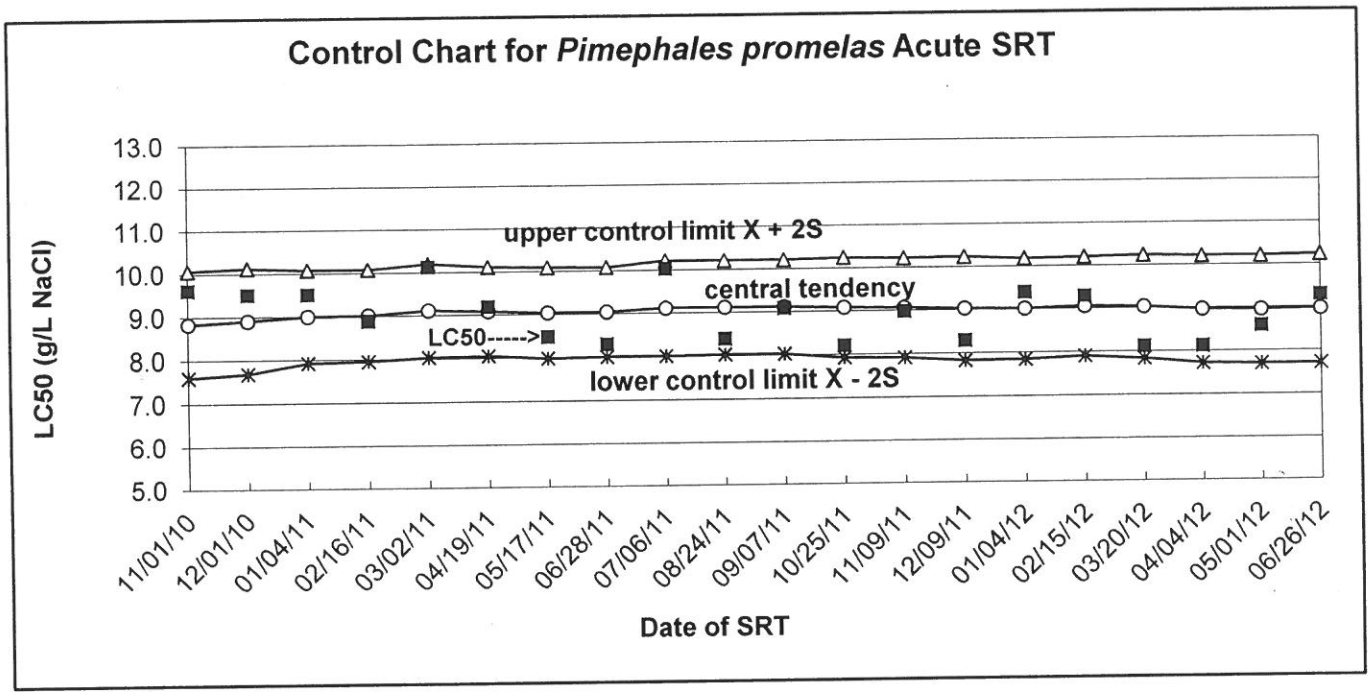
Day	0	1		2		3		4		5		6		~~7~~
Solution	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	~~Old~~
Temperature (°C)	25.4	25.3	24.9 to 25.2	25.3	24.8 to 25.0	25.3	24.8 to 25.0	25.4	25.0 to 25.2	25.4	25.0 to 25.1	25.3	24.9 to 25.1	—
Dissolved Oxygen (mg/L)	8.1	8.4	8.0	8.5	8.4	8.0	7.9	7.9	7.7	8.3	7.8	8.3	7.8	
pH (s.u.)	7.80	7.70	7.75	7.72	7.81	7.74	7.65	7.50	7.67	7.66	7.79	7.57	7.74	
Conductivity (µmho/cm)	1000	1002	1010	1023	1020	1022	1049	1005	1044	1004	1032	1005	980	
Hardness (mg/L $CaCO_3$)	325			359				257						
Alkalinity (mg/L $CaCO_3$)	171			154				154						

Appendix D

SRT Data

48-H LC50 for *Pimephales promelas* Acute NaCl SRT

Date of Test	LC50	Cumulative LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	LC50 Method[1]
11/01/10	9.6	8.8	0.6	10.1	7.6	0.07	10.7	7.0	SK
12/01/10	9.5	8.9	0.6	10.1	7.7	0.07	10.7	7.1	SK
01/04/11	9.5	9.0	0.5	10.1	7.9	0.06	10.6	7.4	SK
02/16/11	8.9	9.0	0.5	10.1	8.0	0.06	10.6	7.4	SK
03/02/11	10.1	9.1	0.5	10.2	8.0	0.06	10.7	7.5	SK
04/19/11	9.2	9.1	0.5	10.1	8.1	0.06	10.6	7.5	SK
05/17/11	8.5	9.0	0.5	10.1	8.0	0.06	10.6	7.5	SK
06/28/11	8.3	9.0	0.5	10.1	8.0	0.06	10.6	7.5	TSK
07/06/11	10.0	9.1	0.6	10.2	8.0	0.06	10.8	7.5	SK
08/24/11	8.4	9.1	0.5	10.2	8.0	0.06	10.8	7.5	SK
09/07/11	9.1	9.1	0.5	10.2	8.0	0.06	10.8	7.5	SK
10/25/11	8.2	9.1	0.6	10.3	7.9	0.06	10.8	7.3	SK
11/09/11	9.0	9.1	0.6	10.2	7.9	0.06	10.8	7.3	SK
12/09/11	8.3	9.0	0.6	10.2	7.8	0.07	10.8	7.2	SK
01/04/12	9.4	9.0	0.6	10.2	7.8	0.06	10.8	7.3	SK
02/15/12	9.3	9.1	0.6	10.2	7.9	0.06	10.8	7.3	SK
03/20/12	8.1	9.0	0.6	10.2	7.8	0.07	10.8	7.2	SK
04/04/12	8.1	9.0	0.6	10.2	7.7	0.07	10.8	7.1	SK
05/01/12	8.6	9.0	0.6	10.2	7.7	0.07	10.8	7.1	SK
06/26/12	9.3	9.0	0.6	10.2	7.7	0.07	10.9	7.1	SK

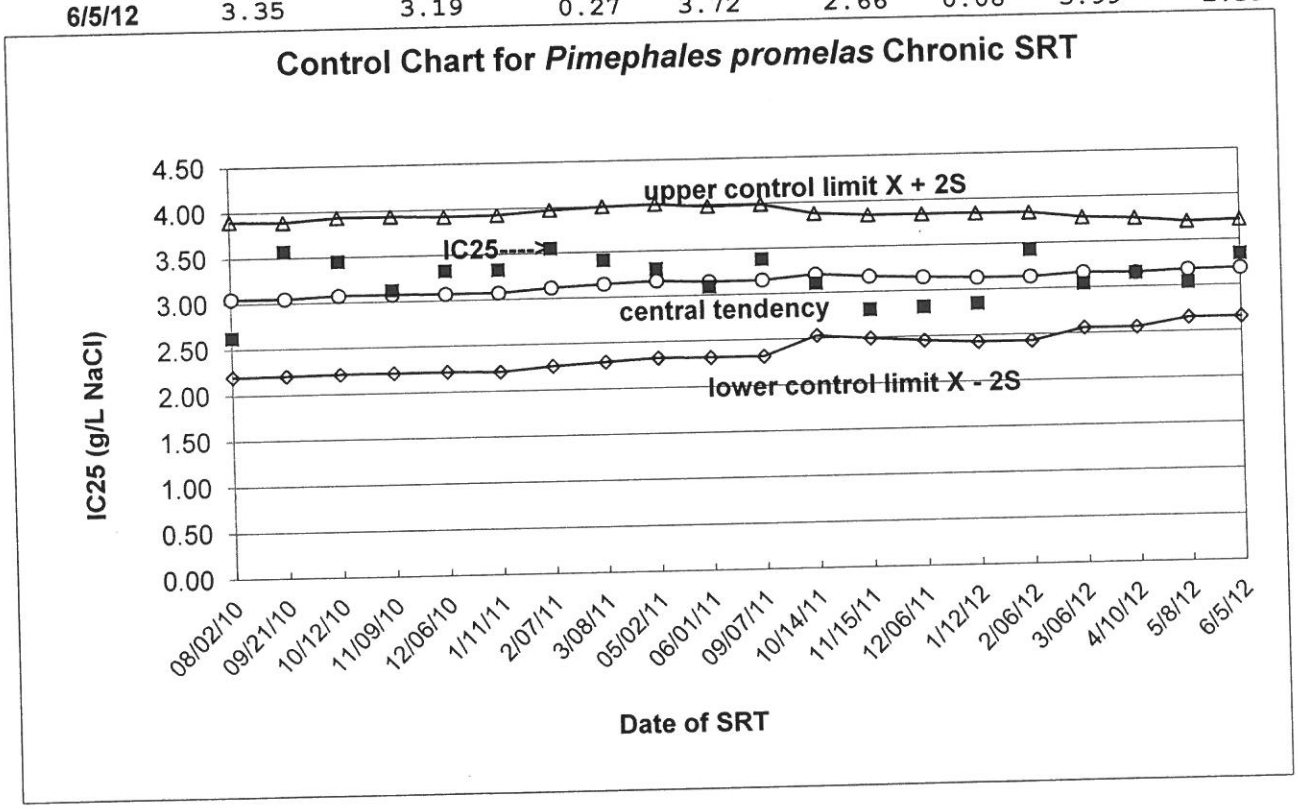


*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
75th percentile of CVs for LC50 = 0.19
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X} \pm 3SD$ = Well outside expected range.

1 - P = Probit method, G = Graphical method, SK = Spearman-Karber method, TSK = Trimmed Spearman-Karber method.

IC25 for *Pimephales promelas* Chronic NaCl SRT

Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD
08/02/10	2.63	3.05	0.43	3.90	2.20	0.14	4.33	1.77
09/21/10	3.57	3.05	0.42	3.89	2.21	0.14	4.31	1.78
10/12/10	3.45	3.08	0.43	3.93	2.22	0.14	4.36	1.79
11/09/10	3.13	3.08	0.43	3.94	2.22	0.14	4.37	1.79
12/06/10	3.33	3.07	0.43	3.92	2.22	0.14	4.35	1.80
1/11/11	3.33	3.07	0.43	3.93	2.21	0.12	4.36	1.78
2/07/11	3.55	3.12	0.43	3.97	2.26	0.14	4.40	1.83
3/08/11	3.41	3.15	0.43	4.00	2.29	0.14	4.43	1.87
05/02/11	3.30	3.17	0.42	4.02	2.32	0.13	4.44	1.90
06/01/11	3.09	3.15	0.42	3.98	2.32	0.13	4.40	1.90
09/07/11	3.39	3.15	0.42	3.99	2.32	0.13	4.41	1.90
10/14/11	3.11	3.20	0.34	3.88	2.53	0.10	4.21	2.20
11/15/11	2.81	3.17	0.34	3.85	2.49	0.11	4.19	2.15
12/06/11	2.82	3.15	0.35	3.84	2.45	0.11	4.19	2.11
1/12/12	2.85	3.13	0.35	3.84	2.43	0.11	4.20	2.07
2/06/12	3.43	3.13	0.35	3.84	2.43	0.11	4.19	2.07
3/06/12	3.05	3.17	0.30	3.78	2.56	0.10	4.08	2.26
4/10/12	3.15	3.16	0.30	3.76	2.56	0.10	4.06	2.26
5/8/12	3.04	3.18	0.26	3.71	2.66	0.08	3.97	2.39
6/5/12	3.35	3.19	0.27	3.72	2.66	0.08	3.99	2.39



*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-2 in EPA/833/R-00/003 .

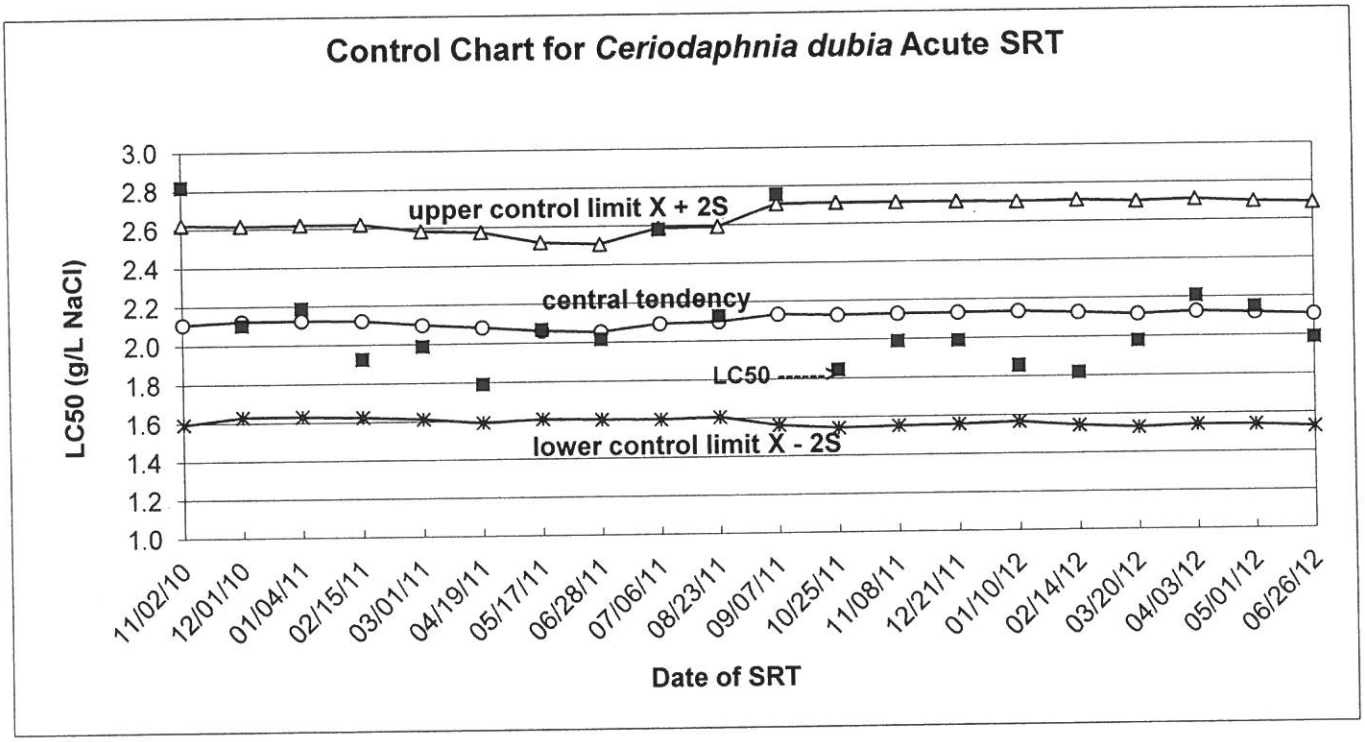
75th percentile of CVs for IC25 = 0.38

Calculated CV should not exceed 75th percentile of CVs.

$\overline{X} \pm 3SD$ = Well outside expected range.

48-H LC50 for *Ceriodaphnia dubia* Acute NaCl SRT

Date of Test	LC50	Cumulative LC50 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD	LC50 Method[1]
11/02/10	2.8	2.1	0.3	2.6	1.6	0.12	2.9	1.3	TSK
12/01/10	2.1	2.1	0.2	2.6	1.6	0.12	2.9	1.4	Probit
01/04/11	2.2	2.1	0.2	2.6	1.6	0.12	2.9	1.4	TSK
02/15/11	1.9	2.1	0.2	2.6	1.6	0.12	2.9	1.4	Probit
03/01/11	2.0	2.1	0.2	2.6	1.6	0.12	2.8	1.4	TSK
04/19/11	1.8	2.1	0.2	2.6	1.6	0.12	2.8	1.3	Probit
05/17/11	2.1	2.1	0.2	2.5	1.6	0.11	2.7	1.4	SK
06/28/11	2.0	2.1	0.2	2.5	1.6	0.11	2.7	1.4	Probit
07/06/11	2.6	2.1	0.2	2.6	1.6	0.12	2.8	1.4	SK
08/23/11	2.1	2.1	0.2	2.6	1.6	0.12	2.8	1.4	SK
09/07/11	2.8	2.1	0.3	2.7	1.6	0.13	3.0	1.3	Probit
10/25/11	1.8	2.1	0.3	2.7	1.5	0.14	3.0	1.3	Probit
11/08/11	2.0	2.1	0.3	2.7	1.6	0.14	3.0	1.3	TSK
12/21/11	2.0	2.1	0.3	2.7	1.6	0.14	3.0	1.3	SK
01/10/12	1.9	2.1	0.3	2.7	1.6	0.13	3.0	1.3	Probit
02/14/12	1.8	2.1	0.3	2.7	1.5	0.14	3.0	1.3	SK
03/20/12	2.0	2.1	0.3	2.7	1.5	0.14	3.0	1.2	TSK
04/03/12	2.2	2.1	0.3	2.7	1.5	0.14	3.0	1.3	Probit
05/01/12	2.2	2.1	0.3	2.7	1.5	0.14	3.0	1.3	Probit
06/26/12	2.0	2.1	0.3	2.7	1.5	0.14	3.0	1.2	SK

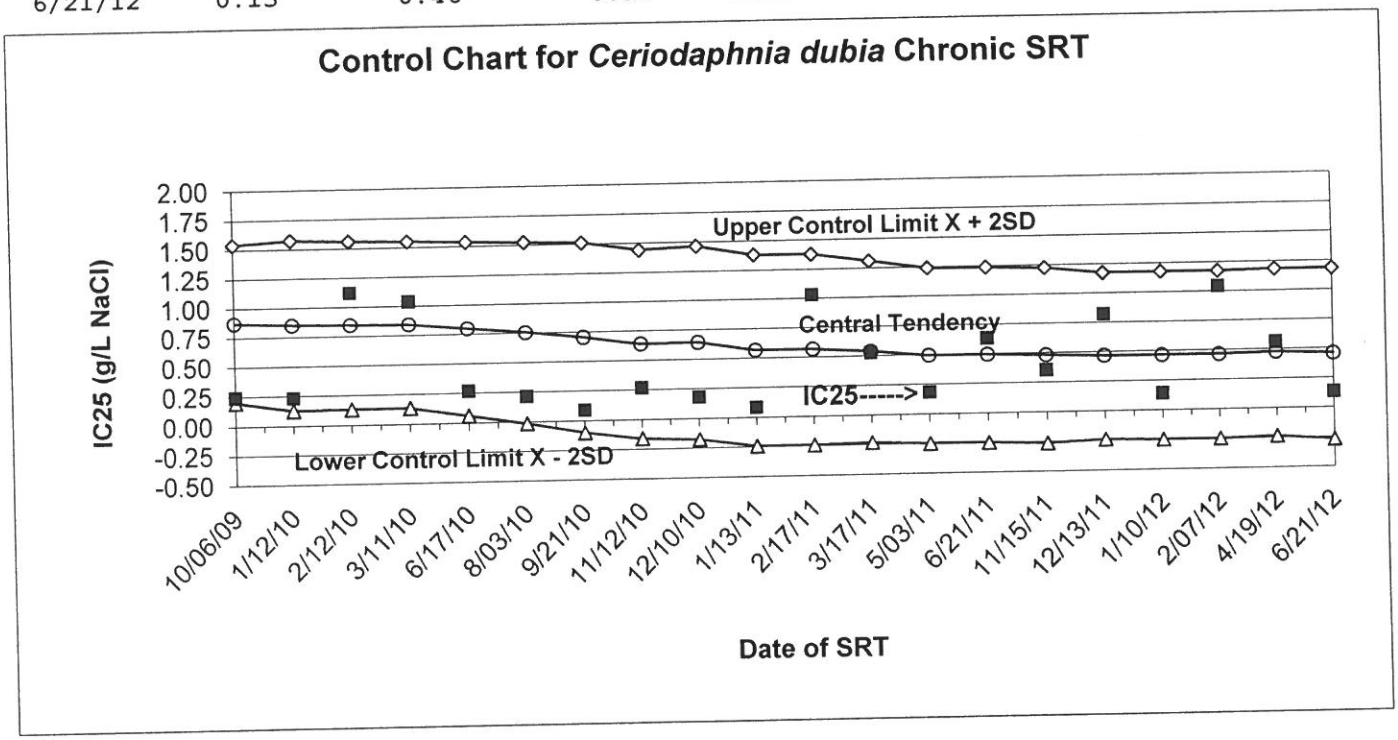


*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-3 in EPA/833/R-00/003 .
75th percentile of CVs for LC50 = 0.29
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X} \pm 3SD$ = Well outside expected range.

1 - P = Probit method, G = Graphical method, SK = Spearman-Karber method, TSK = Trimmed Spearman-Karber method.

IC25 for *Ceriodaphnia dubia* Chronic NaCl SRT

Date of Test	IC25	Cumulative IC25 Mean	SD	X+2SD	X-2SD	CV*	X+3SD	X-3SD
10/06/09	0.24	0.87	0.34	1.54	0.20	0.39	1.88	-0.14
1/12/10	0.23	0.85	0.36	1.58	0.13	0.42	1.94	-0.23
2/12/10	1.12	0.85	0.36	1.56	0.13	0.42	1.92	-0.22
3/11/10	1.04	0.84	0.35	1.55	0.13	0.42	1.91	-0.22
6/17/10	0.27	0.80	0.37	1.54	0.06	0.46	1.91	-0.31
8/03/10	0.21	0.76	0.38	1.53	-0.01	0.51	1.91	-0.40
9/21/10	0.09	0.71	0.40	1.51	-0.10	0.57	1.92	-0.50
11/12/10	0.27	0.64	0.40	1.44	-0.16	0.62	1.84	-0.56
12/10/10	0.18	0.64	0.41	1.46	-0.18	0.64	1.88	-0.59
1/13/11	0.09	0.57	0.41	1.38	-0.24	0.71	1.79	-0.65
2/17/11	1.03	0.57	0.41	1.38	-0.24	0.71	1.78	-0.65
3/17/11	0.53	0.54	0.39	1.31	-0.23	0.71	1.70	-0.62
5/03/11	0.18	0.50	0.37	1.24	-0.25	0.75	1.61	-0.62
6/21/11	0.63	0.50	0.37	1.24	-0.25	0.75	1.61	-0.62
11/15/11	0.35	0.48	0.37	1.23	-0.26	0.77	1.60	-0.63
12/13/11	0.82	0.47	0.35	1.17	-0.24	0.75	1.53	-0.59
1/10/12	0.14	0.46	0.36	1.18	-0.25	0.77	1.53	-0.60
2/07/12	1.05	0.46	0.36	1.18	-0.25	0.77	1.53	-0.60
4/19/12	0.56	0.48	0.35	1.19	-0.23	0.75	1.54	-0.59
6/21/12	0.13	0.46	0.36	1.18	-0.27	0.79	1.55	-0.63



*- compare calculated CV to 75th percentile of CVs reported nationally in Table 3-2 in EPA/833/R-00/003 .
75th percentile of CVs for IC25 = 0.45
Calculated CV should not exceed 75th percentile of CVs.
$\overline{X} \pm 3SD$ = Well outside expected range.