



MACTEC
ENGINEERING AND CONSULTING, INC.
BIOTOX LABORATORY
KENNESAW, GEORGIA

CHRONIC TOXICITY TESTING REPORT

**SILT STOP 707a PRODUCT
WOODSTOCK, GEORGIA**

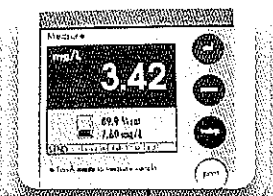
Test Period: May 5 – 9, 2007

Prepared for:

**Applied Polymer Systems, Inc.
Woodstock, Georgia**

May 14, 2007

Project Number: 6126-07-0005



**REPORT FOR CHRONIC TOXICITY TESTING OF
APPLIED POLYMER SYSTEMS, INC.
SILT STOP 707a PRODUCT**

WOODSTOCK, GEORGIA

TEST PERIOD: May 5 – 9, 2007

Prepared for:

**Applied Polymer Systems, Inc.
Woodstock, Georgia**

Prepared by:

**BioTox Laboratory
MACTEC Engineering and Consulting, Inc.
Kennesaw, Georgia**

May 14, 2007



— engineering and constructing a better tomorrow

May 14, 2007

Mr. Steven R. Iwinski
Applied Polymer Systems, Inc.
519 Industrial Drive
Woodstock, GA 30189

Subject: **Applied Polymer Systems, Inc. Chronic Toxicity Testing on 707a Product,
May 2-9, 2007
MACTEC Project No. 6126-07-0005**

Dear Mr. Iwinski:

MACTEC Engineering and Consulting, Inc. (MACTEC), BioTox Laboratory has completed 7-day chronic toxicity testing on samples of Silt Stop 707a. Toxicity testing was initiated by MACTEC on May 2, 2007 to determine the IC₂₅ (the Inhibition Concentration of the sample at which there is a 25% reduction in survival and growth of the fathead minnow, *Pimephales promelas*, when compared to the laboratory control) and the NOEC (the No Observed Effect Concentration, or the highest concentration tested that did not exhibit chronic toxicity). Test samples were prepared by adding 420 milligrams (mg) of polymer to 1.0 liter (L) of laboratory formulated water and allowed to mix on a stir plate until fully dissolved (approximately 4 hours). This stock test sample was then diluted with laboratory formulated water to the following test concentrations: 25.25 ppm, 52.5 ppm, 105 ppm, 210 ppm, and 420 ppm. Fathead minnows were exposed to the individual test concentrations, which were renewed with a fresh polymer solution daily, for a period of 7-days. After 7-days, the survival and growth endpoints were determined.


Test results indicated chronic toxicity to fathead minnow survival and growth at the 420 ppm concentrations. Based on the concentrations analyzed, the statistically determined IC₂₅ for survival was 258.9 ppm and the NOEC was 210 ppm. The statistically determined IC₂₅ for growth was 224.2 ppm and the NOEC was 210 ppm.


Results are summarized in the accompanying report. Detailed testing documentation, including chemical and physical measurements (Appendix A), fathead minnow documentation (Appendix B), and polymer sample test data/reference toxicant data (Appendix C).

We appreciate the opportunity to provide these environmental services to the Applied Polymer Systems, and we look forward to working with you again in the future. If there are any questions, please do not hesitate to contact Dale McPherson or Paul Brafford at (770) 421-3400.

Sincerely,

MACTEC ENGINEERING AND CONSULTING, INC.


R. Dale McPherson
BioTox Laboratory Manager


W. Paul Brafford, CHMM
BioTox Laboratory Director

Attachment: Data Report

TEST SUMMARY

I. Client

Client: Applied Polymer Systems, Inc.
519 Industrial Drive
Woodstock, GA 30189

Product Tested: Silt Stop 707a (MACTEC Lab ID 07-050-01)

II. Laboratory Accreditation

Laboratory: BioTox Laboratory
Mactec Engineering and Consulting, Inc.
3200 Town Point Drive N.W., Suite 100
Kennesaw, Georgia 30144

Accreditor: State of Florida, Department of Health
Bureau of Laboratories

Accreditation ID: Lab ID. E87477

Category: Non-Potable Water – Whole Effluent Toxicity

Effective: October 18 through June 30, 2007

III. Tests Conducted

Test: Fathead minnow, *Pimephales promelas*
Chronic Definitive Larval Survival and Growth Test
EPA-821-R-02-013, 2002

Test Dates: May 2 through 9, 2007

Source/Age of Organisms: Fathead minnow: ABS, ID# CS1090050207 / < 48 hours

Test Concentrations: Control, 25.25 ppm, 52.5 ppm, 105 ppm, 210 ppm, and 420 ppm

Dilution/Control Water: Moderately hard synthetic freshwater (reagent grade chemicals and Milli-Q)

Deviation From Test Protocol: None

IV. Results

Survival and Growth Data for the Fathead minnow, *Pimephales promelas*, Exposed for 7-days to Applied Polymer Systems, Inc. Silt Stop 707a Product, May 2-9, 2007.

Concentration (ppm)	Survival (%)	Growth (mg)
Laboratory Control ^a	100.0	0.591
25.25	80.0	0.378
52.5	100.0	0.495
105	97.5	0.496
210	100.0	0.532
420	0.0	0.000
IC ₂₅ (ppm) ^b	258.9	224.2
NOEC (ppm) ^c	210	210

Prepared by: *40A 5/10/07*
Checked by: *EDS 5/16/07*

^a Laboratory Control – Moderately hard synthetic freshwater.

^b Inhibition Concentration – concentration at which there was a 25% reduction in survival/growth from the Laboratory Control

^c No Observed Effect Concentration - the highest concentration tested that did not exhibit chronic toxicity

V. Summary

Test results indicated chronic toxicity to fathead minnow survival and growth at the 420 ppm concentrations. Based on the concentrations analyzed, the statistically determined IC₂₅ for survival was 258.9 ppm and the NOEC was 210 ppm. The statistically determined IC₂₅ for growth was 224.2 ppm and the NOEC was 210 ppm.

VI. Quality Assurance

Pimephales promelas, KCl Reference Toxicant, RT11CPP, 5/1/2007

Survival: 7-day NOEC = 500 mg/L KCl

7-day NOEC Range of Acceptability = 6 to 660 mg/L KCl (ACCEPTABLE)

Growth: 7-day IC₂₅ = 540 mg/L KCl

7-day IC₂₅ Range of Acceptability = 350 to 710 mg/L KCl (ACCEPTABLE)

GLOSSARY AND ABBREVIATIONS

Acute	Involving a stimulus severe enough to rapidly induce a response; in toxicity tests, a response observed in 96 hours or less typically is considered acute.
Chronic	Involving a stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. A chronic effect can be lethality, growth, reduced reproduction, etc.
Chronic Value	A numeric value representing the geometric mean of the numeric values of concentrations analyzed as the NOEC (No Observed Effect Concentration) and the LOEC (Lowest Observed Effect Concentration) by chronic toxicity testing. The chronic value is an estimate of the toxicant concentration that will be the actual no effect concentration based on the chronic effect tested.
Critical Value	Minimum numeric value for a toxicity test endpoint (i.e., survival, growth, or reproduction) below which a given test result will be statistically significantly different from the control value.
DMW	Diluted Mineral Water
EC	Effective concentration, a point estimate of the toxicant concentration that would cause an adverse response such as death, immobilization, or serious incapacitation.
Ft-c	Foot candles - a measure of <u>light</u> intensity
Graphical Method	Log concentration versus percent mortality method. Toxicity test data are plotted on 2-cycle semi-log graph paper. The logarithmic axis (y axis) is used for percent effluent concentration, and the linear axis (x axis) is used for percent mortality. The graph provides a reasonably accurate estimate of the LC ₅₀ , but does not provide a confidence interval.
IC	Inhibition Concentration, a point estimate of the toxicant concentration that would cause a given percent reduction in a biological measurement such as fecundity or growth.
LC	Lethal Concentration, identical to EC when the observed response is death.
LC ₅₀	The toxicant concentration that is lethal to 50 percent of exposed organisms at a specific time of observation.
LCL	Lower 95% Confidence Limit
LOEC	Lowest-Observed-Effect-Concentration, the lowest concentration of toxicant to which organisms are exposed that causes adverse effects.
LWC	Lab Water Control, moderately hard synthetic freshwater prepared from MILLIPORE MILLI-Q [®] water and reagent grade chemicals.

NOEC	No-Observed-Effect-Concentration, the highest concentration of toxicant to which organisms are exposed that causes no observable adverse effects.
Probit Analysis	Probit Analysis consists of a group of statistical methods used to analyze data from concentration-response experiments, and provides an estimate of the LC ₅₀ and the precision of this estimate. In Probit Analysis, the percentages of affected organisms are converted to Probits (probability units), and the effluent concentrations are converted to logarithms. The relationship between the Probits and the logarithmic values of the concentrations is approximately linear. A Probit regression line drawn through the data points is used to estimate the LC ₅₀ and its precision estimate. To use Probit Analysis, at least two partial mortalities must be obtained in the toxicity test.
RWC	Receiving Water Control
UCL	Upper 95% Confidence Limit
$\mu\text{E}/\text{m}^2/\text{s}$	Micro-ergs per square meter per second - a measure of <u>light</u> intensity

REFERENCES

- Gulley, D.D., and WEST, Inc. 1996. TOXSTAT version 3.5. Fish Physiology and Toxicology Laboratory. Department of Zoology and Physiology. University of Wyoming. Laramie, Wyoming.
- U.S. Environmental Protection Agency. 1979. Methods for Chemical Analysis of Water And Wastes. Environmental Monitoring Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. EPA 600/4-79-020.
- U.S. Environmental Protection Agency. 1999. Errata for Effluent and Receiving Water Toxicity Testing Manuals: Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms; Short-Term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms; and Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. U.S. Environmental Protection Agency, Office of Research and Development, Duluth, MN. EPA/600/R-98/182.
- U.S. Environmental Protection Agency. 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. Office of Water (4303T), U.S. Environmental Protection Agency, Washington, DC. EPA-821-R-02-013.

TABLES

**Summary of Effluent Toxicity Test Conditions for the Fathead minnow (*Pimephales promelas*)
Chronic Survival Test (EPA 821-R-02-013)**

1. Test type:	Method 1000.0, 7-day Chronic Static Renewal
2. Temperature (°C):	24.0-25.0°C
3. Light quality:	Cool White Fluorescent
4. Light intensity:	10-20 uE/m ² /s (50-100 ft-c)
5. Photoperiod:	16-hr light, 8-hr dark
6. Test chamber size:	600 mL
7. Test solution volume:	250 mL/replicate
8. Age of fathead minnow:	< 48 hours
9. No. fish per test chamber:	10
10. No. replicate chambers per concentration:	4
11. No. fish per concentration:	20
12. Feeding regime:	Fed <i>Artemia</i> twice daily
13. Aeration:	None
14. Dilution water:	Moderately hard synthetic freshwater (reagent grade chemicals and Milli-Q)
15. Effluent concentrations (ppm):	Control, 25.25, 52.5, 105, 210, and 420
16. Dilution factor:	0.5
17. Test duration:	7-days
18. Effect measured:	Survival/mortality - no movement on gentle prodding Growth - mg dry weight
19. Test acceptability:	80% or greater survival in controls
20. Sample handling:	Whole polymer samples held at room temperature.
21. Sample volume required:	2.0 L of 420 ppm / day
22. Chemical parameters:	pH, DO, total residual chlorine, total alkalinity, total hardness, conductivity, temperature

Initial Chemical Characterization of Applied Polymer Systems, Inc. Silt Stop 707a Product and Control Water Used in Chronic Toxicity Tests, May 2-9, 2007.

Parameter	707a (420 ppm)	Laboratory Control ^a
Dissolved Oxygen (mg/L)	8.11	7.82
pH	4.39	7.57
Total Alkalinity as CaCO ₃ (mg/L)	<5.00 ^d	57.5
Total Hardness as CaCO ₃ (mg/L)	246.0	58.0
Conductivity @ 25°C (µmhos/cm)	373	170
Total Residual Chlorine ^b (mg/L)	0.023	<0.020
Total Ammonia-nitrogen ^b (mg/L)	0.582 ^c	<0.020

Determined according to EPA 600/4-79-020, 1979.

Prepared by: *ADN 5/10/07*
 Checked by: *ESJ 5-11-07*

^a Laboratory Control = Moderately hard synthetic freshwater (20% Perrier and 80% Milli-Q)

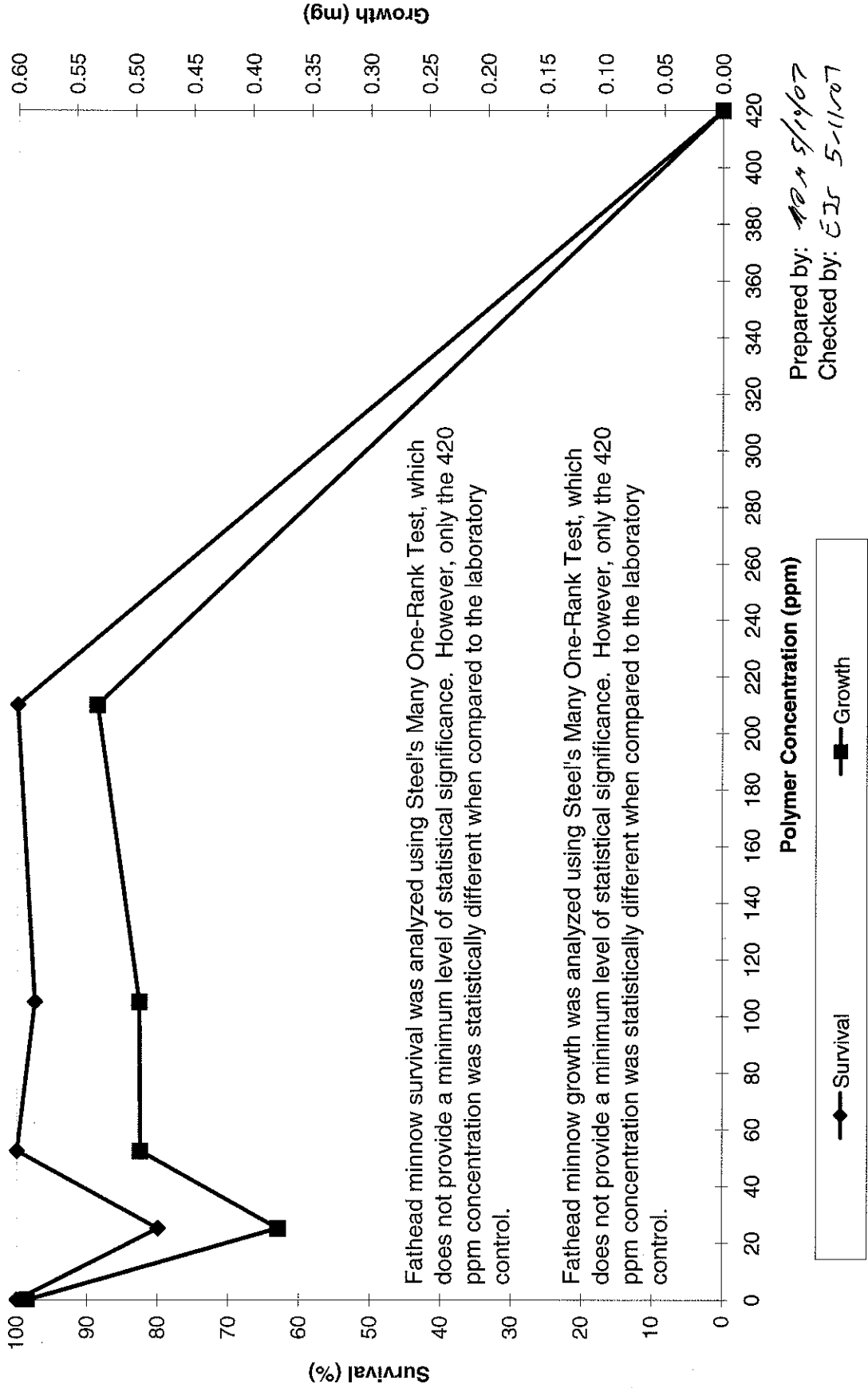
^b Determined by Hach Colorimetric Test Kit in conjunction with spectrophotometer

^c An interference was noted (cloudy white flocculant) upon addition of the Nessler's Reagent to the sample. The typical "orange" coloration that develops when ammonia is present was not noted. Therefore, the results should be interpreted with caution as a false positive result is likely.

^d Alkalinity is measured by the addition of sulfuric acid until a pH of 4.50 is reached, the initial pH of the sample was at ~4.30 results may not reflect the actual alkalinity of the solution.

FIGURES

**Figure 1. Applied Polymer Systems, Inc. Silt Stop 707a Polymer
Fathead Minnow Chronic Toxicity Test
5/2-9/07**



APPENDICES

APPENDIX A

Chemical and Physical Data

MACTEC ENGINEERING AND CONSULTING, INC. Document No. 099

BIOTOX LABORATORY

DAILY WATER QUALITY DATA FOR CHRONIC TEST

CLIENT: Applied Polymer AERATION REQUIRED: no ORGANISM ID: L71090050207
 LOCATION: Windsorlock, GA TEST CHAMBER SIZE: 600 mL D.O. METER ID: 001
 TEST TYPE: Definitive / Screening TEST SOLUTION VOLUME: 250 mL pH METER ID: 001
 NPDES NUMBER: _____ DILUTION/CONTROL ID: LW01050107 SPECTROPHOTOMETER ID: 001

DATE/ANALYST	<u>8/21/10</u>	<u>8/24/10</u>	<u>8/25/10</u>	<u>8/25/10</u>	<u>8/26/10</u>	<u>8/27/10</u>	<u>8/27/10</u>	COMMENTS
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CONTROL: <u>LWC</u>								
D.O.	INITIAL	<u>7.82</u>	<u>8.01</u>	<u>7.60</u>	<u>7.87</u>	<u>7.92</u>	<u>7.83</u>	<u>7.74</u>
	FINAL	<u>7.37</u>	<u>6.26</u>	<u>6.31</u>	<u>6.43</u>	<u>6.32</u>	<u>7.30</u>	<u>7.36</u>
pH	INITIAL	<u>7.57</u>	<u>7.73</u>	<u>7.81</u>	<u>7.83</u>	<u>7.85</u>	<u>7.91</u>	<u>7.73</u>
	FINAL	<u>7.96</u>	<u>7.43</u>	<u>7.57</u>	<u>7.62</u>	<u>7.77</u>	<u>7.59</u>	<u>7.52</u>
ALKALINITY		<u>57.5</u>						
HARDNESS		<u>58.0</u>						
*CONDUCTIVITY		<u>170</u>						
TRC mg/l		<u>0.620</u>						
NH3 mg/l		<u>0.620</u>						

CONCENTRATION: <u>26.25 ppm</u>								
D.O.	INITIAL	<u>8.12</u>	<u>8.07</u>	<u>7.44</u>	<u>7.77</u>	<u>7.84</u>	<u>7.92</u>	<u>7.75</u>
	FINAL	<u>6.77</u>	<u>6.92</u>	<u>6.82</u>	<u>6.07</u>	<u>6.11</u>	<u>7.09</u>	<u>7.59</u>
pH	INITIAL	<u>7.99</u>	<u>7.51</u>	<u>7.59</u>	<u>7.54</u>	<u>7.55</u>	<u>7.60</u>	<u>7.58</u>
	FINAL	<u>7.63</u>	<u>7.68</u>	<u>7.70</u>	<u>7.69</u>	<u>7.72</u>	<u>7.58</u>	<u>7.77</u>

CONCENTRATION: <u>52.5 ppm</u>								
D.O.	INITIAL	<u>8.19</u>	<u>8.17</u>	<u>7.58</u>	<u>7.64</u>	<u>7.63</u>	<u>7.97</u>	<u>7.68</u>
	FINAL	<u>7.07</u>	<u>5.60</u>	<u>6.22</u>	<u>6.31</u>	<u>6.18</u>	<u>6.80</u>	<u>7.60</u>
pH	INITIAL	<u>7.45</u>	<u>7.39</u>	<u>7.23</u>	<u>7.31</u>	<u>7.37</u>	<u>7.43</u>	<u>7.50</u>
	FINAL	<u>7.71</u>	<u>7.54</u>	<u>7.60</u>	<u>7.58</u>	<u>7.50</u>	<u>7.59</u>	<u>7.76</u>

CONCENTRATION: <u>105 ppm</u>								
D.O.	INITIAL	<u>8.18</u>	<u>8.21</u>	<u>7.59</u>	<u>7.87</u>	<u>7.77</u>	<u>7.84</u>	<u>7.78</u>
	FINAL	<u>6.90</u>	<u>6.63</u>	<u>6.22</u>	<u>6.86</u>	<u>6.90</u>	<u>6.45</u>	<u>7.00</u>
pH	INITIAL	<u>7.17</u>	<u>7.00</u>	<u>7.00</u>	<u>7.06</u>	<u>7.09</u>	<u>6.92</u>	<u>7.29</u>
	FINAL	<u>7.67</u>	<u>7.42</u>	<u>7.30</u>	<u>7.41</u>	<u>7.33</u>	<u>7.55</u>	<u>7.71</u>

CONCENTRATION: <u>210 ppm</u>								
D.O.	INITIAL	<u>8.11</u>	<u>8.20</u>	<u>7.46</u>	<u>7.92</u>	<u>7.68</u>	<u>7.63</u>	<u>7.81</u>
	FINAL	<u>7.38</u>	<u>5.76</u>	<u>6.41</u>	<u>6.32</u>	<u>6.46</u>	<u>5.96</u>	<u>6.49</u>
pH	INITIAL	<u>6.73</u>	<u>6.43</u>	<u>6.14</u>	<u>6.26</u>	<u>6.20</u>	<u>6.50</u>	<u>6.68</u>
	FINAL	<u>7.66</u>	<u>7.10</u>	<u>7.14</u>	<u>7.10</u>	<u>7.02</u>	<u>8.725</u>	<u>7.39</u>

CONCENTRATION: <u>420 ppm</u> <u>65558</u>								
D.O.	INITIAL	<u>8.11</u>						
	FINAL	<u>7.33</u>						
pH	INITIAL	<u>4.39</u>						
	FINAL	<u>6.61</u>						

100% Effluent								
ALKALINITY		<u>65.00</u>						
HARDNESS		<u>246.0</u>						
*CONDUCTIVITY		<u>373</u>						
TRC mg/L		<u>0.027</u>						
NH3 mg/L		<u>0.582</u>						

Lab Sample ID: 07-050-01 07-050-01 07-050-01
 Day 0-2 Day 3-4 Day 5-7

D.O. = Dissolved Oxygen
 ALKALINITY and HARDNESS are reported as mg/L as CaCO₃
 TRC mg/L Total Residual Chlorine milligrams per liter
 *Conductivity in micro siemens per centimeter (µs/cm)
 NH₃ mg/L ammonia nitrogen milligrams per liter

Reviewed by: ESJ
 Date: 5/11/07

MACTEC ENGINEERING AND CONSULTING, INC.
BIOTOX LABORATORY
TOTAL RESIDUAL CHLORINE BENCH SHEET

Client: Applied Polymer Curve Reference: CL208006 Reagent Reference: DPD Accuvac Vials: 573
 Analyst: ES Standard Reference: 591
 Instrument ID: S-001 Wavelength: 530

Sample ID	Date	Absorbance	Concentration (mg/L)	Cell Correction Factor	Standard/Blank Correction	Final Concentration (mg/L)
D.I. Blank	5/6/07	-0.001	-0.027	0	0.027	<0.020
0.498 mg/L Cl std	↓	0.2516	0.515	↓	↓	0.542
DMW		0.003	-0.017			<0.020
LWC		0.005	-0.012			<0.020
07050-01 420ppm	↓	0.010	-0.004	↓	↓	0.023
D.I. Blank	5/20/07	0.04	-0.003	0	0.003	<0.020
0.498 mg/L Cl std	↓	0.239	0.500	↓	↓	0.503
DMW 07-050-01 420ppm		0.015	0.008			<0.020

LWC

Chlorine (mg/L) = [(mg/L Curve X Cell Correction) + (Standard + Blank Correction)]

Reviewed by: 401
Date: 5/11/07

APPENDIX B

Organism Document

MACTEC Engineering and Consulting, Inc.
BioTox Laboratory

Pimephales promelas Culture Source Document

Larvae ID No.	Hatch Date	Source	Age of Larvae at Test Start	Water Type/Temp. (°C)	Food Type
CS1090050207	5/1/07	ABS, Inc.	<48 hrs	LWC/25.0	<i>Artemia</i>

APPENDIX C

Test Data Sheets

**Fathead minnow
Polymer Test**

MACTEC Engineering and Consulting, Inc. BioTox Laboratory

7-day Fathead Minnow (*P. promelas*) Larval Survival and Growth Test

Client: Applied Polymer P. promelas ID: 5109050207 Artemia ID: C51044091506
 Discharger: _____ Incubator ID: 003 Dilution Water ID: LWC01050101
 Location: _____ Shelf Number: 002 Test Type: Screening / Definitive

		Surviving Organisms (alive/dead)								
		Day								
		0	1	2	3	4	5	6	7	
*Feeding Record Init.:		<u>NDM</u>	<u>NDM</u>	<u>EJS</u>	<u>EJS</u>	<u>EJS</u>	<u>EJS</u>	<u>EJS</u>	<u>EJS</u>	
Time one/Time two:		<u>1000/1900</u>	<u>0900/1600</u>	<u>0800/1400</u>	<u>0700/1500</u>	<u>1000/1600</u>	<u>0800/1400</u>	<u>0900/1500</u>		
Effluent Concentration	Date:	<u>5/2/07</u>	<u>5/2/07</u>	<u>5/2/07</u>	<u>5/3/07</u>	<u>5/6/07</u>	<u>5/7/07</u>	<u>5/8/07</u>	<u>5/9/07</u>	
	Time:	<u>1645</u>	<u>1400</u>	<u>1100</u>	<u>1200</u>	<u>1230</u>	<u>0630</u>	<u>1130</u>	<u>1240</u>	
	Rep. #	Init: <u>NDM</u>	Init: <u>NDM</u>	Init: <u>EJS</u>	Init: <u>EJS</u>	Init: <u>EJS</u>	Init: <u>EJS</u>	Init: <u>EJS</u>	Init: <u>NDM</u>	% Survival
LWC 0.0 ppm	1	✓10	10	10	10	10	10	10	10	100
	2	✓10	10	10	10	10	10	10	10	
	3	✓10	10	10	10	10	10	10	10	
	4	✓10	10	10	10	10	10	10	10	
	Temp (°C)	<u>24.5</u>	<u>25.0</u>	<u>25.0</u>	<u>24.5</u>	<u>24.0</u>	<u>24.5</u>	<u>24.5</u>	<u>24.0</u>	
26.25 ppm	1	-10	10	10	10	7/3	7	7	7	80
	2	-10	10	10	10	10	10	10	10	
	3	✓10	10	10	9/1	9	9	9	9	
	4	✓10	10	9x10	8/2	7/1	6/1	6	6	
	Temp (°C)	<u>24.5</u>	<u>25.0</u>	<u>24.5</u>	<u>24.5</u>	<u>24.5</u>	<u>24.5</u>	<u>24.0</u>	<u>24.0</u>	
52.5 ppm	1	✓10	10	10	10	10	10	10	10	100
	2	✓10	10	10	10	10	10	10	10	
	3	✓10	10	10	10	10	10	10	10	
	4	✓10	10	10	10	10	10	10	10	
	Temp (°C)	<u>24.0</u>	<u>25.0</u>	<u>25.0</u>	<u>24.5</u>	<u>24.5</u>	<u>24.5</u>	<u>24.5</u>	<u>24.0</u>	
105 ppm	1	✓10	10	10	10	9/1	9	9	9	97.5
	2	✓10	10	10	10	10	10	10	10	
	3	✓10	10	10	10	10	10	10	10	
	4	✓10	10	10	10	10	10	10	10	
	Temp (°C)	<u>24.0</u>	<u>25.0</u>	<u>25.0</u>	<u>24.5</u>	<u>24.5</u>	<u>24.5</u>	<u>24.0</u>	<u>24.0</u>	
210 ppm	1	✓10	10	10	10	10	10	10	10	100
	2	✓10	10	10	10	10	10	10	10	
	3	✓10	10	10	10	10	10	10	10	
	4	✓10	10	10	10	10	10	10	10	
	Temp (°C)	<u>24.0</u>	<u>24.5</u>	<u>24.5</u>	<u>24.5</u>	<u>24.5</u>	<u>24.5</u>	<u>24.0</u>	<u>24.0</u>	
420 ppm	1	✓10	0/10							0.0
	2	✓10	0/10							
	3	✓10	0/10							
	4	10	0/10							
	Temp (°C)	<u>24.5</u>	<u>25.0</u>							

Thermometer ID: 004

Init. = Analysts initials

Rep. = Concentration replicate

* NDM 5/9/07

*If feeding twice daily, add 0.15 grams *Artemia* nauplei 6 hours apart
 If feeding three times daily add 0.10 grams *Artemia* 4 hours apart.

Reviewed By: EJS

Date: 5/11/07

Title: Applied Polymer P. promelas chronic survival 5/2/07
File: appps050107 Transform: ARC SINE(SQUARE ROOT(Y))

GRP	IDENTIFICATION	MEAN	SMOOTHED MEAN	CONCENTRATION
1	LWC - 0.0 ppm	1.4120	1.4120	0.0000
2	26.25 ppm	1.1346	1.3325	26.2500
3	52.5 ppm	1.4120	1.3325	52.5000
4	105 ppm	1.3713	1.3325	105.0000
5	210 ppm	1.4120	1.3325	210.0000
6	420 ppm	0.1588	0.1588	420.0000

ICp estimate with p = 25 is 258.9278

Bootstrap results using 480 iterations:

Mean = 258.9696 Standard Deviation = 3.7136
95% Confidence Interval: (251.2737 , 266.6589)
Adjusted Interval: (246.6813 , 271.2976)

Title: Applied Polymer P. promelas chronic survival 5/2/07
File: appps050107 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's Test for Normality

D = 0.1923
W = 0.7270

Critical W = 0.8840 (alpha = 0.01 , N = 24)
W = 0.9160 (alpha = 0.05 , N = 24)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Warning - The first three homogeneity tests are sensitive to non-normality and should not be performed with this data as is.

Title: Applied Polymer P. promelas chronic survival 5/2/07
File: appps050107 Transform: ARC SINE(SQUARE ROOT(Y))

Hartley's Test for Homogeneity of Variance
Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Title: Applied Polymer P. promelas chronic survival 5/2/07
File: appps050107 Transform: ARC SINE(SQUARE ROOT(Y))
Number of Groups: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	LWC - 0.0 ppm	1	1.0000	1.4120
1	LWC - 0.0 ppm	2	1.0000	1.4120
1	LWC - 0.0 ppm	3	1.0000	1.4120
1	LWC - 0.0 ppm	4	1.0000	1.4120
2	26.25 ppm	1	0.7000	0.9912
2	26.25 ppm	2	1.0000	1.4120
2	26.25 ppm	3	0.9000	1.2490
2	26.25 ppm	4	0.6000	0.8861
3	52.5 ppm	1	1.0000	1.4120
3	52.5 ppm	2	1.0000	1.4120
3	52.5 ppm	3	1.0000	1.4120
3	52.5 ppm	4	1.0000	1.4120
4	105 ppm	1	0.9000	1.2490
4	105 ppm	2	1.0000	1.4120
4	105 ppm	3	1.0000	1.4120
4	105 ppm	4	1.0000	1.4120
5	210 ppm	1	1.0000	1.4120
5	210 ppm	2	1.0000	1.4120
5	210 ppm	3	1.0000	1.4120
5	210 ppm	4	1.0000	1.4120
6	420 ppm	1	0.0000	0.1588
6	420 ppm	2	0.0000	0.1588
6	420 ppm	3	0.0000	0.1588
6	420 ppm	4	0.0000	0.1588

Read by *WJH 5/10/07*
Reviewed by *EJS 5/11/07*

Title: Applied Polymer P. promelas chronic survival 5/2/07
 File: appps050107 Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	LWC - 0.0 ppm	4	1.4120	1.4120	1.4120
2	26.25 ppm	4	0.8861	1.4120	1.1346
3	52.5 ppm	4	1.4120	1.4120	1.4120
4	105 ppm	4	1.2490	1.4120	1.3713
5	210 ppm	4	1.4120	1.4120	1.4120
6	420 ppm	4	0.1588	0.1588	0.1588

Title: Applied Polymer P. promelas chronic survival 5/2/07
 File: appps050107 Transform: ARC SINE(SQUARE ROOT(Y))

Summary Statistics on Transformed Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	LWC - 0.0 ppm	0.0000	0.0000	0.0000	0.0000
2	26.25 ppm	0.0575	0.2397	0.1199	21.1288
3	52.5 ppm	0.0000	0.0000	0.0000	0.0000
4	105 ppm	0.0066	0.0815	0.0407	5.9424
5	210 ppm	0.0000	0.0000	0.0000	0.0000
6	420 ppm	0.0000	0.0000	0.0000	0.0000

Title: Applied Polymer P. promelas chronic survival 5/2/07

File: appps050107

Transform:

ARC SINE(SQUARE ROOT(Y))

Steel's Many-One Rank Test

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Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	LWC - 0.0 ppm	1.4120				
2	26.25 ppm	1.1346	12.00	10.00	4.00	
3	52.5 ppm	1.4120	18.00	10.00	4.00	
4	105 ppm	1.3713	16.00	10.00	4.00	
5	210 ppm	1.4120	18.00	10.00	4.00	
6	420 ppm	0.1588	10.00	10.00	4.00	*

Critical values are 1 tailed (k = 5)

Review by ASD 5/10/07
Reviewed by EJS 5/11/07

MACTEC ENGINEERING AND CONSULTING, INC.
BIOTOX LABORATORY

Laboratory weight data for *P. promelas* larval survival and growth test

Client: Applied Polymer
Discharge: _____
Location: _____
Test Type: Definitive / Screening

Test Dates: 5/2-9-07
Organism ID: C57090050207
Oven ID: 001
Blance ID: 001

Drying Temp.: 55°C
Drying Time: 22.5 hours
Analyst(s): EJS, MDA

Concentration of Effluent	Replicate No.	Weight of boat (grams)	Dry weight boat and larvae (grams)	Total dry weight of larvae (grams)	Original Number of larvae [day 0]	Mean dry weight per rep original number of larvae (*mg)	Mean dry weight per conc. original number larvae (*mg)	Oven Times	
								In Date:	Out Date:
LWC	1	0.96378	0.97061					1320	1400
	2	0.95943	0.96491						
	3	0.96449	0.97102						
	4	0.96091	0.96570						
25.25ppm	1	0.95221	0.95515						
	2	0.96684	0.97165						
	3	0.95798	0.96303						
	4	0.95958	0.96189						
58.5ppm	1	0.95756	0.96311						
	2	0.95879	0.96367						
	3	0.95702	0.96178						
	4	0.95562	0.96022						
105ppm	1	0.95750	0.96176						
	2	0.95624	0.96233						
	3	0.95442	0.95924						
	4	0.95677	0.96146						
210ppm	1	0.96355	0.96919						
	2	0.95647	0.96180						
	3	0.96120	0.96639						
	4	0.96163	0.96674						
420ppm	1								
	2								
	3								
	4								

*milligrams

Reviewed by: MDA

Date: 5/10/07

MACTEC ENGINEERING & CONSULTING, INC.
BIOTOX LABORATORY
Laboratory Weight Data for *P. promelas* Larval Survival and Growth Test

Client: Appleid Polymer
 Discharge: _____
 Location: _____
 Test Type: Definitive

Test Dates: 5/2-9/07
 Organism ID: CS1090050207
 Oven ID: 1
 Balance ID: 1

Drying Temp.: 55.0 degress C
 Drying Time: 24.5 hours
 Analyst(s): EJS, RDM

Concentration of Effluent	Replicate No.	Weight of boat (grams)	Dry weight: boat and larvae (grams)	Total dry weight of larvae (grams)	Original Number of larvae [day 0]	Mean dry weight per rep original number of larvae (*mg)	Mean dry weight per conc., original number larvae (*mg)	Oven	Times
								In	Out
								Date:	Date:
								4/17/2007	4/21/2007
LWC	1	0.96378	0.97061	0.00683	10	0.683	0.591	1320	1400
	2	0.95943	0.96491	0.00548	10	0.548		1320	1400
	3	0.96449	0.97102	0.00653	10	0.653		1320	1400
	4	0.96091	0.96570	0.00479	10	0.479		1320	1400
26.25 ppm	1	0.95221	0.95515	0.00294	10	0.294	0.378	1320	1400
	2	0.96684	0.97165	0.00481	10	0.481		1320	1400
	3	0.95798	0.96303	0.00505	10	0.505		1320	1400
	4	0.95958	0.96189	0.00231	10	0.231		1320	1400
62.5 ppm	1	0.95756	0.96311	0.00555	10	0.555	0.495	1320	1400
	2	0.95879	0.96367	0.00488	10	0.488		1320	1400
	3	0.95702	0.96178	0.00476	10	0.476		1320	1400
	4	0.95562	0.96022	0.00460	10	0.460		1320	1400
105 ppm	1	0.95750	0.96176	0.00426	10	0.426	0.496	1320	1400
	2	0.95624	0.96233	0.00609	10	0.609		1320	1400
	3	0.95442	0.95924	0.00482	10	0.482		1320	1400
	4	0.95677	0.96146	0.00469	10	0.469		1320	1400
210 ppm	1	0.96355	0.96919	0.00564	10	0.564	0.532	1320	1400
	2	0.95647	0.96180	0.00533	10	0.533		1320	1400
	3	0.96120	0.96639	0.00519	10	0.519		1320	1400
	4	0.96163	0.96674	0.00511	10	0.511		1320	1400
420 ppm	1			0.00000	10	0.000	0.000	1320	1400
	2			0.00000	10	0.000		1320	1400
	3			0.00000	10	0.000		1320	1400
	4			0.00000	10	0.000		1320	1400

*milligrams

Prepared by: MD Date: 5/16/07Reviewed by: EJS Date: 5/11/07

Title: Applied Polymer P. promelas Chronic Growth 5/1/07

File: apppg050107

Transform:

NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.1076

W = 0.9574

Critical W = 0.8840 (alpha = 0.01 , N = 24)

W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Applied Polymer P. promelas Chronic Growth 5/1/07

File: apppg050107

Transform:

NO TRANSFORMATION

Number of Groups: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	LWC - 0.0 ppm	1	0.6830	0.6830
1	LWC - 0.0 ppm	2	0.5480	0.5480
1	LWC - 0.0 ppm	3	0.6530	0.6530
1	LWC - 0.0 ppm	4	0.4790	0.4790
2	26.25 ppm	1	0.2940	0.2940
2	26.25 ppm	2	0.4810	0.4810
2	26.25 ppm	3	0.5050	0.5050
2	26.25 ppm	4	0.2310	0.2310
3	62.5 ppm	1	0.5550	0.5550
3	62.5 ppm	2	0.4880	0.4880
3	62.5 ppm	3	0.4760	0.4760
3	62.5 ppm	4	0.4600	0.4600
4	105 ppm	1	0.4260	0.4260
4	105 ppm	2	0.6090	0.6090
4	105 ppm	3	0.4820	0.4820
4	105 ppm	4	0.4690	0.4690
5	210 ppm	1	0.5640	0.5640
5	210 ppm	2	0.5330	0.5330
5	210 ppm	3	0.5190	0.5190
5	210 ppm	4	0.5110	0.5110
6	420 ppm	1	0.0000	0.0000
6	420 ppm	2	0.0000	0.0000
6	420 ppm	3	0.0000	0.0000
6	420 ppm	4	0.0000	0.0000

Prepared by ADA 5/10/07

Reviewed by EJS 5/11/07

Title: Applied Polymer P. promelas Chronic Growth 5/1/07

File: apppg050107

Transform:

NO TRANSFORMATION

Summary Statistics on Data

TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	LWC - 0.0 ppm	4	0.4790	0.6830	0.5908
2	26.25 ppm	4	0.2310	0.5050	0.3778
3	62.5 ppm	4	0.4600	0.5550	0.4948
4	105 ppm	4	0.4260	0.6090	0.4965
5	210 ppm	4	0.5110	0.5640	0.5318
6	420 ppm	4	0.0000	0.0000	0.0000

Title: Applied Polymer P. promelas Chronic Growth 5/1/07

File: apppg050107

Transform:

NO TRANSFORMATION

Summary Statistics on Data

TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	LWC - 0.0 ppm	0.0089	0.0943	0.0472	15.9697
2	26.25 ppm	0.0185	0.1359	0.0679	35.9750
3	62.5 ppm	0.0017	0.0418	0.0209	8.4431
4	105 ppm	0.0062	0.0787	0.0394	15.8560
5	210 ppm	0.0005	0.0233	0.0117	4.3899
6	420 ppm	0.0000	0.0000	0.0000	N/A

Title: Applied Polymer P. promelas Chronic Growth 5/1/07

File: apppg050107

Transform:

NO TRANSFORMATION

Steel's Many-One Rank Test

-

Ho: Control < Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	LWC - 0.0 ppm	0.5908				
2	26.25 ppm	0.3778	12.00	10.00	4.00	
3	62.5 ppm	0.4948	13.00	10.00	4.00	
4	105 ppm	0.4965	13.00	10.00	4.00	
5	210 ppm	0.5318	15.00	10.00	4.00	
6	420 ppm	0.0000	10.00	10.00	4.00	*

Critical values are 1 tailed (k = 5)

Proposed by *MS 5/1/07*

Revised by *ESS 5/1/07*

Title: Applied Polymer P. promelas Chronic Growth 5/1/07
File: apppg050107 Transform: NO TRANSFORMATION

GRP	IDENTIFICATION	MEAN	SMOOTHED MEAN	CONCENTRATION
1	LWC - 0.0 ppm	0.5908	0.5908	0.0000
2	26.25 ppm	0.3778	0.4752	26.2500
3	62.5 ppm	0.4948	0.4752	62.5000
4	105 ppm	0.4965	0.4752	105.0000
5	210 ppm	0.5318	0.4752	210.0000
6	420 ppm	0.0000	0.0000	420.0000

ICp estimate with p = 25 is 224.1970

Bootstrap estimate did not exist for 99 iterations.
Bootstrap results using 381 iterations:

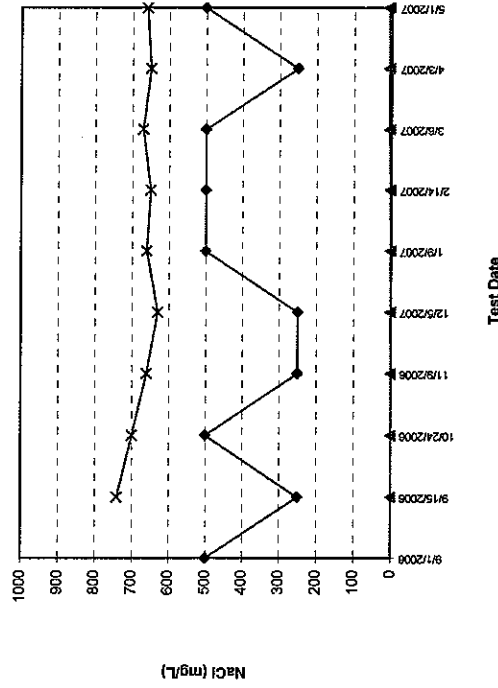
Mean = 229.3261 Standard Deviation = 11.9008
95% Confidence Interval: (210.8929 , 253.1588)
Adjusted Interval: (202.9104 , 270.5359)

**Fathead minnow
Reference Toxicant Data Sheets**

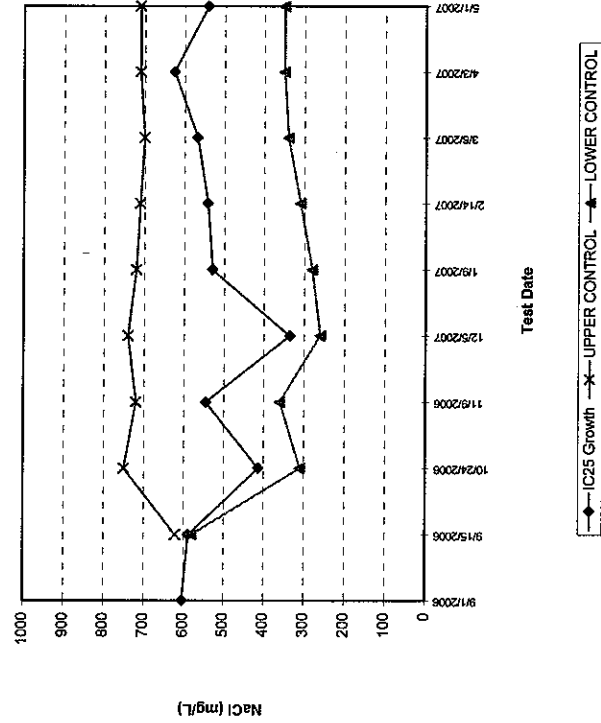
P. promelas Chronic KCl Reference Toxicant Data

TESTING NO.	INITIS	DATE	NOEC Surv.	CUM MEAN (CUM SD)	UPPER CONTROL (+2x CUM SD)	LOWER CONTROL (-2x CUM SD)	IC25 Growth (C25)	CUM MEAN (CUM SD)	CUM SD	UPPER CONTROL (+2x CUM SD)	LOWER CONTROL (-2x CUM SD)
1	EJS	9/1/2006	500	500	740	0	603	600	10	620	580
3	EJS	9/15/2006	250	380	700	0	588	600	110	750	310
4	EJS	10/24/2006	500	420	660	0	413	530	90	720	360
5	EJS	11/9/2006	250	380	630	1	545	500	120	740	260
6	EJS	12/5/2007	250	350	660	2	529	500	110	720	280
7	EJS	1/8/2007	500	380	650	3	541	510	100	710	310
8	EJS	2/14/2007	500	390	670	4	567	520	90	700	340
9	RDM	3/6/2007	500	410	650	5	625	530	90	710	350
10	EJS	4/3/2007	250	390	660	6	540	530	90	710	350
11	RDM	5/1/2007	500	400	660						

Chronic Fathead Minnow KCl Reference Toxicant Control Chart -- Survival



Chronic Fathead Minnow KCl Reference Toxicant Control Chart -- Growth



Reviewed by: EJS 5/1/07