

**REPORT FOR CHRONIC TOXICITY TESTING OF
APPLIED POLYMER SYSTEMS, INC.
SHOCK LOG PRODUCT**

WOODSTOCK, GEORGIA

TEST PERIOD: DECEMBER 7-14, 2004

Prepared for:

APPLIED POLYMER SYSTEMS, INC.
Woodstock, Georgia

January 2005

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Prepared for:

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

Prepared by:

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

January 2005

January 6, 2005

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

Subject: **Chronic Toxicity Testing of Applied Polymer Systems, Inc. Shock Log,
December 7-14, 2004
MACTEC Project No. 12000-0-2046.01**

Dear Mr. Iwinski:

MACTEC Engineering and Consulting, Inc. (MACTEC), BioTox Laboratory has completed 7-day chronic toxicity testing on samples of Shock Log. Toxicity testing was initiated by MACTEC on December 7, 2004 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 1,680 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 1 hour). This stock test sample was aerated for 15 minutes then diluted with laboratory formulated water to the following test concentrations: 6.56 parts per million (ppm), 13.13 ppm, 26.25 ppm, 52.5 ppm, 105 ppm, 210 ppm, 420 ppm, 840 ppm, and 1680 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 840 and 1680 ppm test concentrations. Based on the concentrations analyzed, the statistically determined IC₂₅ for survival was 298 ppm and the NOEC was 420 ppm. The statistically determined IC₂₅ for growth was 25 ppm and the NOEC was 420 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).

MACTEC appreciates the opportunity to provide these testing services to you and Applied Polymer Systems, and we look forward to serving your future needs. If there are any questions, please do not hesitate to contact Mr. Andrew S. Peiken at (770) 421-7057.

Sincerely,

LAW ENGINEERING AND ENVIRONMENTAL SERVICES, INC.

Andrew S. Peiken, C.E.
BioTox Laboratory Manager

Margaret E. Tanner
Environmental Engineer

Attachment: Data Report

TEST SUMMARY

I. Client

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

Product Tested: Shock Log (MACTEC Lab ID 04-0282-04)

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: July 1, 2004 through June 30, 2005

III. Tests Conducted

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

Test Dates: December 7-14, 2004

Source/Age of Organisms: ABS, ID No. CS951120704 / <48 hrs.

Test Concentrations: Control, 6.56, 13.13, 26.25, 52.5, 105, 210, 420, 840, and 1680 ppm

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

Deviation From Test Protocol: Chronic toxicity testing was conducted using nine test concentrations (as opposed to five per the test method) and three test replicates (as opposed to four per the test method). Daily water quality monitoring was performed on the laboratory control, the 13.13 ppm, the 210 ppm, and the 1680 ppm polymer test concentrations (as opposed to all test concentrations).

IV. Results

Survival and Growth Data for the Fathead minnow, *Pimephales promelas*, Exposed for 7-days to Applied Polymer Systems, Inc. Shock Log Product, December 7-14, 2004.

Concentration (ppm)	Survival (%)	Growth (mg)
Laboratory Control ^a	90	0.264
6.56	100	0.410
13.13	80	0.296
26.25	73.3	0.230
52.5	66.7	0.211
105	83	0.275
210	73.3	0.266
420	66.7	0.254
840	50	0.181
1,680	0	0.0
IC₂₅ (ppm) ^b	298	25
NOEC (ppm) ^c	420	420

Calculated by: ASP1/5/05
 Checked by: SEC 1/5/05

^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 840 and 1680 ppm test concentrations. Based on the concentrations analyzed, the statistically determined IC₂₅ for survival was 298 ppm and the NOEC was 420 ppm. The statistically determined IC₂₅ for growth was 25 ppm and the NOEC was 420 ppm.

VI. Quality Assurance

Pimephales promelas, NaCl Reference Toxicant No. 159, 11/3/04

Survival: 7-day IC₂₅ = 5,136 mg/L NaCl (**PASS**)
 7-day IC₂₅ Range of Acceptability = 1,550 to 5,830 mg/L NaCl
 Growth: 7-day IC₂₅ = 3,292 mg/L NaCl (**PASS**)
 7-day IC₂₅ Range of Acceptability = 1,240 to 4,080 mg/L NaCl

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 ^R 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.
UCL	Upper 95% Confidence Limit
μE/m ² /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. 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.

**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):	24.0-25.5°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:	400 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:	3
11. No. fish per concentration:	30
12. Feeding regime:	Fed <i>Artemia</i> twice daily
13. Aeration:	None during testing. The 1,680 ppm stock solution was gently aerated immediately after the 24-hour mixing period.
14. Dilution water:	Moderately hard synthetic freshwater (reagent grade chemicals and Milli-Q)
15. Effluent concentrations (ppm):	Control, 6.56, 13.13, 26.25, 52.5, 105, 210, 420, 840, and 1,680
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 L of 1,680 ppm / day
22. Chemical parameters:	pH, DO, total residual chlorine, total alkalinity, total hardness, conductivity, temperature

Initial Chemical Characterization of Applied Polymer Systems, Inc. Shock Log Product and Control Water Used in Chronic Toxicity Tests, December 7-14, 2004.

Parameter	Shock Log (1,680 ppm)	Laboratory Control^a
Dissolved Oxygen (mg/L)	7.28	7.24
pH	5.46	7.95
Total Alkalinity as CaCO ₃ (mg/L)	198.5	61.0
Total Hardness as CaCO ₃ (mg/L)	913	73.0
Conductivity @ 25°C (µmhos/cm)	1,100	305
Total Residual Chlorine ^b (mg/L)	<0.01	<0.01
Total Ammonia-nitrogen ^b (mg/L)	0.25	<0.01

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

Entered by: ASP 1/6/05
Checked by: SEC 1/6/05

^a Laboratory Control = Moderately hard synthetic freshwater (reagent grade chemicals and Milli-Q)

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

APPENDICES

FIGURES

APPENDIX A

Chemical and Physical Data

APPENDIX B

Fathead minnow Documents

MACTEC Engineering and Environmental Services, 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
CS951120704	12/6/04	ABS	<48 hrs	LWC/25.0	<i>Artemia</i>

APPENDIX C

Test Data Sheets

**Fathead minnow
Polymer Test**

Fathead minnow
Reference Toxicant Data Sheets