

**REPORT FOR ACUTE TOXICITY TESTING OF
APPLIED POLYMER SYSTEMS, INC.
APS#712**

WOODSTOCK, GEORGIA

TEST PERIOD: August 15-17, 2003

Prepared for:

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

Prepared by:

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

September 2003

September 4, 2003

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

Subject: **Applied Polymer Systems, Inc. Acute Toxicity Testing on APS#712 Product,
August 15-17, 2003
MACTEC Project No. 12000-0-2046.01**

Dear Mr. Iwinski:

Mactec Engineering and Consulting, Inc. (MACTEC), BioTox Laboratory has completed 48-hour acute toxicity testing on samples of APS#712 received by us August 15, 2003. Toxicity testing using the water flea, *Ceriodaphnia dubia*, and the fathead minnow, *Pimephales promelas*, was initiated by MACTEC on August 15, 2003 to determine the LC₅₀ (the Lethality Concentration of the sample at which 50% of the test organisms die) and the NOAEC (the No Observed Acute Effect Concentration, or the highest concentration tested that did not exhibit acute toxicity). Samples were prepared by adding 6,720 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 30 minutes).

Test results indicated acute mortality to the water flea in the APS#712 polymer sample at the 1,680 ppm, 3,360 ppm, and 6,720 ppm concentrations. The statistically determined LC₅₀ for the sample was 1,617 ppm and the NOAEC was 840 ppm.

Test results indicated no acute mortality to the fathead minnow in the APS#712 polymer sample at any of the test concentrations. The statistically determined LC₅₀ for the sample was >6,720 ppm and the NOAEC was 6,720 ppm.

Results are summarized in the accompanying report (41 total pages). All test results contained herein comply with the requirements of the National Environmental Laboratory Accreditation Conference (NELAC). A summary of test conditions, as well as chemical and physical data, are located in Appendix A (10 total pages). Test organism source data are located in Appendix B (3 total pages). Raw laboratory data and statistical analyses results are located in Appendix C (18 total pages).

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) 421-7027.

Sincerely,

MACTEC ENGINEERING AND CONSULTING, INC.

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

Margaret E. Tanner
Senior Environmental Engineer

Attachment: Data Report

TEST SUMMARY

I. Client

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

Product Tested: APS#712 (MACTEC ID #03-0121-01)

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: July 1, 2003 through June 30, 2004

III. Tests Conducted

Test: Water Flea, *Ceriodaphnia dubia*
48-hr. Acute Static Toxicity Test
EPA-821-R-02-012, Fifth Edition, October 2002

Fathead Minnow, *Pimephales promelas*
48-hr. Acute Static Toxicity Test
EPA-821-R-02-012, Fifth Edition, October 2002

Test Dates (Times): Water Flea: August 15 (1430) – 17 (1010), 2003
Fathead Minnow: August 15 (1400) – 17 (1000), 2003

Source, Age of Organisms: Water Flea: In-house Cultures, CERM826080103 / <24hr
Fathead Minnow: ARO, CS897080503 / 10 days

Test Concentrations: Control, 420, 840, 1,680, 3,360, and 6,720 ppm

Dilution/Control Water: Water Flea: Diluted Mineral Water (20% Perrier)
Fathead Minnow: Moderately hard synthetic freshwater (reagent grade chemicals and Milli-Q)

Deviation From Test Protocol: None

IV. Results**Survival Data for Water Flea, *Ceriodaphnia dubia*, Exposed for 48-hr to Applied Polymer Systems, Inc., APS#712 Product, August 15-17, 2003.**

Concentration (ppm)	Survival (%) at 48-hours
Laboratory Control ^a	100
420	95
840	90
1,680	65
3,360	0
6,720	0
LC₅₀^b	1,617 ppm
NOAEC^c	840 ppm

Calculated by: ASP 9/4/03

Checked by: MRF 9/4/03

^a Laboratory Control – Diluted Mineral Water^b Lethal Concentration at which 50% mortality occurs.^c No Observable Acute Effect Concentration.**Survival Data for Fathead Minnow, *Pimephales promelas*, Exposed for 48-hr to Applied Polymer Systems, Inc., APS#712 Product, August 15-17, 2003.**

Concentration (ppm)	Survival (%) at 48-hours
Laboratory Control ^a	100
420	100
840	100
1,680	100
3,360	100
6,720	100
LC₅₀^b	>6,720 ppm
NOAEC^c	6,720 ppm

Calculated by: ASP 9/4/03

Checked by: MRF 9/4/03

^a Laboratory Control – Moderately Hard Synthetic Water^b Lethal Concentration at which 50% mortality occurs.^c No Observable Acute Effect Concentration.

V. Summary

Test results indicated acute mortality to the water flea in the APS#712 polymer sample at the 1,680 ppm, 3,360 ppm, and 6,720 ppm concentrations. The statistically determined LC₅₀ for the sample was 1,617 ppm and the NOAEC was 840 ppm.

Test results indicated no acute mortality to the fathead minnow in the APS#712 polymer sample at any of the test concentrations. The statistically determined LC₅₀ for the sample was >6,720 ppm and the NOAEC was 6,720 ppm.

VI. Quality Assurance

Ceriodaphnia dubia, NaCl Reference Toxicant, 127, 6/30/03

Survival: 48-hr LC₅₀ = 2,722 mg/L NaCl (**PASS**)
48-hr LC₅₀ Range of Acceptability = 1,970 to 3,130 mg/L NaCl

Pimephales promelas, NaCl Reference Toxicant, 118, 7/2/03

Survival: 48-hr LC₅₀ = 8,000 mg/L NaCl (**PASS**)
48-hr LC₅₀ Range of Acceptability = 6,430 to 12,390 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.
RWC	Receiving Water Control
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.
- Peltier, W., and C.I. Weber. eds. 1991. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. Fourth Edition. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. EPA 600/4-90/027F.
- 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.

Summary of Effluent Toxicity Test Conditions for the Fathead Minnow (*Pimephales promelas*) Acute Survival Test (EPA-821-R-02-012)

1. Test type:	48-hr Static Acute
2. Temperature (°C):	20.0-22.0°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:	250 ml
7. Test solution volume:	200 ml/replicate
8. Age of minnows:	10 days
9. No. fish per test chamber:	10
10. No. replicate chambers per concentration:	2
11. No. fish per concentration:	20
12. Feeding regime:	Fed brine shrimp 2 hours prior to test start
13. Aeration:	None
14. Dilution water:	Moderately hard synthetic freshwater (reagent grade chemicals and Milli-Q)
15. Effluent concentrations (ppm):	Control, 420, 840, 1,680, 3,360, and 6,720
16. Dilution factor:	0.5
17. Test duration	48-hr
18. Effect measured:	Mortality - no movement on gentle prodding
19. Test acceptability:	90% or greater survival in controls
20. Sample handling:	Whole polymer samples held at room temperature.
21. Sample volume required:	1 L
22. Chemical parameters:	pH, DO, total residual chlorine, total alkalinity, total hardness, conductivity, temperature

Summary of Effluent Toxicity Test Conditions for the water flea (*Ceriodaphnia dubia*) Acute Survival Test (EPA-821-R-02-012)

1. Test type:	48-hr Static Acute
2. Temperature (°C):	20.0-21.0°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:	250 ml
7. Test solution volume:	100 ml/replicate
8. Age of neonates:	< 24 hours
9. No. neonates per test chamber:	10
10. No. replicate chambers per concentration:	2
11. No. neonates per concentration:	20
12. Feeding regime:	Fed YCT/selenastrum 2 hours prior to test start
13. Aeration:	None
14. Dilution water:	Moderately hard synthetic freshwater (reagent grade chemicals and Milli-Q)
15. Effluent concentrations (ppm):	Control, 420, 840, 1,680, 3,360, and 6,720
16. Dilution factor:	0.5
17. Test duration	48-hr
18. Effect measured:	Mortality - no movement on gentle prodding
19. Test acceptability:	90% or greater survival in controls
20. Sample handling:	Whole polymer samples held at room temperature.
21. Sample volume required:	1 L
22. Chemical parameters:	pH, DO, total residual chlorine, total alkalinity, total hardness, conductivity, temperature

Initial Chemical Characterization of Applied Polymer Systems, Inc. APS#712 Product and Control Water Used in Acute Toxicity Tests, August 15-17, 2003.

Constituent	APS#712 (6,720 ppm)	DMW ^a	LWC ^b
Dissolved Oxygen (mg/L)	7.32	7.95	7.62
pH	6.57	6.98	6.52
Total Alkalinity as CaCO ₃ (mg/L)	75	67.5	62.5
Total Hardness as CaCO ₃ (mg/L)	1150	81.0	87.0
Conductivity @ 25°C (µmhos/cm)	1220	183	292
Total Residual Chlorine ^c (mg/L)	<0.01	<0.01	<0.01
Total Ammonia-nitrogen ^c (mg/L)	7.2 ^d	<0.01	<0.01

Determined according to EPA 600/4-79-020, 1979.
ASP 9/4/03

Entered by:

Checked by: MRF 9/4/03

^a Control = Diluted Mineral Water (20% Perrier and Milli-Q)

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

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

^d A flocculant (interference) was noted upon addition of the test reagents. Results should be interpreted with caution.

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
CS897080503	8/4/03	ARO	10 days	LWC (20.0)	Brine shrimp

Mactec Engineering and Consulting, Inc.
BioTox Laboratory

Ceriodaphnia dubia Culture Source Document

Brood Board ID No.	Brood Board Start Date	Source	Age of Neonates at Test Start	Water Type/Temp. (°C)	Food Type
CERM826080103	8/1/03	In-house Cultures	< 24 hrs	DMW (20.0)	YCT/selenastrum