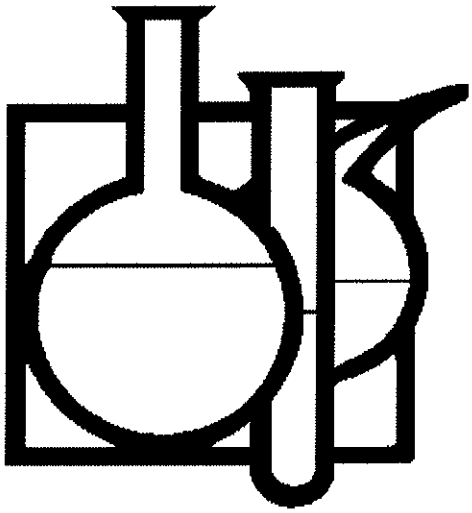

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Americo Manufacturing Company

Toxicity Report for
Fusion Brown, Island Brown and Green Filters
March 2012

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Completed: 16 April 2012

Chronic Toxicity Screening Report
Survival and Growth Effects on Fathead Minnows, *Pimephales promelas*
EPA/600/4-89/001 Method 1000.0
Americo Manufacturing Company

Fusion Brown Filter
Island Brown Filter
Green Filter

Americo Manufacturing Company
Acworth, GA
16 April 2012

Introduction

On March 13, 2012 through March 20, 2012 Guardian Systems Inc. located in Leeds, Alabama performed a 7-Day Chronic Toxicity Test for Americo Manufacturing Company located in Acworth, GA. This test was performed to aid in the determination of toxicity of Americo's following filters: Fusion Brown, Island Brown and Green, on *Pimephales promelas*, fathead minnows. The chronic toxicity test followed USEPA/600/4-89/001 Method 1000.0, "Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth Test". All deviations from this method are noted within this report. The objective of this procedure was to determine the chronic toxicity of the membrane under daily-renewal conditions. Chronic toxicity was determined and measured utilizing mortality and growth as the effect by exposing the fathead minnow to water containing strips of the filters: Fusion Brown, Island Brown and Green.

Test Organisms

Test organisms used for this test were fathead minnows, *Pimephales promelas* that were less than 24 hours old. The fathead minnow is widely distributed in North America. It is a popular bait fish, and the ease with which it is propagated has led to its widespread introduction both within and outside the native range of the species. The presumed native distribution extends from the Great Slave Lake in the northwest to New Brunswick, in Eastern Canada, southward through the Mississippi Valley in the United States, to southern Chihuahua in Mexico. The fathead minnow is found in a wide range of habitats. This species is most abundant in brooks, small streams, creeks, ponds, and small lakes. The fathead is omnivorous. They feed on algae, organic detritus, planktonic organisms, aquatic insects, worms, small crustaceans and other animals. Young organisms are often more sensitive to toxicants than adults. For this reason, the use of early life stages such as juvenile fish is recommended for testing.

Pimephales promelas larvae were obtained from Aquatox, Inc., a culture facility located in Hot Springs, Arkansas. Larvae used had hatched less than 24 hours prior to test initiation. All animals were progeny of animals originally obtained from the EPA. The minnows exhibited no abnormal behavior or disease.

Sample and Dilution Water Sources and Sampling Methods

The membrane was submitted to Guardian Systems by Kris Paniettiere of Americo. Manufacturing Co. The sample was received February 28, 2012. The filter was secured by Guardian Systems personnel until test initiation. Prior to and during the testing period the Filters were covered and protected from contamination. The filters were cut into strips for submersion in the test chambers. For each chamber, a test strip was submerged to a depth of 5 cm. The strips were suspended to ensure maximum surface area exposed, without creating danger for the minnows.

Synthetic moderately hard water was prepared according to EPA/600/4-89/001 section 7 on March 1st, March 13th and March 15, 2012. This water was used for control preparation, dilution water, and reference toxicant tests. The following chemical parameters were measured and recorded pH, Dissolved oxygen, specific conductivity, total alkalinity, hardness, and ammonia. All results are included in this report.

Test Methods for Chronic Toxicity

The test performed was a 7-Day Chronic Toxicity test using static, renewal conditions for the duration of seven (7) days. The test started at 15:30 on March 13, 2012 and ended at 14:40 on March 20, 2012. The test followed the procedures dictated by the EPA and those incorporated into Guardian Systems Inc. standard operating procedures. All deviations from the method are included in this report.

Quality Assurance

A reference toxicant test was performed to determine and ensure proper sensitivity of the test organisms. Reagent grade Sodium Chloride (NaCl) was used as the standard reference toxicant. The reference test was performed in accordance with the EPA acute and chronic method, using the same dilution/control water that was used in the EPDM toxicity test. Data and calculation sheets are provided for the reference toxicant test.

Results and Conclusions

The toxicity test was started at 15:30 on March 13, 2012. The organisms were checked for mortality daily around 1545. The control organisms and test organisms were alive for the duration of the test. At the end of the seven days the test organisms exposed to the filter exhibited 0% mortality.

On March 20, 2012 the minnows were removed from the test chambers to be dried and weighed. After drying 18 hrs at 110°c, each replicate was then weighed to obtain the average dry weight per larvae. The following results were obtained:

Control A		Fusion Brown Filter	
C1	0.405	Fusion Brown - 1	0.604
C2	0.529	Fusion Brown - 2	0.708
C3	0.512	Fusion Brown - 3	0.625
C4	0.557	Fusion Brown - 4	0.801
	Mean = 0.501		Mean = 0.685

Control A		Island Brown Filter	
C1	0.405	Island Brown - 1	0.747
C2	0.529	Island Brown - 2	0.825
C3	0.512	Island Brown - 3	0.829
C4	0.557	Island Brown - 4	0.734
	Mean = 0.501		Mean = 0.784

Control A		Green Filter	
C1	0.405	Green - 1	0.739
C2	0.529	Green - 2	0.820
C3	0.512	Green - 3	0.632
C4	0.557	Green - 4	0.575
	Mean = 0.501		Mean = 0.692

As the enclosed statistical data indicated, there is no significant difference between the growth rate of the control and the test group at the 95 % confidence level. Therefore, for this test there is no significant toxic effect on growth of the fathead minnows by Americo Manufacturing Company's filters at the approximate concentration: 0.514 m^3/L for the Fusions Brown, 0.339 m^3/L for the Island Brown and 0.595 m^3/L, in the terms of survival and growth.

SUMMARY OF EFFLUENT TOXICITY TEST CONDITIONS FOR THE FATHEAD MINNOW (*PIMEPHALES PROMELAS*) LARVAL SURVIVAL AND GROWTH TEST

1. Test Type: Daily renewal.
2. Temperature (C): 25 ± 1 C
3. Light quality: Ambient Laboratory illumination.
4. Light intensity: 10-20 uE/M2/s (50-100 ft-c) (ambient Laboratory levels).
5. Photoperiod: 16 h light, 8 h darkness.
6. Test chamber size: 800 ml
7. Test solution volume: 350 ml
8. Renewal of test concentrations: Daily.
9. Age of test organisms: Newly hatched larvae less than 24 h old.
10. No. larvae per test chamber: 10
11. No replicate chambers per concentration: 4
12. No. larvae per concentration: 40
13. Feeding regime: Fed 0.15 ml newly hatched (less than 24h old) brine shrimp nauplii twice daily,6 h between feedings (at the beginning of the work day prior to renewal, and at the end of the work day following renewal). Sufficient nauplii added to provide an excess. Not fed during the final 12 h of the test.
14. Cleaning: Siphoned daily, immediately before test solution renewal.
15. Aeration: None.

16. Dilution water: Synthetic, moderately hard water.

17. Effluent concentrations: Dilution water with 0.514 m^3/L for the Fusion Brown, 0.339 m^3/L for the Island Brown and 0.595 m^3/L for the Green Filter surface area submerged and a control.

18. Test duration: 7 days.

19. Endpoints: Survival and growth (weight).

SUMMARY OF EFFLUENT TOXICITY TEST CONDITIONS FOR FATHEAD MINNOW (PIMEPHALES PROMELAS) LARVAL SURVIVAL AND GROWTH TEST (CONT.)

20. Test acceptability: 80% or greater survival in controls; average dry weight surviving controls equals or exceeds 0.25 mg.

21. Sampling requirement: One square meter.

Exceptions and Modifications to EPA Fathead Minnow Survival and Growth Test EPA/600/4-89/001 Method 1000.0

The 7-day chronic fathead minnow survival and growth test was performed in accordance with method 1000.0 for single concentration and a control except for the following:

- 350 ml of control water was placed into each of four replicate 800 ml plastic beakers for the test series and four replicates for the control series. This gave a total solution depth of 5.5 cm.

- Strips of the filters were suspended 0.05 cm above the bottom of the test chamber. The strips were submerged to a depth of 5 cm. For a total submerged surface area of 0.514 m^3/L water for the Fusions Brown. For a total submerged surface area of 0.339 m^3/L water for the Island Brown filter. For a total submerged surface area of 0.595 m^3/L ml water for the Green filter.

- The control test chambers were treated exactly the same as the filter test chambers but with no filter strips being submerged.

- Daily renewal of the test solutions and the control solutions included fresh liner strips for the test solution.

SOP for EPA/600/4-89/001 Method 1000.0
Fathead Minnow (Pimephales promelas) Larval Survival and Growth Test

Single Concentration and Control Screening Test

- Samples for test are received and chain of custody documentation completed, aliquot of sample is poured off for routine chemical analysis of raw sample. Test sample is then filtered through a 60-um plankton net.

- A portion of the sample is diluted if necessary to the proper concentration with moderately hard water. This dilution water is also used for the control solution and reference toxicant tests. The remaining raw sample is refrigerated to 4.0^0 C for use the next day.

- The effluent test solution and control solution are temperature equilibrated to 25 ± 1^0 C. Aliquots of each solution are poured off for routine chemical analysis. pH and dissolved oxygen are measured and recorded for test solution and the control solution. D.O. range of 40% - 100% saturation is established (using single bubble aeration if necessary) prior to test initiation.

- For each test solution and control 350 ml is poured into each of four replicate 800 ml disposable plastic beakers. The beakers are labeled and randomized.

- Using an inverted 50 ml pipette fitted with a rubber bulb, fathead larvae are placed two at a time into each test chamber until each chamber contains ten organisms. Care is taken to avoid adding excess additional water with the organisms.

- The fish are fed 0.15 ml of newly hatched and rinsed brine shrimp nauplii per test chamber.

- The test chambers are placed into the environmental chamber and covered with Plexiglas sheets to prevent excess evaporation and outside contamination.

- Temperature in the environmental chamber is monitored and recorded daily for two positions inside the chamber.

- At the beginning of the next work day the larvae are fed 0.15 ml of newly hatched brine shrimp nauplii that have been rinsed with deionized water.

- Fresh test and control solutions are temperature equilibrated to 25 ± 1^0 C. Aliquots

of each solution are poured off for routine chemical analysis. pH and dissolved oxygen are measured and recorded for the test solution and the control. Greater than 40% oxygen saturation is required; if single bubble aeration is required all concentrations and control are aerated.

- After the solutions are temperature equilibrated, the test chambers are cleaned using a siphon tube fitted with a polished glass end, the flow is regulated with an adjustable clamp. Test containers are placed on a light box to better see the larvae. The remaining brine shrimp are suctioned and placed into a white bottomed container to ease the retrieval and return of inadvertently suctioned fish.

- Test and control solution renewal immediately follows chamber cleaning. The water level is lowered to a depth of 7 to 10 mm. Fresh test solution is added slowly by carefully pouring down the side of the test container to avoid excessive turbulence.

- The number of live larvae in each chamber is recorded daily and the dead larvae are discarded.

- After solution renewal the larvae are fed 0.15 ml of newly hatched brine shrimp nauplii per test chamber that have been rinsed with deionized water.

- The test chambers are placed in the environmental chamber in random order and covered with Plexiglas sheets.

- The test is terminated after seven days of exposure. At test termination, the surviving larvae in each replicate are counted and prepared as a group for dry weight determination.

- Weighing boats for each replicate are labeled and weighed.

- Immediately prior to dry weight analysis, each group of larvae is rinsed with deionized water to remove food particles, transferred to a weigh boat with known recorded weight, and dried at 110^0 C for a minimum of 6 hours.

- Immediately upon removal from the drying oven, the weighing boats are placed in a desiccator until weighed, to prevent absorption of moisture from the air. All weights are measured to the nearest 0.01 mg.

Reference:

Environmental Protection Agency 1989. "Short-Term Methods for Estimation of Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms"' EPA/600/4-89/001