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# **AQUATIC TOXICITY SCREENING OF FIRE FIGHTING AGENTS**

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**Interim Report, 2004**

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14. ABSTRACT

**This series of laboratory experiments was initiated to determine the expected median lethal concentration (LC50) of various fire fighting agents using the Fathead Minnow (*Pimephales promelas*) as the screening organism. Aquatic toxicity screening offers an inexpensive, efficient and reliable method for determining the toxic effects of a substance on a given organism. Toxicity screens were conducted for eight agents between Feb 2 and Jul 14, 2004, in which the minnows were exposed to five concentrations of the fire fighting agent while a simultaneous test was performed with five concentrations of Ansul Ansulite Aqueous Film Forming Foam (AFFF), the reference toxicant. The aquatic toxicity screening consisted of an acute, static, range-finding test conducted over a 48-hour period. Dissolved oxygen, pH and temperatures were monitored throughout the experiment. The agents screened included eight agents. Arctic Fire, FEM-12, FEM-12C and FEM-12SC are specifically formulated for Class D metal fires. PolyOx 301 is a specially designed additive to increase the throw distance of water. The remaining agents were designed to extinguish Class B hydrocarbon fires. The calculated LC50s for the eight agents screened ranged between 27 and 675 ppm. Traditional MIL SPEC AFFFs have an average LC50 greater than or equal to 700 ppm.**

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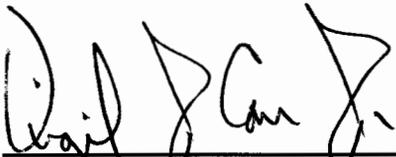
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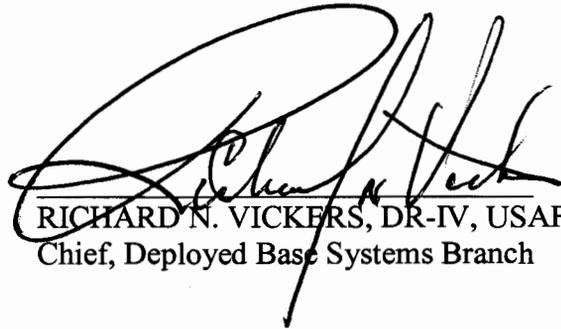
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## SUMMARY

Due to overwhelming requests submitted to the Department of Defense and Federal Aviation Administration, the Air Force Research Laboratory (AFRL/MLQD) issued an invitation to screen various fire fighting agent formulations submitted by outside companies in an effort to evaluate the toxicity of commercial and experimental foam concentrates against current Military Specification MIL-F-24385F Fire Extinguishing Agent, Aqueous Film Forming Foam (AFFF) Liquid Concentrate, For Fresh and Sea Water (MIL SPEC AFFF).

This series was conducted under a joint program with the Federal Aviation Administration as a means to centralize the screening and information made available on commercial and experimental fire fighting agents. Agents that were considered for further evaluation must show a decreased level of toxicity compared to the MIL SPEC AFFF, which has a known aquatic toxicity between 700-950 ppm.

## METHODS

Between February 2 and July 14, 2004, toxicity screening on seven fire fighting agents and one additive was conducted at the AFRL to determine the expected median lethal concentration (LC50) using the Fathead Minnow (*Pimephales promelas*) as the test organism. Eight commercial agents were screened including Arctic Fire, FEM-12, FEM-12C, FEM-12SC, FlameOut, FlameOut Foam, Hawk Super B and PolyOx 301. Arctic Fire, FEM-12, FEM-12C and FEM-12SC were specifically formulated for Class D metal fires. PolyOx 301 was a specially designed additive to increase the throw distance of water. The remaining agents were designed to extinguish Class B hydrocarbon fires.

The Fathead minnows were subjected to a 48-hour, acute, static, range-finding aquatic toxicity test (ASTM E729-96). Five concentrations of each test agent and Ansul Ansulite AFFF were tested with a total of 40 organisms exposed to each toxicant concentration. During the 48-hour experiment, dissolved oxygen concentration, pH and temperature were monitored to assure optimal water quality conditions. LC50 (median lethal concentration) results were generated using Tidepool Scientific ToxCalc™ Bioassay Calculator software. The LC50 data were calculated using the Trimmed Spearman-Kärber Method and the results are given in parts per million (ppm).

## RESULTS

Dissolved oxygen concentrations and temperature remained within acceptable limits for all nine test agents (Appendix B), however, the pH of FEM-12, FEM-12C, FEM-12SC and Arctic Fire varied from the neutral range (6-8), potentially causing a negative effect on the toxicity results. FEM-12, 12C and 12SC had lower, acidic pHs of 4.48, 2.2 and 4.67, respectively. Arctic Fire had a higher alkaline pH of 10.01. Both manufacturers were notified of the pH of their products and asked for more neutral pH formulations for retesting.

FEM-12SC showed the lowest toxicity at 675 ppm. FEM-12C could not be analyzed with the ToxCalc because after 24 hours the test organisms in all concentrations had expired and there was insufficient data to calculate a LC50. Therefore, FEM-12C had the greatest toxicity of the agents tested in 2004. The unusually high mortality was probably a direct result of the low pH of the agent. FEM-12 was moderately toxic at 201ppm.

The first round of testing with Arctic Fire showed a high toxicity of 44 ppm. Additional analysis of the foam concentrate showed that the pH (10.01) was higher than the neutral pH indicated by the manufacturer. The manufacturer sent a second unopened sample for testing, which had a more neutral pH. This significantly improved the LC50 (44 to 407 ppm), indicating that the high level of toxicity was most likely due to pH.

All three Class B agents showed high levels of toxicity. Hawk Super B (no dye) exhibited the second greatest toxicity at 27 ppm, while both versions of FlameOut had LC50s around 85 ppm.

The PolyOx additive itself had a low toxicity (672 ppm) and raised the toxicity of Ansul AFFF slightly when mixed (522 vs. 700 ppm).

## **SECTION I: INTRODUCTION**

### **BACKGROUND**

Fire fighting foams are used as the primary suppression agent to extinguish liquid hydrocarbon fuel fires involving aircraft operations. Several types of foam exist including protein, fluoroprotein and aqueous film-forming foams (AFFF). Currently, AFFF is the most effective fire fighting foam available and is the primary agent for hydrocarbon fuel fires. The general composition of AFFF is water, fluoroalkyl surfactants, non-fluorinated surfactants and organic solvent.

Due to overwhelming requests submitted to the Department of Defense (DoD) and Federal Aviation Administration (FAA), the Air Force Research Laboratory (AFRL) issued an invitation to test various fire fighting agent formulations submitted by outside companies in an effort to evaluate the toxicity of commercial and experimental foam concentrates against the current MIL SPEC AFFF.

This test series was conducted under a joint program with the FAA as a means to centralize the screening and information made available on commercial and experimental fire fighting agents. Agents that were considered for further evaluation must show a decreased level of toxicity compared to the MIL SPEC Ansul AFFF, which has a known aquatic toxicity between 700-950 ppm. This report summarizes the aquatic toxicity screening conducted during the 2004 calendar year.

### **PURPOSE**

The purpose of this screening was to quantify the aquatic toxicity of new fire fighting agents using an inexpensive, efficient, reliable method for comparison to current MIL SPEC AFFF.

### **SCOPE**

A series of screenings were conducted to determine the median lethal concentration (LC50) of seven fire fighting agent concentrates and one additive using ASTM E-729 – 96 (Reapproved 2002) Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates and Amphibians using the Fathead Minnow (*Pimephales promelas*) as the test organism. The tests and subsequent analysis were conducted at the Air Force Research Laboratory, Fire Research Group, Tyndall AFB, Florida.

## **SECTION II: METHODS**

### **PROCEDURES**

Nine independent toxicity tests were completed between February 2, 2004 and July 14, 2004 using the Fathead Minnow as the test organism. The ASTM E 729-96 Standard specifies that all organisms in a test should be as uniform as possible in age and size. To provide a consistent age class, Aquatic Research Organisms, Hampton, New Hampshire, at the beginning of each trial, provided 19-day-old laboratory-reared fish. Dissolved oxygen levels were maintained between 60 and 100% of saturation and the fish did not incur a temperature fluctuation of more than 3°C in any 12-hour period. The minnows were subjected to a 48-hour, acute, static, range-finding toxicity test. A range-finding test, according to the United States Environmental Protection Agency (USEPA), consists of scaled-down, abbreviated static acute test in which groups of organisms are exposed to several widely-spaced sample dilutions in a logarithmic series. Forty fish (twenty each, in duplicate chambers) were exposed to varying concentrations of foam.

Upon arrival, the shipping water was analyzed for dissolved oxygen (DO) concentration, pH and temperature to assure that no extreme changes in the water quality occurred during transport (Appendix A). The test water was also analyzed for DO, pH and temperature. A water filtration system installed by USFilter was used as the source of purified water, which was oxygenated for 48 hours prior to the test.

Five concentrations of the test agents were used during the trials, ranging from 220,000-22.0 ppm or 3,000-0.3 ppm, depending on the manufacturer recommendation for normal proportioning. Each concentration was one order of magnitude lower than the previous, starting with the recommended use concentration. Five concentrations (30,000- 3.0 ppm) of Ansul Ansulite AFFF were run simultaneously as a reference toxicant with each trial of an agent. Two chambers of fish with no agent added were used as a blank control. Each test chamber contained one liter of water and 20 fish, with each concentration having a duplicate test chamber. Therefore, forty fish were exposed to each concentration. Measurements, including pH, DO concentrations and temperature were taken at times 0, 24 and 48-hours and recorded on data sheets (Appendix B). The fish were not fed 24-hours prior to or during the experiment.

Eight commercial agents were screened including Arctic Fire, FEM-12, FEM-12C, FEM-12SC, FlameOut, FlameOut Foam, Hawk Super B and PolyOx 301. Arctic Fire, FEM-12, FEM-12C and FEM-12SC were specifically formulated for Class D metal fires. PolyOx 301 was a specially designed additive to increase the throw distance of water. The remaining agents were designed to extinguish Class B hydrocarbon fires. Table 1 shows the manufacturer contact information for each agent tested during 2004.

**Table 1. Manufacturer Contact Information.**

<b>AGENT</b>	<b>MANUFACTURER</b>	<b>ADDRESS</b>	<b>PHONE</b>
Arctic Fire	Safety Strategies, Inc	PO Box 8058 State College, PA 16803	1-814-234-1198
FEM-12, FEM-12C, FEM-12SC	TLI Group LTD	PO Box 774 35 Kennedy Drive Carver, MA 02330	1-508-523-6732
FlameOut, FlameOut Foam	Summit Environmental Corporation, Inc	133 E. Tyler St Longview, TX 75601	1-800-552-7841
Hawk Super B	Hawk International, Inc	PO Box 590 Gig Harbor, WA 98335	1-253-851-3444
PolyOx 301	Dow Chemical Co.	2030 Dow Center Midland, MI 48674	1-800-258-2436

## ANALYSIS

LC50 results were generated using the Tidepool Scientific ToxCalc™ Bioassay Calculator software. The data were calculated using the Trimmed Spearman-Kärber Method and the results shown in parts per million (ppm). Program output for each agent tested is shown in Appendix C.

### SECTION III: RESULTS

Dissolved oxygen concentrations and temperature remained within acceptable limits for all nine test agents (Appendix B), however, the pH of FEM-12, FEM-12C, FEM-12SC and Arctic Fire varied from the neutral range (6-8), potentially causing a negative effect on the toxicity results. FEM-12, 12C and 12SC had lower, acidic pHs of 4.48, 2.2 and 4.67, respectively. Arctic Fire had a higher alkaline pH of 10.01. Both manufacturers were notified of the pH of their products and asked for more neutral pH formulations for retesting.

Table 2 shows the toxicity results for the ten trials listed in ppm, with lower numbers indicating greater toxicity. FEM-12SC showed the lowest toxicity at 675 ppm. FEM-12SC was not completely soluble in water at the manufacturer's recommended concentration, even though the MSDS provided by the manufacturer indicated that it was completely soluble. Some portion of the highest concentration remained out of solution at the bottom of the test chamber. The lack of thorough mixing was not an issue since the concentration experienced 100% mortality within the first few minutes of screening. The four lower concentrations were completely soluble.

**Table 2. Summary of Aquatic Toxicity Results for the Eight Agents Tested.**

DATE	AGENT NAME	END USE	AGENT (ppm)
2/4/04	PolyOx 301	Additive	672
2/4/04	PolyOx 301 and AFFF	Additive	522
2/25/04	Hawk Super B (no dye)	Class B	27
4/14/04	FEM-12	Class D	201
4/14/04	FEM-12C	Class D	NA
6/8/04	FlameOut	Class B	87
6/8/04	FlameOut Foam	Class B	84
6/23/04	Arctic Fire <sup>1</sup>	Class D	44
6/23/04	FEM-12SC	Class D	675
7/14/04	Arctic Fire <sup>2</sup>	Class D	407

1. Arctic Fire with pH=10.5

2. Arctic Fire with pH=8.5

FEM-12C could not be analyzed with the ToxCalc because after 24 hours the test organisms in all concentrations had expired and there was insufficient data to calculate a LC50. Therefore, FEM-12C had the greatest toxicity of the agents tested in 2004. The unusually high mortality was probably a direct result of the low pH of the agent. FEM-12 was moderately toxic at 201 ppm.

The first toxicity screen performed with Arctic Fire had an unusual reaction with the fish. After the fish died, the remains turned to a gel-like consistency. This result was observed with another agent from a previous year and the source was an excessively high pH. Additional analysis of the foam concentrate showed that the pH (10.01) was higher than the neutral pH indicated by the manufacturer. The manufacturer then explained that the

foam container was opened a few years ago and the agent had been sitting for an extended period of time, which led to the rise in pH. The manufacturer sent a second unopened sample for testing, which had a more neutral pH. This significantly improved the LC50 (44 to 407 ppm), indicating that the high level of toxicity was most likely due to pH.

All three Class B agents showed high levels of toxicity. Hawk Super B (no dye) exhibited the second greatest toxicity at 27 ppm, while both versions of FlameOut had LC50s around 85 ppm.

The PolyOx additive itself had a low toxicity (672 ppm) and raised the toxicity of Ansul AFFF slightly when mixed (522 vs. 700 ppm).

**APPENDIX A: DATA SHEETS – QUALITY CONTROL  
MEASUREMENTS**

**AQUATIC TOXICITY TEST – POLYOX 301**

DATE: 2 February 04

TEST TYPE: Acute, static, range-finding

TEST AGENT: PolyOx 301

TEST AGENT CONC: 0.3%, 3.0x10<sup>-2</sup>%, 3.0x10<sup>-3</sup>%, 3.0x10<sup>-4</sup>%, 3.0x10<sup>-5</sup>%

TEST SPECIES: *Pimephales promelas*, Fathead Minnow

SPECIES SOURCE: Aquatic Research Organisms

DATE HATCHED: 15 January 04

TEMPERATURE OF SHIPPING WATER: 24°C

TEMPERATURE OF CHAMBER WATER: 22°C

DILUTION H<sub>2</sub>O: Deionized Water, aerated 48 hours

**AQUATIC TOXICITY TEST – POLYOX 301**

DATE: 2 February 04

TEST TYPE: Acute, static, range-finding

TEST AGENT: PolyOx 301 and Ansul AFFF

TEST AGENT CONC: 3.3%, 0.33%,  $3.3 \times 10^{-2}\%$ ,  $3.3 \times 10^{-3}\%$ ,  $3.3 \times 10^{-4}\%$

TEST SPECIES: *Pimephales promelas*, Fathead Minnow

SPECIES SOURCE: Aquatic Research Organisms

DATE HATCHED: 15 January 04

TEMPERATURE OF SHIPPING WATER: 24°C

TEMPERATURE OF CHAMBER WATER: 22°C

DILUTION H<sub>2</sub>O: Deionized Water, aerated 48 hours

**AQUATIC TOXICITY TEST – HAWK SUPER B (NO DYE)**

DATE: 25 February 04

TEST TYPE: Acute, static, range-finding

TEST AGENT: Hawk Super B (No Dye)

TEST AGENT CONC: 3.0%, 0.30%,  $3.0 \times 10^{-2}\%$ ,  $3.0 \times 10^{-3}\%$ ,  $3.0 \times 10^{-4}\%$

TEST SPECIES: *Pimephales promelas*, Fathead Minnow

SPECIES SOURCE: Aquatic Research Organisms

DATE HATCHED: 2 February 04

TEMPERATURE OF SHIPPING WATER: 22°C

TEMPERATURE OF CHAMBER WATER: 22°C

DILUTION H<sub>2</sub>O: Deionized Water, aerated 48 hours

**AQUATIC TOXICITY TEST – FEM-12**

DATE: 14 April 04

TEST TYPE: Acute, static, range-finding

TEST AGENT: FEM-12

TEST AGENT CONC: 22%, 2.2%, 0.22%,  $2.2 \times 10^{-2}\%$ ,  $2.2 \times 10^{-3}\%$ ,

TEST SPECIES: *Pimephales promelas*, Fathead Minnow

SPECIES SOURCE: Aquatic Research Organisms

DATE HATCHED: 25 March 04

TEMPERATURE OF SHIPPING WATER: 24°C

TEMPERATURE OF CHAMBER WATER: 22°C

DILUTION H<sub>2</sub>O: Deionized Water, aerated 48 hours

**AQUATIC TOXICITY TEST – FEM-12C**

DATE: 14 April 04

TEST TYPE: Acute, static, range-finding

TEST AGENT: FEM-12C

TEST AGENT CONC: 3.3%, 0.33%,  $3.3 \times 10^{-2}\%$ ,  $3.3 \times 10^{-3}\%$ ,  $3.3 \times 10^{-4}\%$

TEST SPECIES: *Pimephales promelas*, Fathead Minnow

SPECIES SOURCE: Aquatic Research Organisms

DATE HATCHED: 25 March 04

TEMPERATURE OF SHIPPING WATER: 24°C

TEMPERATURE OF CHAMBER WATER: 22°C

DILUTION H<sub>2</sub>O: Deionized Water, aerated 48 hours

**AQUATIC TOXICITY TEST – FLAMEOUT**

DATE: 8 June 04

TEST TYPE: Acute, static, range-finding

TEST AGENT: FlameOut

TEST AGENT CONC: 3.0%, 0.30%, 3.0x10<sup>-2</sup>%, 3.0x10<sup>-3</sup>%, 3.0x10<sup>-4</sup>%

TEST SPECIES: *Pimephales promelas*, Fathead Minnow

SPECIES SOURCE: Aquatic Research Organisms

DATE HATCHED: 20 May 04

TEMPERATURE OF SHIPPING WATER: 24°C

TEMPERATURE OF CHAMBER WATER: 22°C

DILUTION H<sub>2</sub>O: Deionized Water, aerated 48 hours

**AQUATIC TOXICITY TEST – FLAMEOUT FOAM**

DATE: 8 June 04

TEST TYPE: Acute, static, range-finding

TEST AGENT: FlameOut Foam

TEST AGENT CONC: 3.0%, 0.30%, 3.0x10<sup>-2</sup>%, 3.0x10<sup>-3</sup>%, 3.0x10<sup>-4</sup>%

TEST SPECIES: *Pimephales promelas*, Fathead Minnow

SPECIES SOURCE: Aquatic Research Organisms

DATE HATCHED: 20 May 04

TEMPERATURE OF SHIPPING WATER: 24°C

TEMPERATURE OF CHAMBER WATER: 22°C

DILUTION H<sub>2</sub>O: Deionized Water, aerated 48 hours

**AQUATIC TOXICITY TEST – ARCTIC FIRE**

DATE: 23 June 04

TEST TYPE: Acute, static, range-finding

TEST AGENT: Arctic Fire

TEST AGENT CONC: 15.0%, 1.5%, 0.15%,  $1.5 \times 10^{-2}\%$ ,  $1.5 \times 10^{-3}\%$

TEST SPECIES: *Pimephales promelas*, Fathead Minnow

SPECIES SOURCE: Aquatic Research Organisms

DATE HATCHED: 2 June 04

TEMPERATURE OF SHIPPING WATER: 22°C

TEMPERATURE OF CHAMBER WATER: 22°C

DILUTION H<sub>2</sub>O: Deionized Water, aerated 48 hours

**AQUATIC TOXICITY TEST – FEM-12SC**

DATE: 23 June 04

TEST TYPE: Acute, static, range-finding

TEST AGENT: FEM-12SC

TEST AGENT CONC: 22%, 2.2%, 0.22%,  $2.2 \times 10^{-2}\%$ ,  $2.2 \times 10^{-3}\%$

TEST SPECIES: *Pimephales promelas*, Fathead Minnow

SPECIES SOURCE: Aquatic Research Organisms

DATE HATCHED: 2 June 04

TEMPERATURE OF SHIPPING WATER: 22°C

TEMPERATURE OF CHAMBER WATER: 22°C

DILUTION H<sub>2</sub>O: Deionized Water, aerated 48 hours

**AQUATIC TOXICITY TEST – ARCTIC FIRE**

DATE: 14 July 04

TEST TYPE: Acute, static, range-finding

TEST AGENT: Arctic Fire

TEST AGENT CONC: 15.0%, 1.50%, 0.15%, 1.5x10<sup>-2</sup>%, 1.5x10<sup>-3</sup>%

TEST SPECIES: *Pimephales promelas*, Fathead Minnow

SPECIES SOURCE: Aquatic Research Organisms

DATE HATCHED: 24 June 04

TEMPERATURE OF SHIPPING WATER: 24°C

TEMPERATURE OF CHAMBER WATER: 22°C

DILUTION H<sub>2</sub>O: Deionized Water, aerated 48 hours

**APPENDIX B: DATA SHEETS – EXPERIMENTAL  
MEASUREMENTS**

Hawk Super B (No Dye)

TEST AGENT: 25 February 2004

	t = 0				t = 24				t = 48			
	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp
Control 1	0/20	7.7	8.34	21.4	0/20	7.9	7.9	19.3	0/20	7.6	8	18.9
Control 2	0/20	7.6	8.52	21.3	0/20	7.8	7.81	19.2	1/20	7.5	7.92	18.8
Test Agent												
3 ppm	0/20	7.5	8.3	21.4	0/20	7.7	7.83	19.2	0/20	7.6	7.82	18.8
Duplicate	0/20	7.5	8.39	21.3	0/20	7.5	7.86	19.2	0/20	7.4	7.9	18.7
30 ppm	0/20	7.5	8.38	21.3	1/20	7.3	7.49	19.2	2/20	7.1	7.71	18.8
Duplicate	0/20	7.5	8.38	21.3	20/20	6.9	7.37	19.1				
300 ppm	20/20	7.3	8.28	21.4								
Duplicate	20/20	7.2	8.36	21.3								
3,000 ppm	20/20	6.6	8.31	21.4								
Duplicate	20/20	6.5	8.31	21.3								
30,000 ppm	20/20	6.9	8.15	21.3								
Duplicate	20/20	7.2	8.21	21.3								
AFFF												
3 ppm	0/20	6.1	8.42	21.3	2/20	6.2	7.99	19.2	3/20	6.3	7.95	18.8
Duplicate	0/20	6	8.27	21.3	3/20	6.1	8.16	19.2	4/20	6.1	7.82	18.7
30 ppm	0/20	6	8.37	21.3	1/20	6	8.17	19.2	8/20	5.8	7.99	18.8
Duplicate	0/20	6	8.31	21.3	5/20	6	8.02	19.2	10/20	5.9	7.79	18.7
300 ppm	0/20	6	8.39	21.3	1/20	5.9	7.92	19.2	5/20	5.5	7.61	18.8
Duplicate	0/20	6	8.43	21.3	1/20	6.4	7.73	19.2	1/20	6.1	6.86	18.7
3,000 ppm	0/20	5.9	8.53	21.3	20/20							
Duplicate	2/20	5.9	8.48	21.3	20/20							
30,000 ppm	20/20	7.3	8.4	21.4								
Duplicate	20/20	7.4	8.45	21.4								

PolyOx

TEST AGENT: 2 February 2004

	t = 0				t = 16				t = 48			
	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp
Control 1	0/20	7.6	8.75	21.7	0/20	7.2	7.98	21.3	0/20	7.1	7.53	22
Control 2	0/20	7	8.75	21.6	0/20	6.8	8.1	21.2	0/20	7.1	7.37	22
Test Agent												
.3 ppm	0/20	7.1	8.49	21.8	0/20	7.1	7.96	21.3	0/20	6.9	7.41	22
Duplicate	0/20	6.9	8.41	21.7	0/20	6.9	7.97	21.2	0/20	6.9	7.41	22
3 ppm	0/20	7.1	8.25	21.8	0/20	7	7.79	21.2	0/20	7.1	7.32	22
Duplicate	0/20	7.1	8.19	21.8	0/20	7.1	7.7	21.2	0/20	7.2	7.37	21.9
30 ppm	0/20	7.2	7.91	21.9	0/20	7.1	7.47	21.3	0/20	7.3	7.21	22.1
Duplicate	1/20	7.4	7.89	21.8	1/20	7.2	7.49	21.2	1/20	7.4	7.04	21.9
300 ppm	0/20	7.6	7.44	21.9	0/20	7.3	7.94	21.3	1/20	7.3	6.56	22
Duplicate	4/20	7.8	7.06	21.9	4/20	7.3	7.31	21.2	4/20	7.3	7.55	22
3,000 ppm	0/20	9.5	7.2	22.2	19/20	9.1	6.66	21.3	20/20	8.3	6.4	22
Duplicate	0/20	9.7	7.2	22.4	20/20	9.1	6.89	21.3	20/20	8.3	6.4	22
AFFF												
3 ppm												
Duplicate												
30 ppm												
Duplicate												
300 ppm												
Duplicate												
3,000 ppm												
Duplicate												
30,000 ppm												
Duplicate												

FEM-12

TEST AGENT: 14 April 2004

	t = 0				t = 24				t = 48			
	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp
Control 1	0/20	6.57	9.72	17	0/20	6.67	8.06	19.4	1/20	6.66	7.98	18.8
Control 2	0/20	6.7	9.52	17	1/20	6.78	8.23	19.5	1/20	6.81	8.08	18.7
Test Agent												
22 ppm	0/20	6.81	9.39	16.9	0/20	6.78	7	19.4	0/20	6.8	7.13	18.6
Duplicate	0/20	6.74	9.3	16.8	0/20	6.76	6.67	19.4	0/20	6.83	6.96	18.6
220 ppm	0/20	6.81	9.35	16.7	4/20	6.63	5.5	19.4	13/20	6.63	2.56	18.6
Duplicate	0/20	6.73	9.31	16.7	5/20	6.65	5.25	19.4	9/20	6.66	2.61	18.6
2,200 ppm	19/20	6.46	9.39	16.8	20/20							
Duplicate	20/20	6.46	9.33	16.7								
22,000 ppm	20/20	4.7	9.53	16.7								
Duplicate	20/20	4.75	9.56	16.6								
220,000 ppm	20/20	4.48	9.6	16.7								
Duplicate												
AFFF												
3 ppm	0/20	7	9.22	16.8	0/20	6.86	7.4	19.4	0/20	6.83	7.4	18.5
Duplicate	0/20	6.9	9.17	16.7	0/20	6.77	7.43	19.4	1/20	6.67	7.43	18.5
30 ppm	0/20	6.9	9.04	16.7	0/20	6.48	7.34	19.3	0/20	6.59	7.34	18.5
Duplicate	0/20	7	9.12	16.6	1/20	6.53	7.32	19.3	1/20	6.47	7.32	18.4
300 ppm	0/20	6.2	8.95	16.7	0/20	6.14	7.14	19.5	11/20	5.5	7.14	18.6
Duplicate	0/20	6.4	8.92	16.6	1/20	6.01	7.2	19.4	11/20	5.2	7.2	18.6
3,000 ppm	4/20	6.3	8.75	16.7	20/20							
Duplicate	2/20	6.2	8.75	16.7	20/20							
30,000 ppm	20/20	6.9	8.86	16.9								
Duplicate	20/20	6.9	8.87	16.9								

FEM-12C

TEST AGENT: 14 April 2004

	t = 0				t = 24				t = 48			
	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp
Control 1	0/20	6.57	9.72	17	0/20	6.67	8.06	19.4	1/20	6.66	7.98	18.8
Control 2	0/20	6.7	9.52	17	1/20	6.78	8.23	19.5	1/20	6.81	8.08	18.7
Test Agent												
22 ppm	0/20	4.3	9.02	16.8	20/20		8.04	20.2				
Duplicate	3/20	4.4	9.12	16.7	20/20		8.56	19.9				
220 ppm	20/20	3.7	9.32	16.7								
Duplicate	20/20	3.5	9.28	16.6								
2,200 ppm	20/20	2.8	9.26	16.7								
Duplicate	20/20	2.8	9.25	16.6								
22,000 ppm	20/20	2.2	9.41	16.6								
Duplicate												
220,000 ppm												
Duplicate												
AFFF												
3 ppm	0/20	7	9.22	16.8	0/20	6.86	7.4	19.4	0/20	6.83	7.4	18.5
Duplicate	0/20	6.9	9.17	16.7	0/20	6.77	7.43	19.4	1/20	6.67	7.43	18.5
30 ppm	0/20	6.9	9.04	16.7	0/20	6.48	7.34	19.3	0/20	6.59	7.34	18.5
Duplicate	0/20	7	9.12	16.6	1/20	6.53	7.32	19.3	1/20	6.47	7.32	18.4
300 ppm	0/20	6.2	8.95	16.7	0/20	6.14	7.14	19.5	11/20	5.5	7.14	18.6
Duplicate	0/20	6.4	8.92	16.6	1/20	6.01	7.2	19.4	11/20	5.2	7.2	18.6
3,000 ppm	4/20	6.3	8.75	16.7	20/20							
Duplicate	2/20	6.2	8.75	16.7	20/20							
30,000 ppm	20/20	6.9	8.86	16.9								
Duplicate	20/20	6.9	8.87	16.9								

FlameOut

TEST AGENT: 8 June 2004

	t = 0				t = 24				t = 48			
	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp
Control 1	0/20	7.19	9.37	23.2	0/20	7.08	7.56	23.7	0/20	6.62	5.77	23.2
Control 2	0/20	7.18	9.31	23.2	0/20	7.09	7.6	23.6	1/20	6.63	5.96	23.2
Test Agent												
3 ppm	0/20	7.05	9.9	22.9	0/20	6.63	7.64	23.2	0/20	6.84	5.84	22.9
Duplicate	0/20	7.01	9.78	22.8	0/20	6.71	8	23.3	0/20	6.95	6.25	22.9
30 ppm	0/20	6.96	10.01	22.8	0/20	6.74	7.68	23.3	1/20	6.88	5.66	22.8
Duplicate	0/20	7.13	8.87	22.9	0/20	6.83	7.78	23.4	1/20	6.55	5.35	22.9
300 ppm	20/20	6.84	9.61	22.8								
Duplicate	20/20	6.93	9.4	22.9								
3,000 ppm	20/20	7.76	9.47	22.8								
Duplicate	20/20	7.55	9.65	22.8								
30,000 ppm	20/20	7.75	10.14	22.9								
Duplicate	20/20	7.88	9.37	22.8								
AFFF												
3 ppm	0/20	7.12	9.39	23.1	0/20	7	7.43	23.4	0/20	6.62	5.85	23.1
Duplicate	0/20	7	9.44	23	0/20	6.9	7.67	23.3	0/20	6.65	6.01	23
30 ppm	0/20	7.07	9.44	23	0/20	6.97	7.59	23.3	0/20	6.61	5.67	23
Duplicate	0/20	7.02	9.06	22.9	0/20	6.9	7.27	23.2	1/20	6.56	5.29	22.9
300 ppm	0/20	6.9	9.67	22.9	0/20	6.77	7.59	23.2	0/20	6.52	5.21	23
Duplicate	0/20	7.07	8.88	23	0/20	6.76	7.67	23.2	1/20	6.42	5.79	23
3,000 ppm	20/20	6.69	8.91	22.9								
Duplicate	18/20	6.67	9.17	22.9	20/20	6.44	4.02	23.2				
30,000 ppm	20/20	6.76	9.61	23								
Duplicate	20/20	6.87	9.28	23.1								

FlameOut Foam

TEST AGENT: 8 June 2004

	t = 0				t = 24				t = 48			
	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp
Control 1	0/20	7.19	9.37	23.2	0/20	7.08	7.56	23.7	0/20	6.62	5.77	23.2
Control 2	0/20	7.18	9.31	23.2	0/20	7.09	7.6	23.6	1/20	6.63	5.96	23.2
Test Agent												
3 ppm	0/20	7.02	9.12	22.9	0/20	6.61	6.64	23.3	1/20	6.44	5.18	22.9
Duplicate	0/20	6.89	9.61	22.9	0/20	6.62	6.24	23.3	0/20	6.63	5.97	22.9
30 ppm	0/20	7	9.64	22.9	0/20	6.71	5.9	23.2	0/20	6.69	5.49	22.9
Duplicate	0/20	6.94	9.73	22.9	2/20	6.66	5.44	23.2	3/20	6.67	5.38	22.8
300 ppm	11/20	6.94	9.76	22.9	20/20	6.58	2.65	23.2				
Duplicate	16/20	6.94	9.79	22.9	20/20	6.55	3.93	23.2				
3,000 ppm	20/20	7.14	9.67	22.9								
Duplicate	20/20	7.04	10.53	22.9								
30,000 ppm	20/20	7.56	11.09	23								
Duplicate	20/20	7.66	9.99	23								
AFFF												
3 ppm	0/20	7.12	9.39	23.1	0/20	7	7.43	23.4	0/20	6.62	5.85	23.1
Duplicate	0/20	7	9.44	23	0/20	6.9	7.67	23.3	0/20	6.65	6.01	23
30 ppm	0/20	7.07	9.44	23	0/20	6.97	7.59	23.3	0/20	6.61	5.67	23
Duplicate	0/20	7.02	9.06	22.9	0/20	6.9	7.27	23.2	1/20	6.56	5.29	22.9
300 ppm	0/20	6.9	9.67	22.9	0/20	6.77	7.59	23.2	0/20	6.52	5.21	23
Duplicate	0/20	7.07	8.88	23	0/20	6.76	7.67	23.2	1/20	6.42	5.79	23
3,000 ppm	20/20	6.69	8.91	22.9								
Duplicate	18/20	6.67	9.17	22.9	20/20	6.44	4.02	23.2				
30,000 ppm	20/20	6.76	9.61	23								
Duplicate	20/20	6.87	9.28	23.1								

## Arctic Fire

TEST AGENT: 23 June 2004

	t = 0				t = 24				t = 48			
	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp
Control 1	0/20	6.48	8.13	23.4	0/20	6.88	5.18	22.6	3/20	6.76	4.39	23.2
Control 2	0/20	6.81	8.11	23.3	0/20	6.71	5.01	22.5	0/20	6.66	4.48	23.1
Test Agent												
15 ppm	0/20	7.21	7.96	23	0/20	6.95	5.21	22.4	2/20	6.92	3.26	22.9
Duplicate	0/20	7.29	7.96	23	0/20	6.93	5.09	22.3	3/20	6.96	3.19	22.9
150 ppm	1/20	7.38	7.94	23	20/20	6.87	3.53	22.4				
Duplicate	2/20	7.33	7.94	22.9	20/20	6.83	3.71	22.2				
1,500 ppm	20/20	8.48	8.15	23								
Duplicate	20/20	8.38	8.06	22.9								
15,000 ppm	20/20	9.64	8.14	22.9								
Duplicate	20/20	9.58	8.12	22.8								
150,000 ppm	20/20	10.01	7.99	22.8								
Duplicate	20/20	9.97	8.02	22.7								
AFFF												
3 ppm	0/20	6.73	7.94	23.3	0/20	7.01	4.81	22.5	2/20	6.94	4.3	23.1
Duplicate	0/20	6.89	7.95	23.2	0/20	6.92	4.65	22.4	0/20	6.82	4.06	23
30 ppm	0/20	6.97	8.05	23.3	0/20	6.83	4.76	22.7	1/20	6.68	3.91	23.2
Duplicate	0/20	6.92	8.04	23.2	0/20	6.95	4.81	22.5	0/20	6.75	4.09	23.1
300 ppm	0/20	6.93	7.98	23.3	0/20	6.9	4.52	22.7	0/20	6.78	3.72	23.3
Duplicate	0/20	6.97	7.99	23.2	1/20	6.89	4.53	22.6	1/20	6.77	3.77	23.2
3,000 ppm	16/20	6.91	8.13	23.3	20/20	6.61	3.84	22.8				
Duplicate	20/20	6.92	8.07	23.2	20/20	6.63	3.76	22.7				
30,000 ppm	20/20	6.95	8.1	23.4								
Duplicate	20/20	7.01	8.08	23.4								

## FEM-12SC

TEST AGENT: 23 June 2004

	t = 0				t = 24				t = 48			
	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp
Control 1	0/20	6.48	8.13	23.4	0/20	6.88	5.18	22.6	3/20	6.76	4.39	23.2
Control 2	0/20	6.81	8.11	23.3	0/20	6.71	5.01	22.5	0/20	6.66	4.48	23.1
Test Agent												
22 ppm	0/20	7.17	8.22	23.2	0/20	7.05	5.25	22.3	0/20	7.05	2.76	22.8
Duplicate	0/20	7.21	8.21	23.2	0/20	7.07	5.66	22.3	2/20	6.99	2.78	22.8
220 ppm	0/20	6.4	8.11	23.3	1/20	6.8	3.69	22.3	2/20	7.11	2.22	22.9
Duplicate	0/20	6.48	8.14	23.2	1/20	6.87	4.01	22.3	1/20	7.13	2.23	22.8
2,200 ppm	0/20	5.41	8.15	23.2	20/20	5.83	0.3	22.7				
Duplicate	0/20	5.21	8.11	23.1	20/20	5.58	0.29	22.7				
22,000 ppm	20/20	5.15	8.53	23.1								
Duplicate	20/20	5.13	8.44	23.1								
220,000 ppm	20/20	4.71	10.5	22.8								
Duplicate	20/20	4.67	11.03	22.7								
AFFF												
3 ppm	0/20	6.73	7.94	23.3	0/20	7.01	4.81	22.5	2/20	6.94	4.3	23.1
Duplicate	0/20	6.89	7.95	23.2	0/20	6.92	4.65	22.4	0/20	6.82	4.06	23
30 ppm	0/20	6.97	8.05	23.3	0/20	6.83	4.76	22.7	1/20	6.68	3.91	23.2
Duplicate	0/20	6.92	8.04	23.2	0/20	6.95	4.81	22.5	0/20	6.75	4.09	23.1
300 ppm	0/20	6.93	7.98	23.3	0/20	6.9	4.52	22.7	0/20	6.78	3.72	23.3
Duplicate	0/20	6.97	7.99	23.2	1/20	6.89	4.53	22.6	1/20	6.77	3.77	23.2
3,000 ppm	16/20	6.91	8.13	23.3	20/20	6.61	3.84	22.8				
Duplicate	20/20	6.92	8.07	23.2	20/20	6.63	3.76	22.7				
30,000 ppm	20/20	6.95	8.1	23.4								
Duplicate	20/20	7.01	8.08	23.4								

## Arctic Fire

TEST AGENT: 14 July 2004

	t = 0				t = 24				t = 48			
	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp	Mortality	pH	DO	Temp
Control 1	0/20	7.35	7.73	22.6	0/20	7.05	2.23	23.4	3/20	7.05	1.56	22.7
Control 2	0/20	7.32	7.80	22.6	0/20	7.08	2.22	23.4	0/20	7.08	1.55	22.7
Test Agent												
3 ppm	0/20	7.05	7.75	22.4	1/20	6.86	2.22	23.3	2/20	6.86	1.42	22.8
Duplicate	0/20	7.02	7.74	22.3	0/20	6.60	2.17	23.2	2/20	6.60	1.38	22.6
30 ppm	0/20	6.92	7.73	22.4	3/20	6.71	2.19	23.2	3/20	6.71	1.41	22.6
Duplicate	0/20	7.00	7.70	22.3	2/20	6.76	2.17	23.1	2/20	6.76	1.39	22.5
300 ppm	20/20	6.98	7.79	22.3								
Duplicate	20/20	7.00	7.85	22.3								
3,000 ppm	20/20	7.39	7.84	22.3								
Duplicate	20/20	7.34	7.83	22.1								
30,000 ppm	20/20	8.31	8.04	22.1								
Duplicate	20/20	8.32	8.04	22.1								
AFFF												
3 ppm	0/20	7.38	7.69	22.5	1/20	7.12	2.24	23.2	1/20	7.13	1.52	22.6
Duplicate	0/20	7.36	7.75	22.5	0/20	7.14	2.25	23.2	0/20	7.15	1.49	22.5
30 ppm	0/20	7.28	7.72	22.4	0/20	7.01	2.2	23.2	0/20	6.91	1.44	22.5
Duplicate	0/20	7.20	7.76	22.4	0/20	6.92	2.19	23.2	2/20	6.86	1.46	22.5
300 ppm	0/20	7.19	7.68	22.5	0/20	6.93	2.19	23.2	1/20	6.86	1.45	22.5
Duplicate	0/20	7.22	7.74	22.4	0/20	6.95	2.17	23.2	0/20	6.68	1.43	22.6
3,000 ppm	8/20	6.84	7.73	22.5	20/20	6.47	2.07	23.2				
Duplicate	12/20	6.87	7.76	22.5	20/20	6.45	2.04	23.2				
30,000 ppm	20/20	6.76	7.78	22.6								
Duplicate	20/20	6.73	7.81	22.6								

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**APPENDIX C: OUTPUT DATA PRODUCED BY TIDEPool SCIENTIFIC  
TOXCALC™ BIOASSAY CALCULATOR SOFTWARE**

# AQUATIC TOXICITY TEST – POLYOX 301

DATE: 4 February 04

## Acute Fish Test-48 Hr Survival

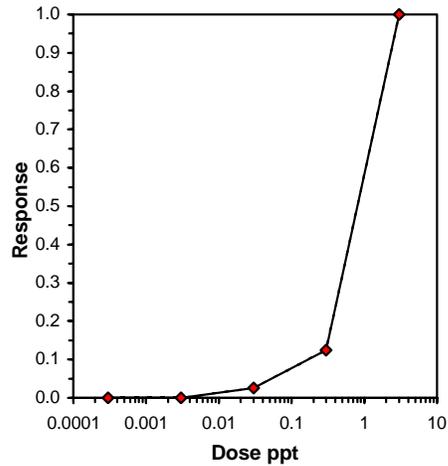
Start Date: 2/4/2004      Test ID: 1      Sample ID: Fire Fighting Foam  
 End Date: 2/6/2004      Lab ID: Air Force Research Lab      Sample Type: PolyOx  
 Sample Date:      Protocol: EPAA 91-EPA Acute      Test Species: PP-Pimephales promelas  
 Comments:

Conc-ppt	1	2
B-Control	1.0000	1.0000
0.0003	1.0000	1.0000
0.003	1.0000	1.0000
0.03	1.0000	0.9500
0.3	0.9500	0.8000
3	0.0000	0.0000

Conc-ppt	Mean	N-Mean	Transform: Arcsin Square Root				N	Number Resp	Total Number
			Mean	Min	Max	CV%			
B-Control	1.0000	1.0000	1.4588	1.4588	1.4588	0.000	2	0	40
0.0003	1.0000	1.0000	1.4588	1.4588	1.4588	0.000	2	0	40
0.003	1.0000	1.0000	1.4588	1.4588	1.4588	0.000	2	0	40
0.03	0.9750	0.9750	1.4020	1.3453	1.4588	5.723	2	1	40
0.3	0.8750	0.8750	1.2262	1.1071	1.3453	13.732	2	5	40
3	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Trimmed Spearman-Kärber			
Trim Level	EC50	95% CL	
0.0%	0.6716	0.5146	0.8765
5.0%	0.7551	0.5720	0.9969
10.0%	0.7984	0.5440	1.1719
20.0%	0.8048	0.6877	0.9419
Auto-0.0%	0.6716	0.5146	0.8765



# AQUATIC TOXICITY TEST – POLYOX 301 AND AFFF

DATE: 4 February 04

## Acute Fish Test-48 Hr Survival

Start Date: 2/4/2004      Test ID: 1      Sample ID: Fire Fighting Foam  
 End Date: 2/6/2004      Lab ID: Air Force Research Lab      Sample Type: PolyOx and Ansul AFFF  
 Sample Date:      Protocol: EPAA 91-EPA Acute      Test Species: PP-Pimephales promelas

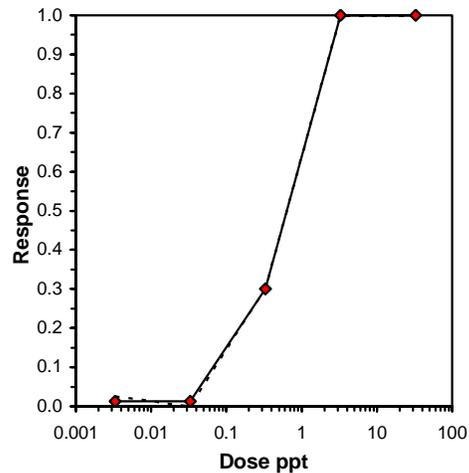
Comments:

Conc-ppt	1	2
B-Control	1.0000	1.0000
0.0033	1.0000	0.9500
0.033	1.0000	1.0000
0.33	0.6500	0.7500
3.3	0.0000	0.0000
33	0.0000	

Conc-ppt	Mean	N-Mean	Transform: Arcsin Square Root					N	Number Resp	Total Number
			Mean	Min	Max	CV%				
B-Control	1.0000	1.0000	1.4588	1.4588	1.4588	0.000	2	0	40	
0.0033	0.9750	0.9750	1.4020	1.3453	1.4588	5.723	2	1	40	
0.033	1.0000	1.0000	1.4588	1.4588	1.4588	0.000	2	0	40	
0.33	0.7000	0.7000	0.9925	0.9377	1.0472	7.798	2	12	40	
3.3	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40	
33	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	1	20	20	

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Trimmed Spearman-Kärber			
Trim Level	EC50	95% CL	
0.0%			
5.0%	0.5408	0.3719	0.7865
10.0%	0.5662	0.3673	0.8729
20.0%	0.6126	0.3192	1.1754
Auto-1.3%	0.5216	0.3695	0.7363



**AQUATIC TOXICITY TEST – HAWK SUPER B, NO DYE**  
**DATE: 25 February 04**

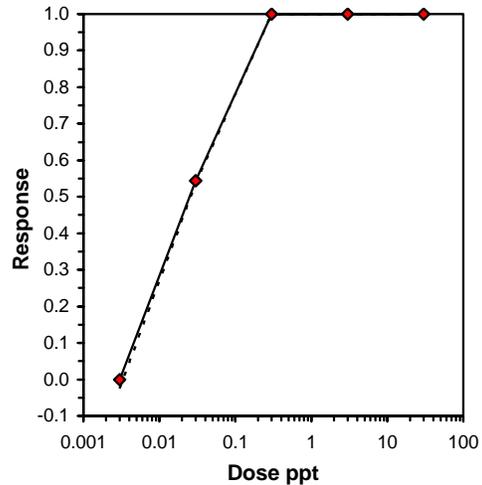
Acute Fish Test-48 Hr Survival					
Start Date:	2/25/2004	Test ID:	1	Sample ID:	Fire Fighting Foam
End Date:	2/27/2004	Lab ID:	Air Force Research Lab	Sample Type:	Hawk Super B (w/o dye)
Sample Date:		Protocol:	EPAA 91-EPA Acute	Test Species:	PP-Pimephales promelas
Comments:					

Conc-ppt	1	2
B-Control	1.0000	0.9500
0.003	1.0000	1.0000
0.03	0.9000	0.0000
0.3	0.0000	0.0000
3	0.0000	0.0000
30	0.0000	0.0000

Conc-ppt	Mean	N-Mean	Transform: Arcsin Square Root				N	Number Resp	Total Number
			Mean	Min	Max	CV%			
B-Control	0.9750	1.0000	1.4020	1.3453	1.4588	5.723	2	1	40
0.003	1.0000	1.0256	1.4588	1.4588	1.4588	0.000	2	0	40
0.03	0.4500	0.4615	0.6805	0.1120	1.2490	118.139	2	22	40
0.3	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40
3	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40
30	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Trimmed Spearman-Kärber			
Trim Level	EC50	95% CL	
0.0%	0.0271	0.0189	0.0389
5.0%	0.0268	0.0179	0.0401
10.0%	0.0265	0.0169	0.0416
20.0%	0.0260	0.0145	0.0468
Auto-0.0%	0.0271	0.0189	0.0389



# AQUATIC TOXICITY TEST – FEM-12

DATE: 14 April 04

## Acute Fish Test-48 Hr Survival

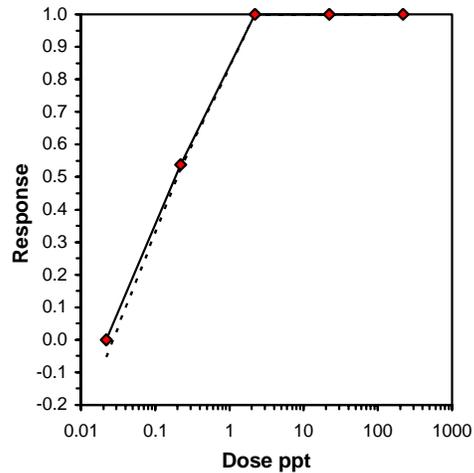
Start Date: 4/14/2004      Test ID: 1      Sample ID: Fire Fighting Foam  
 End Date: 4/16/2004      Lab ID: Air Force Research Laboratory      Sample Type: FEM-12  
 Sample Date:      Protocol: EPAA 91-EPA Acute      Test Species: PP-Pimephales promelas  
 Comments:

Conc-ppt	1	2
B-Control	0.9500	0.9500
0.022	1.0000	1.0000
0.22	0.3500	0.5500
2.2	0.0000	0.0000
22	0.0000	0.0000
220	0.0000	0.0000

Conc-ppt	Transform: Arcsin Square Root							Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N		
B-Control	0.9500	1.0000	1.3453	1.3453	1.3453	0.000	2	2	40
0.022	1.0000	1.0526	1.4588	1.4588	1.4588	0.000	2	0	40
0.22	0.4500	0.4737	0.7343	0.6331	0.8355	19.494	2	22	40
2.2	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40
22	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40
220	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

Trimmed Spearman-Kärber			
Trim Level	EC50	95% CL	
0.0%	0.2014	0.1401	0.2895
5.0%	0.1996	0.1334	0.2985
10.0%	0.1978	0.1260	0.3105
20.0%	0.1944	0.1078	0.3506
Auto-0.0%	0.2014	0.1401	0.2895



# AQUATIC TOXICITY TEST – FLAMEOUT

DATE: 8 June 04

## Acute Fish Test-48 Hr Survival

Start Date: 6/8/2004      Test ID: 1      Sample ID: Fire Fighting Foam  
 End Date: 6/10/2004      Lab ID: Air Force Research Lab      Sample Type: Flame Out  
 Sample Date:      Protocol: EPAA 91-EPA Acute      Test Species: PP-Pimephales promelas  
 Comments:

Conc-ppt	1	2
B-Control	1.0000	0.9500
0.003	1.0000	1.0000
0.03	0.9500	0.9500
0.3	0.0000	0.0000
3	0.0000	0.0000
30	0.0000	0.0000

Conc-ppt	Mean	N-Mean	Transform: Arcsin Square Root					N	Number Resp	Total Number
			Mean	Min	Max	CV%				
B-Control	0.9750	1.0000	1.4020	1.3453	1.4588	5.723	2	1	40	
0.003	1.0000	1.0256	1.4588	1.4588	1.4588	0.000	2	0	40	
0.03	0.9500	0.9744	1.3453	1.3453	1.3453	0.000	2	2	40	
0.3	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40	
3	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40	
30	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40	

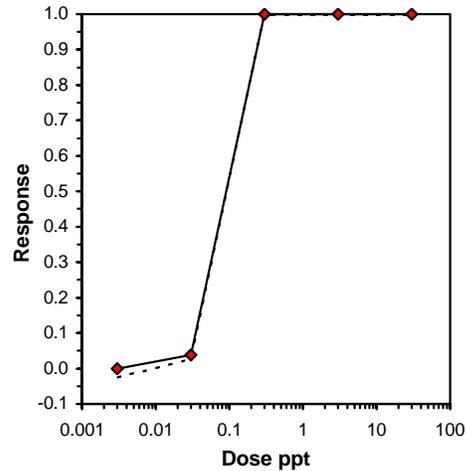
### Auxiliary Tests

Normality of the data set cannot be confirmed  
 Equality of variance cannot be confirmed

Statistic      Critical      Skew      Kurt

### Trimmed Spearman-Kärber

Trim Level	EC50	95% CL	
0.0%	0.0869	0.0756	0.0999
5.0%	0.0907	0.0841	0.0977
10.0%	0.0907	0.0841	0.0977
20.0%	0.0907	0.0841	0.0977
Auto-0.0%	0.0869	0.0756	0.0999



# AQUATIC TOXICITY TEST – FLAMEOUT FOAM

DATE: 8 June 04

## Acute Fish Test-48 Hr Survival

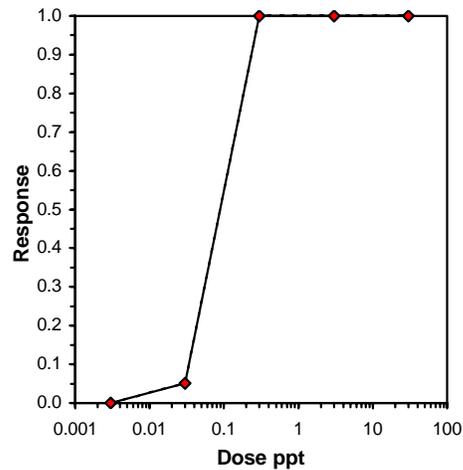
Start Date: 6/8/2004      Test ID: 1      Sample ID: Fire Fighting Foam  
 End Date: 6/10/2004      Lab ID: Air Force Research Lab      Sample Type: Flame Out Foam  
 Sample Date:      Protocol: EPAA 91-EPA Acute      Test Species: PP-Pimephales promelas  
 Comments:

Conc-ppt	1	2
B-Control	1.0000	0.9500
0.003	0.9500	1.0000
0.03	1.0000	0.8500
0.3	0.0000	0.0000
3	0.0000	0.0000
30	0.0000	0.0000

Conc-ppt	Mean	N-Mean	Transform: Arcsin Square Root					N	Number Resp	Total Number
			Mean	Min	Max	CV%				
B-Control	0.9750	1.0000	1.4020	1.3453	1.4588	5.723	2	1	40	
0.003	0.9750	1.0000	1.4020	1.3453	1.4588	5.723	2	1	40	
0.03	0.9250	0.9487	1.3159	1.1731	1.4588	15.350	2	3	40	
0.3	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40	
3	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40	
30	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40	

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Bartlett's Test indicates equal variances (p = 0.66)	0.844553	9.210351		

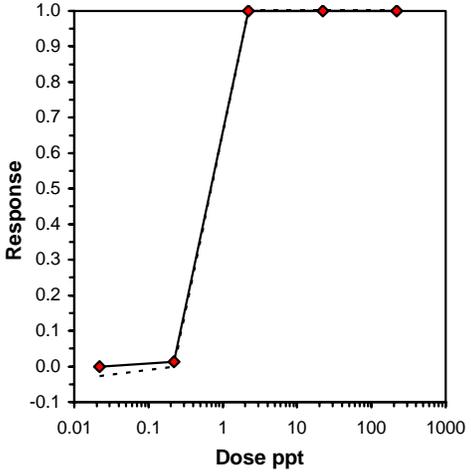
Trimmed Spearman-Kärber			
Trim Level	EC50	95% CL	
0.0%	0.0843	0.0718	0.0990
5.0%	0.0891	0.0685	0.1160
10.0%	0.0891	0.0815	0.0975
20.0%	0.0891	0.0815	0.0975
Auto-0.0%	0.0843	0.0718	0.0990



# AQUATIC TOXICITY TEST – FEM-12SC

DATE: 23 June 04

Acute Fish Test-48 Hr Survival									
Start Date:	6/23/2004	Test ID:	1	Sample ID:	Fire Fighting Foam				
End Date:	6/25/2004	Lab ID:	Air Force Research Laboratory	Sample Type:	FEM-12SC				
Sample Date:		Protocol:	EPAA 91-EPA Acute	Test Species:	PP-Pimephales promelas				
Comments:									
Conc-ppt	1	2							
B-Control	0.8500	1.0000							
0.022	1.0000	0.9000							
0.22	0.9000	0.9500							
2.2	0.0000	0.0000							
22	0.0000	0.0000							
220	0.0000	0.0000							
Transform: Arcsin Square Root									
Conc-ppt	Mean	N-Mean	Mean	Min	Max	CV%	N	Number Resp	Total Number
B-Control	0.9250	1.0000	1.3159	1.1731	1.4588	15.350	2	3	40
0.022	0.9500	1.0270	1.3539	1.2490	1.4588	10.953	2	2	40
0.22	0.9250	1.0000	1.2972	1.2490	1.3453	5.246	2	3	40
2.2	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40
22	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40
220	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40
Auxiliary Tests									
Normality of the data set cannot be confirmed				Statistic		Critical		Skew Kurt	
Bartlett's Test indicates equal variances ( $p = 0.71$ )				0.695805		9.210351			
Trimmed Spearman-Kärber									
Trim Level	EC50	95% CL							
0.0%	0.6747	0.6206	0.7334						
5.0%	0.6850	0.6562	0.7150						
10.0%	0.6850	0.6562	0.7150						
20.0%	0.6850	0.6562	0.7150						
Auto-0.0%	0.6747	0.6206	0.7334						



# AQUATIC TOXICITY TEST – ARCTIC FIRE

DATE: 23 June 04

## Acute Fish Test-48 Hr Survival

Start Date: 6/23/2004      Test ID: 1      Sample ID: Fire Fighting Foam  
 End Date: 6/25/2004      Lab ID: Air Force Research Laboratory      Sample Type: Arctic Fire  
 Sample Date:      Protocol: EPAA 91-EPA Acute      Test Species: PP-Pimephales promelas

Comments:

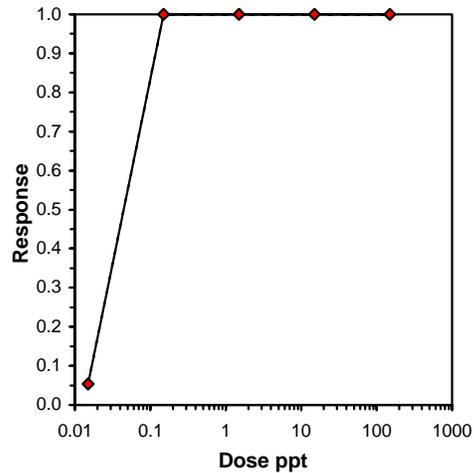
Conc-ppt	1	2
B-Control	0.8500	1.0000
0.015	0.9000	0.8500
0.15	0.0000	0.0000
1.5	0.0000	0.0000
15	0.0000	0.0000
150	0.0000	0.0000

Conc-ppt	Mean	N-Mean	Transform: Arcsin Square Root					N	Number Resp	Total Number
			Mean	Min	Max	CV%				
B-Control	0.9250	1.0000	1.3159	1.1731	1.4588	15.350	2	3	40	
0.015	0.8750	0.9459	1.2111	1.1731	1.2490	4.434	2	5	40	
0.15	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40	
1.5	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40	
15	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40	
150	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40	

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
F-Test indicates equal variances (p = 0.33)	14.14691	16212.46		

### Trimmed Spearman-Kärber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%	0.0444	0.0405	0.0487
20.0%	0.0444	0.0405	0.0487
Auto-5.4%	0.0444	0.0405	0.0487



## AQUATIC TOXICITY TEST – ARCTIC FIRE

Acute Fish Test-48 Hr Survival			
Start Date:	7/14/2004	Test ID:	1
End Date:	7/16/2004	Lab ID:	Air Force Research Lab
Sample Date:		Protocol:	EPAA 91-EPA Acute
Comments:		Sample ID:	Fire Fighting Foam
		Sample Type:	Arctic Fire
		Test Species:	PP-Pimephales promelas

Conc-ppt	1	2
B-Control	0.8500	1.0000
0.015	0.9000	1.0000
0.15	0.8500	0.9000
1.5	0.0000	0.0000
15	0.0000	0.0000
150	0.0000	0.0000

Conc-ppt	Mean	N-Mean	Transform: Arcsin Square Root				N	Number Resp	Total Number
			Mean	Min	Max	CV%			
B-Control	0.9250	1.0000	1.3159	1.1731	1.4588	15.350	2	3	40
0.015	0.9500	1.0270	1.3539	1.2490	1.4588	10.953	2	2	40
0.15	0.8750	0.9459	1.2111	1.1731	1.2490	4.434	2	5	40
1.5	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40
15	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40
150	0.0000	0.0000	0.1120	0.1120	0.1120	0.000	2	40	40

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Bartlett's Test indicates equal variances (p = 0.62)	0.969105	9.210351		

Trimmed Spearman-Kärber			
Trim Level	EC50	95% CL	
0.0%	0.4068	0.3393	0.4879
5.0%	0.4347	0.3358	0.5629
10.0%	0.4369	0.3936	0.4849
20.0%	0.4369	0.3936	0.4849
Auto-0.0%	0.4068	0.3393	0.4879

