

DEPARTMENT OF THE NAVY

NAVAL RESEARCH LABORATORY

WASHINGTON, D.C. 20375-5000

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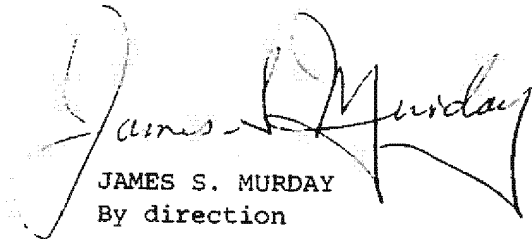
26 April 1991

From: Commanding Officer, Naval Research Laboratory
To: Commander, Naval Sea System Command (SEA 5122P)
Subj: RESULTS OF QUALIFICATION OF AFFF LIQUID CONCENTRATE FIRE
EXTINGUISHING AGENTS MANUFACTURED BY THE 3M COMPANY

Ref: (a) 3M ltr of 15 Oct 90

Encl: (1) Two copies of subject report.

1. In accordance with reference (a), the subject products have been tested in order to determine their compliance with the requirements of MIL-F-24385E(SH). A report covering the work is forwarded herewith as enclosure (1).


JAMES S. MURDAY
By direction

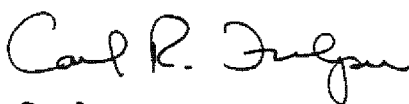
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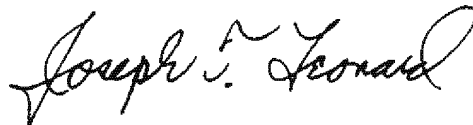
Subj: RESULTS OF QUALIFICATION OF AFFF LIQUID CONCENTRATE FIRE
EXTINGUISHING AGENTS MANUFACTURED BY THE 3M COMPANY

- Ref: (a) Military Specification, "Fire Extinguishing Agent,
Aqueous Film-Forming Foam (AFFF) Liquid Concentrate,
for Fresh and Sea Water," MIL-F-24385E(SH), November
1990.
- (b) Qualified Products List, "Products Qualified under
Military Specification MIL-F-24385, Fire Extinguishing
Agent, Aqueous Film-Forming Foam (AFFF) Liquid
Concentrate, for Fresh and Sea Water," QPL-24385-20,
September 1990.

1. AFFF concentrates manufactured by the 3M Company and designated
as FC-203CF (3% type 3) and FC-206CF (6% type 6) were tested in
accordance with the procedures outlined in reference (a).
2. The test data obtained are summarized in the attached tables.
3. Both candidate AFFF concentrates met the requirements of
reference (a).



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Attachments

- 1) Tables 1-10
- 2) "Results of 96-hour, Fundulus heteroclitus Flow-Through Acute
Toxicity Tests on Test Materials from the Naval Research
Laboratory," EA Engineering, Science and Technology, March 1991.

Table 1. Concentrate Chemical and Physical Characteristics
Test Results (Paragraph 3.3)

<u>Characteristic Measured</u>	<u>Specifications Requirements</u>		<u>Results</u>	
	<u>Type 3</u>	<u>Type 6</u>	<u>Type 3 FC-203CF</u>	<u>Type 6 FC-206CF</u>
Refractive Index, minimum	1.3630	1.3580	1.3660	1.3589
Viscosity (centistokes)				
maximum @ 5° C	20.0	10.0	7.3	3.3
minimum @ 25° C	2.0	2.0	3.9	2.1
Hydrogen Ion Concentration (pH)	7.0-8.5	7.0-8.5	8.3	8.1
Spreading Coefficient, minimum	3.0	3.0	4.4	6.7
Foamability, minimum				
Fresh Water; Expansion Ratio	6.0	6.0	6.7	7.4
25% Drain Time (minutes)	2.5	2.5	6.4	5.6
Sea Water; Expansion Ratio	6.0	6.0	7.6	7.6
25% Drain Time (minutes)	2.5	2.5	5.0	4.6
Film Formation and Sealability	No Sustained Ignition	No Sustained Ignition	Flashed-NS	No ignition
Fresh Water; Half Strength	(for all solutions)	(for all solutions)	No ignition	No ignition
Full Strength			No ignition	Flashed-NS
Sea Water; Half Strength			No ignition	No ignition
Full Strength			No ignition	No ignition
Quintuple Strength				
Corrosion				
General; maximum				
-Steel (milli in./yr)	1.5	1.5	<1.5	<1.5
-Cu-Ni (milli in./yr)	1.0	1.0	<1.0	<1.0
-Ni-Cu (milli in./yr)	1.0	1.0	<1.0	<1.0
-Bronze (milligrams)	100	100	<100	<100
Localized CRES -Steel	No pits	No pits	No pits	No pits
Total Halides, maximum (ppm)	500	250	55.6	31.9
Dry Chemical Compatibility				
Burnback Resistance, minimum (s)	360	360	416 ⁽¹⁾	690
Environmental Impact				
Toxicity, LC ₅₀ , minimum (mg/l)	500	1000	1449	3613
COD, maximum (mg/l)	1,000,000	500,000	585,000	295,000
BOD ₂₀ /COD, minimum	0.7	0.7	0.7	0.7

(1) 25% burnback never achieved. Burnback pan pulled at 387 s. Foam blanket then closed in on hole and fire was completely extinguished at 416 s.

NS No Sustained Ignition

NI No Ignition

Table 2. Stability Test Results (Paragraph 3.3.2)

<u>Property Measured</u>	<u>Specification Requirement (all solutions)</u>	<u>Results</u>			
		<u>Type 3</u>		<u>Type 6</u>	
		<u>Fresh</u>	<u>Sea</u>	<u>Fresh</u>	<u>Sea</u>
Spreading Coefficient, minimum					
Concentrates (fresh water only)	3.0	4.9	---	6.9	---
Pre-mixed Solution (fresh water only)	3.0	6.2	---	7.4	---
Foamability, minimum					
Concentrates; Full Strength (fresh and sea)					
Expansion Ratio	6.0	7.4	7.2	6.7	6.3
25% Drain Time (minutes)	2.5	5.3	4.7	5.6	4.7
Pre-mixed Solutions; Full Strength (fresh/sea)					
Expansion Ratio	6.0	6.7	7.2	6.3 ⁽¹⁾	6.0 ⁽¹⁾
25% Drain Time (minutes)	2.5	5.2	5.3	4.9	4.8
Film Formation and Sealability					
Concentrates; Half Strength	No Sustained Ignition	NI	NI	NI	NI
Full Strength	No Sustained Ignition	NI	NI	NI	NI
Pre-mixed Solutions; Half Strength	No Sustained Ignition	NI	NI	NI	NI
Full Strength	No Sustained Ignition	NI	NI	NI	NI
Fire Performance, 2.6 m ² (28 ft ²)					
Concentrates; Half Strength (fresh/sea)					
-Extinguishment Time, maximum (s)	45	40	42	33	35
-Burnback Time, minimum (s)	300	421	413	465	390
Concentrates; Full Strength (fresh/sea)					
-Extinguishment Time, maximum (s)	30	26	28	22	19
-Burnback Time, minimum (s)	360	502	427	530	480
Pre-mixed Solutions; Half Strength (fresh/sea)					
-Extinguishment Time, maximum (s)	45	42	43	44	35
-Burnback Time, minimum (s)	300	442	511	500	499
Pre-mixed Solutions; Full Strength (fresh/sea)					
-Extinguishment Time, maximum (s)	30	26	21	28	22
-Burnback Time, minimum (s)	360	563	473	545	395
Stratification					
Concentrates	None	None		None	
Pre-mixed Solutions; Half Strength	None	None	None	None	None
Full Strength	None	None	None	None	None
Precipitation, maximum (% volume)					
Concentrates	<0.05	<0.05		<0.05	
Pre-mixed Solutions; Half Strength	<0.05	<0.05	<0.05	<0.05	<0.05
Full Strength	<0.05	<0.05	<0.05	<0.05	<0.05

(1) Expansion Ratio corrected for temperature as per NFPA 412
NI No Ignition

Table 3. Interagent Compatibility Test Results (Paragraph 3.3.3)
for 50/50 Mixture of 3M FC203CE and FC203CF

<u>Property Measured</u>	<u>Specification Requirement (all solutions)</u>	<u>Results</u>			
		<u>Concentrates</u>		<u>Pre-mixed Solutions</u>	
		<u>Fresh</u>	<u>Sea</u>	<u>Fresh</u>	<u>Sea</u>
Foamability, minimum					
Expansion Ratio	6.0	7.7	7.2	6.8	7.0
25% Drain Time (minutes)	2.5	5.4	4.9	4.9	5.3
Film Formation and Sealability	No Sustained Ignition	NI	NI	NI	NI
Fire Performance, 2.6 m ² (28 ft ²)					
Extinguishment Time, maximum (s)	30	30	26	27	24
Burnback Time, minimum (s)	360	525	505	559	545
Stratification	None	None		None	None
Precipitation, maximum (% volume)	<0.05	<0.05		<0.05	<0.05

NI No Ignition

Table 4. Interagent Compatibility Test Results (Paragraph 3.3.3)
for 50/50 Mixture of Ansul AFC-5A and 3M FC203CF

<u>Property Measured</u>	<u>Specification Requirement (all solutions)</u>	<u>Results</u>			
		<u>Concentrates</u>		<u>Pre-mixed Solutions</u>	
		<u>Fresh</u>	<u>Sea</u>	<u>Fresh</u>	<u>Sea</u>
Foamability, minimum					
Expansion Ratio	6.0	7.1	6.2	7.2	6.3 ⁽¹⁾
25% Drain Time (minutes)	2.5	5.4	5.3	5.5	4.8
Film Formation and Sealability	No Sustained Ignition	NI	NI	NI	NI
Fire Performance, 2.6 m ² (28.0 ft ²)					
Extinguishment Time, maximum (s)	30	25	26	23	25
Burnback Time, minimum (s)	360	549	454	583	505
Stratification	None	None		None	None
Precipitation, maximum (% volume)	<0.05	<0.05		<0.05	<0.05

(1) Expansion Ratio for temperature as per NFPA 412
NI No Ignition

Table 5. Interagent Compatibility Test Results (Paragraph 3.3.3)
for 50/50 Mixture of 3M FC206CE and FC206CF

<u>Property Measured</u>	<u>Specification Requirement (all solutions)</u>	<u>Results</u>			
		<u>Concentrates</u>		<u>Pre-mixed Solutions</u>	
		<u>Fresh</u>	<u>Sea</u>	<u>Fresh</u>	<u>Sea</u>
Foamability, minimum					
Expansion Ratio	6.0	7.3	7.2	6.9	6.1 ⁽¹⁾
25% Drain Time (minutes)	2.5	5.0	5.5	4.9	4.8
Film Formation and Sealability	No Sustained Ignition	NI	NI	NI	NI
Fire Performance, 2.6 m ² (28.0 ft ²)					
Extinguishment Time, maximum (s)	30	28	29	26	24
Burnback Time, minimum (s)	360	542	439	548	538
Stratification	None	None		None	None
Precipitation, maximum (% volume)	<0.05	<0.05		<0.05	<0.05

(1) Expansion Ratio corrected for temperature as per NFPA 412
NI No Ignition

Table 6. Interagent Compatibility Test Results (Paragraph 3.3.3)
for 50/50 Mixture of Ansul AFC-5 and 3M FC206CF

<u>Property Measured</u>	<u>Specification Requirement (all solutions)</u>	<u>Results</u>			
		<u>Concentrates</u>		<u>Pre-mixed</u>	<u>Solutions</u>
		<u>Fresh</u>	<u>Sea</u>	<u>Fresh</u>	<u>Sea</u>
Foamability, minimum					
Expansion Ratio	6.0	6.7	6.9	7.4	7.4
25% Drain Time (minutes)	2.5	5.1	6.1	5.8	4.4
Film Formation and Sealability	No Sustained Ignition	NI	NI	NI	NI
Fire Performance, 2.6 m ² (28.0 ft ²)					
Extinguishment Time, maximum (s)	30	29	26	22	23
Burnback Time, minimum (s)	360	450	485	552	468
Stratification	None	None		None	None
Precipitation, maximum (% volume)	<0.05	<0.05		<0.05	<0.05

NI No Ignition

Table 7. Interagent Compatibility Test Results (Paragraph 3.3.3)
for one-third/one-third/one-third Mixture of Ansul AFC-5A, 3M FC203CE, and FC203CF

<u>Property Measured</u>	<u>Specification Requirement (all solutions)</u>	<u>Results</u>			
		<u>Concentrates</u>		<u>Pre-mixed Solutions</u>	
		<u>Fresh</u>	<u>Sea</u>	<u>Fresh</u>	<u>Sea</u>
Foamability, minimum					
Expansion Ratio	6.0	7.0	7.1	7.0	6.8
25% Drain Time (minutes)	2.5	5.1	4.7	4.6	5.1
Film Formation and Sealability	No Sustained Ignition	NI	NI	NI	NI
Fire Performance, 2.6 m ² (28.0 ft ²)					
Extinguishment Time, maximum (s)	30	28	26	25	28
Burnback Time, minimum (s)	360	467	528	(1)	539
Stratification	None	None		None	None
Precipitation, maximum (% volume)	<0.05	<0.05		<0.05	<0.05

NI No Ignition

(1) 25% burnback never obtained. Burnback pan pulled at 368 s. Foam blanket then closed in on hole and fire was completely extinguished at 446 s.

Table 8. Interagent Compatibility Test Results (Paragraph 3.3.3)
for one-third/one-third/one-third Mixture of Ansul AFC-5, 3M FC206CE, and FC206CF

<u>Property Measured</u>	<u>Specification Requirement (all solutions)</u>	<u>Results</u>			
		<u>Concentrates</u>		<u>Pre-mixed Solutions</u>	
		<u>Fresh</u>	<u>Sea</u>	<u>Fresh</u>	<u>Sea</u>
Foamability, minimum					
Expansion Ratio	6.0	6.6	7.0	7.6	7.6
25% Drain Time (minutes)	2.5	5.3	6.0	5.5	4.9
Film Formation and Sealability	No Sustained Ignition	NI	NI	NI	NI
Fire Performance, 2.6 m ² (28.0 ft ²)					
Extinguishment Time, maximum (s)	30	26	23	26	26
Burnback Time, minimum (s)	360	478	463	466	506
Stratification	None	None		None	None
Precipitation, maximum (% volume)	<0.05	<0.05		<0.05	<0.05

NI No Ignition

Table 9. Fire Performance Test Results (Paragraph 3.4)

<u>Property Measured</u>	<u>Specification Requirement (all solutions)</u>	<u>Results</u>	
		<u>Type 3 FC203CF</u>	<u>Type 6 FC206CF</u>
2.6 m ² (28.0 ft ²) Fire Tests			
Extinguishment Time, maximum (s)			
Fresh water, Half Strength	45	38	40
Full Strength	30	21	20
Sea water, Half Strength	45	34	34
Full Strength	30	27	22
Quintuple Strength	55	33	36
Burnback Time, minimum (s)			
Fresh water, Half Strength	300	450	390
Full Strength	360	710	567
Sea water, Half Strength	300	449	475
Full Strength	360	498	621
Quintuple Strength	200	253	370
4.6 m ² (50 ft ²) Fire Tests			
Extinguishment Time, maximum (s)	50	46	37
Burnback Time, minimum (s)	360	402	369
40 s Summation, minimum	320	323	328

Table 10. Cap Removed Torque Test Results (Paragraph 5.1.1.1.1)

<u>Pass/Fail Criteria</u> <u>Maximum Torque (In-lb)</u>	<u>Results</u>	
	<u>Type 3 FC203CF</u>	<u>Type 6 FC206CF</u>
50	30	31



RESULTS OF 96-HOUR *Fundulus heteroclitus*
FLOW-THROUGH ACUTE TOXICITY TESTS
ON TEST MATERIALS FROM THE
NAVAL RESEARCH LABORATORY

Prepared for:

Naval Research Laboratory
Washington, D.C.

Prepared by:

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March 1991

EA Project 70003.10

Report Number 711

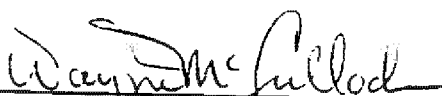
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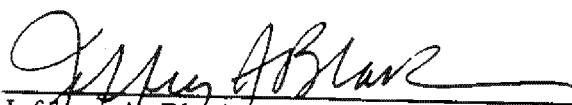
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5 Mar 1991
Date


Jeffrey A. Black, Ph.D.
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6 Mar 1991
Date

March 1991

1. INTRODUCTION

At the request of the Naval Research Laboratory, Washington, D.C., EA Engineering, Science, and Technology, Inc. performed flow-through acute toxicity tests on two formulations (type 3 and type 6) of aqueous film-forming foam (AFFF), fire fighting agent. The objective of these tests was to determine the 96-hour median lethal concentration (LC50) of the test materials to Fundulus heteroclitus (mummichogs) using lethality as the endpoint.

2. METHODS AND MATERIALS

2.1 SAMPLE DESCRIPTION

The test samples were delivered to EA on 14 January, 1991 by courier. One liter bottles each containing type 3 and type 6 aqueous film-forming foam (AFFF) were shipped to EA, logged in, visually inspected against the Chain of Custody Records, and assigned EA accession numbers (EA sample numbers AT1-017 and AT1-018 respectively). Based on initial test results, an additional one liter of type 6 AFFF was requested in order to have sufficient sample volume, and that sample arrived on 5 February 1991 (Sample #AT1-065). Samples were stored at room temperature (20 C) when not in use.

2.2 TEST ORGANISMS

Juvenile mummichogs, Fundulus heteroclitus, constituted the test organisms. The test organisms were 92-96 days old at test initiation. The fish were obtained from cultures maintained at Hampton Roads Sanitation District in Virginia Beach, Virginia.

Two separate lots of F. heteroclitus (lot numbers FU-004 and FU-004A) were received on 10 and 21 January, respectively. The fish were held at 20 C and 20 ppt salinity and were fed flaked food daily until used in testing. The mean acclimation temperatures and salinities for lots FU-004 and FU-004A were: FU-004-- 19.7 ± 0.7 C and 20.8 ± 0.5 ppt and FU-004A-- 20.2 ± 0.8 C and 20.8 ± 0.6 ppt.

There was no mortality observed in either lot of fish during the acclimation period. The mean length and weight of lot FU-004 organisms were $22.58 (\pm 1.52)$ mm and $0.23 (\pm 0.05)$ g, respectively. The mean length and weight of lot FU-004A organisms were $23.49 (\pm 1.67)$ mm and $0.27 (\pm 0.07)$ g, respectively.

2.3 DILUTION WATER

The water used for acclimation, holding, and for the flow-through studies was 20 ppt artificial seawater. Dry synthetic sea salts (Forty Fathoms, Marine Enterprises, Towson, Maryland) were added to dechlorinated tap water allowed to age prior to use. A summary of the chemical characteristics of Forty Fathoms is presented in Table 1. The source of the makeup water was Baltimore municipal water supply, which upon entry into the laboratory was passed through a high capacity activated carbon dechlorination unit which removes chlorine and hexane soluble compounds.

2.4 TOXICITY TEST OPERATIONS AND PERFORMANCE

Test procedures used in the two flow-through studies followed those described in the protocol ATS-FAF-GT-01 (Attachment I) which is contained in EA's Aquatic Toxicology Laboratory Quality Control and Standard Operating Procedures Manual (EA, 1986). EA's protocol is in conformance with guidelines contained in ASTM's E729 procedure, and with EPA (Peltier and Weber, 1985) and Standard Methods (APHA, 1989) documents.

The tests employed Masterflex peristaltic pumps which continuously delivered test solution from the stock solution tanks to splitter assemblies for each test concentration. The fill and dump splitter assemblies divided the solution flow equally to the two replicates chambers for each test concentration. Each test was comprised of five concentrations and a control. The type 3 AFFF test series was 115, 240, 490, 1,000, 2,100 ppm (vol/vol) and control, while the type 6 AFFF test series was 420, 750, 1,350, 2,400, 4,200 ppm and control.

Daily, a fresh 60 liter batch of each test concentration was prepared in a 20 gallon aquarium using 20 ppt Forty Fathoms artificial seawater as dilution water and control. There were a total of six 20 gallon aquaria, one for each test concentration and control. The masterflex pumps were

calibrated to deliver test solution at 40 ml/min to each concentration's splitter assembly, Masterflex silicone tubing was used, and test solution did not come in contact with any pump parts.

The test chambers were 2 1/2 gallon all glass aquaria, which were calibrated to hold 5 L of test solution at the standpipe overflow. Based on the 40 ml/min flow to the two replicate tanks per concentration, there were more than five 90 percent replacement volumes to each test chamber during a 24-hour period. Each test concentration exposure group consisted of two replicate chambers with 10 organisms each.

Mortality observations, pump delivery calibrations and water quality determinations were made daily throughout the 96-hour exposure period, and were recorded on the data sheets. Copies of the original data sheets are included in attachment II. Temperature, dissolved oxygen, pH and salinity were measured in each batch of new test solution daily and for the old test solutions, in at least one replicate of each concentration at 24, 48, 72, and 96 hours. Aeration was provided to the test chambers when the dissolved oxygen-saturation in any of the tanks dropped below 40 percent. The aeration rate was 100 bubbles/minute per EPA guidance. Using the total number of dead organisms per replicate and concentration at the end of the exposure period, the 96-hour median lethal concentration (LC50 value) was calculated by the binomial, moving average, and probit analysis methods as described as Stephan (1977). Copies of the statistical analysis are included in Attachment III.

2.5 REFERENCE TOXICANT TESTS

The reference toxicant used was sodium dodecyl sulfate (SDS). The test species were exposed to the appropriate graded concentration series to determine if the lots of organisms were within the acceptable limits of EA's Aquatic Toxicology Laboratory. The test duration was 24 hour and treatments were not replicated.

2.6 ARCHIVES

Original data sheets, records, memoranda, notes, and computer printouts are archived at EA's laboratory facilities in Sparks, Maryland. Primary data and associated information will be retained for a period of 5 years unless a longer period of time is requested by the Naval Research Laboratory.

3. RESULTS

Both flow-through studies required aeration because dissolved oxygen (DO) levels dropped below 40 percent saturation during the test period. Aeration was initiated on test #076 with the Type 3 AFFF on the afternoon of day 1 of the test when DO's of 3.9 and 28.5 percent saturation were measured in the 2,100 ppm replicates. All test organisms were still alive at that time. Similarly, aeration was initiated on test #094 with Type 6 AFFF on the afternoon of day 1 when DOs reached the trigger value, and all test organisms were alive when aeration was started. All other water quality parameters were within acceptable limits during the tests and a summary of water quality values measured can be found in Table 2.

The results of the flow-through acute toxicity tests are summarized in Table 3. In the acute test (#076) which was conducted with the type 3 sample there was 100 percent survival in the control and the 115, 240, 490, and 1,000 ppm concentrations. There were no surviving organisms in the 2,100 ppm concentration at test termination. Based on the survival data, the 96-hour LC50 was 1449.1 ppm with a 95 percent confidence interval of 1,000-2,100 ppm.

In acute test 91-094, conducted with type 6 sample, there was 100 percent survival in the laboratory control and the 420, 750, 1,350, and 2,400 ppm test concentrations. At test termination there was 25 percent survival of organisms in the 4,200 ppm concentration. The 96-hour LC50 value and 95 percent confidence interval based on the survival data was 3613.0 (2,400-4,200) ppm.

The reference toxicant LC50 value conducted on F. heteroclitus lot FU-004 was 7.1 mg/L. EA does not presently have sufficient data points with F. heteroclitus to calculate a valid acceptability range (minimum 5 values). LC50 values for the previous 3 tests ranged from 11.2 to 15.8 mg/L and are in the same general concentration range as the present test.

TABLE 1 SUMMARY OF WATER QUALITY CHARACTERISTICS ASSOCIATED
WITH FORTY FATHOMS MARINE MIX HYDRATED TO A DENSITY
OF 1.025 USING DISTILLED WATER

<u>Parameter</u>	<u>Molecular Weight</u>	<u>Forty Fathoms (ppm)</u>
Aluminum	27.0	
Antimony	121.7	0.06
Arsenic	74.9	0.0005
Barium	137.3	0.01
Bicarbonate	61.0	0.12
Beryllium	9.0	174.0
Boron	10.8	0.0002
Bromide	79.9	2.1
Cadmium	112.4	62.0
Calcium	40.0	0.009
Carbonate	60.0	410.0
Cerium	140.0	10.0
Chromium	52.0	0.0007
Chloride	35.4	0.02
Cobalt	58.9	18,600
Copper	63.5	0.0025
Fluorine	19.0	0.007
Gallium	69.7	1.9
Germanium	72.6	0.0004
Iodine	126.9	0.00005
Iron	55.8	0.03
Lithium	6.9	0.03
Magnesium	24.3	0.24
Manganese	54.9	1,290
Mercury	200.6	0.008
Molybdenum	95.9	0.0007
Nickel	58.7	0.005
Nitrogen	14.0	0.009
Phosphorus	31.0	0.85
Potassium	39.1	0.04
Silicon	28.1	380
Sodium	23.0	4.5
Strontium	87.6	10,400
Sulfur (SO ₄)	96.1	12.4
Thallium	204.4	2,600
Tin	118.7	0.000007
Titanium	47.9	0.006
Tungsten	183.9	0.004
Vanadium	50.9	0.004
Zinc	65.4	0.0009
		0.24

TABLE 2. SUMMARY OF WATER QUALITY RANGES FOR 96-HOUR FLOW-THROUGH
ACUTE TOXICITY TESTS FOR THE NAVAL RESEARCH LABORATORY

<u>Test Number</u>	<u>EA Accession Number</u>	<u>Temperature (C)</u>	<u>pH</u>	<u>DO (mg/L)</u>	<u>Salinity (ppt)</u>
FT-01-28-91-076	AT1-017	18.5-20.2	7.6-8.5	2.0-8.3(a)	18.5-20.5
FT-02-04-91-094	AT1-018	19.2-20.9	7.6-8.4	<1.0-8.1(b)	19.2-20.6
	AT1-065				

- (a) DO values of 3.9 mg/L and 28.5 percent saturation were measured on Day 1. At this time, the entire test was aerated.
- (b) DO values of <1.0 mg/L were measured in test concentrations on Day 1. All test vessels were aerated at this time.

TABLE 3. RESULTS OF THE 96-HOUR FLOW-THROUGH ACUTE TOXICITY TESTS
USING *Fundulus heteroclitus*

Test number:	FT-01-28-91-076(#076)	FT-02-04-91-094(#076)
Sample Description:	Type 3	Type 6
Accession Number:	AT1-017	AT1-018/065
LC50 values(a):	1449.1 (1000-2100)(b)	3613.0 (2400-4200)

Exposure Concentration (ppm)--Percent Survival

Control--100	Control--100
115--100	420--100
240--100	750--100
490--100	1350--100
1000--100	2400--100
2100-- 0	4200-- 25

- (a) LC50 values were calculated using the binomial method.
(b) Values in parentheses represent 95 percent confidence intervals.

ATTACHMENT I

PROTOCOLS

24. FLOW-THROUGH ACUTE 96-HOUR LC50 ASSAY WITH A FISH

1. TEST OBJECTIVE

To assess the toxicity of a test article to a fish species under flow-through conditions and determine the 96-hour LC50 using lethality as the test end point.

2. TEST ARTICLE

2.1 Description/Identification

Unless otherwise specified, the test article will be supplied by the client. Adequate chemical specifications with special reference to hazardous properties and storage conditions also will be supplied by the client.

2.2 Methods of Synthesis

Information on the methods of synthesis, stability, and data on composition or other characteristics which define the test article are on file with the client.

3. EXPERIMENTAL DESIGN

3.1 Test Organisms

A fish will constitute the test species as defined by project needs.

3.2 Source

Stocks are laboratory reared, obtained from a scientific organism vendor, or field collected using techniques that will be specified in the report.

3.3 Holding Conditions

Upon receipt, fish are transferred to holding tanks which are continuously replenished with dechlorinated municipal tap water, synthetic seawater, or other appropriate dilution water. The fish are acclimated to laboratory conditions for a minimum of 7 days prior to testing. The laboratory is maintained on a 16-hour light, 8-hour dark photoperiod cycle by a programmable electrical timer. All fish stocks are examined regularly throughout the work week. Dead fish, or those displaying abnormal swimming behavior, discoloration, or pronounced lethargy are removed as observed, and recorded on appropriate log sheets. Fish

stocks are fed a commercial fish food a minimum of once daily throughout the work week. They are not fed for 48 hours prior to initiating a test.

3.4 Dilution Water

The source of dilution water is the City of Baltimore Municipal Water System. Upon entry to the laboratory, the water is passed through a high-capacity, activated-carbon filtration system to remove chlorine, detergents, and hexane-soluble organic materials. This water source has proven safe for aquatic organism toxicity testing, as evidenced by maintenance of the multigeneration Daphnia cultures, with no evident loss of fecundity. If requested, reconstituted fresh water, synthetic sea water, or other dilution water may be used.

3.5 Test Concentration Series

The test concentration series to be used for definitive toxicity tests will be selected following preliminary tests to bracket appropriate concentration ranges. A minimum of five concentrations and diluent water controls will be used. If it proves necessary to use a carrier solvent to disperse the test article in aqueous solution, an additional set of solvent controls will be used. All test concentrations and controls will normally be conducted with two replicates. Additional replicates may be used at the client's request.

3.6 Toxicant Delivery System

A solenoid-activated proportional diluter system similar to that described by Peltier and Weber (1985) will constitute the toxicant delivery system. The proportional diluter will be calibrated prior to test initiation and at test termination. Each test concentration should be at least 50 percent of the next highest concentration in the series. Test vessel calibrations, cycle times, flow rates, and other pertinent data will be reported.

3.7 Test Vessels and Test Volume

Test vessels will be of sufficient volume as to not exceed the organism loading ratio and minimum number of daily tank volume replacements.

3.8 Test Organism Number

A minimum of 10 test organisms will be used in each test replicate, constituting a minimum number of 20 fish per treatment group. Fish will be randomly assigned to each test replicate.

3.9 Test Environment

The test vessels will be maintained at a temperature similar to that at which the fish have been acclimated, but within the recommended test range for that species. Test temperature will not vary more than ± 2 C from specified acclimation temperature. The tests will be conducted on a 16-hour light, 8-hour dark photoperiod cycle.

3.10 Analysis of Test Concentrations for Test Article

If required, test solution replicates may be analyzed for chemical analytical verification of achieved test concentrations. The analytical method and number of determinations will be formulated after consultation with the client. Where chemical analyses are necessary, both nominal and actual measured test solution concentrations will be reported.

3.11 Test Observations

Test organisms will be observed at a minimum of 24, 48, 72, and 96 hours after test initiation to determine number of animals killed by test article concentrations. The study will terminate after completion of the 4-day observation period. The study may be extended, however, at the request of the client.

Routine water quality measurements are performed on the test solutions and diluent water controls according to the following schedule: temperature is measured daily from all test chambers; dissolved oxygen, salinity (when applicable), and pH are measured initially from all test chambers as well as at test termination, and from the control, low, medium, and high concentration at 24-, 48-, and 72-hour intervals. Specific conductance, total alkalinity, and total hardness are measured from the diluent water and sample (when applicable). Specific conductance may also be used for concentration monitoring at the request of the client. Test solutions will not be aerated unless dissolved oxygen concentrations fall below 40 percent of saturation. Analytical methods will be conducted according to APHA et al. (1985) and EPA (1979).

Dead fish are removed when observed. At the end of the 96-hour test period, a minimum of 30 surviving fish are removed from the test containers, placed in labeled specimen bags, and frozen. The fish are subsequently thawed, measured to the nearest mm (standard length), patted dry, and weighed to the nearest 0.01 g. The average length and weight of the test fish are reported, along with their respective ranges and standard deviations.

ETC-T-101

EA Engineering, Science,
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Effective May 1981

Revision 3, August 1986

3.12 Data Analysis

The LC50 values and associated statistics will be derived by the probit analysis, moving average, and binomial test methods as described by Stephan (1977). Depending on the nature of the data, other methods may be used including the probit approximation method of Litchfield and Wilcoxon (1949), SAS probit analysis (SAS Institute 1979), or graphical interpolation using the log concentration vs. percent lethality as described by APHA et al. (1985). The methods used will be specified in the report.

4. FINAL REPORT

The final report will be prepared to contain the following information:

- . Objectives and procedures stated in the approved protocol, including any changes made to the original protocol
- . Identity of the test articles by name or code number and their strength (i.e., quality/purity) and a description of any pretreatment
- . Source of the dilution water, its chemical characteristics, and a description of any pretreatment
- . Test concentration series used and duration of the assay
- . Mean standard lengths and weights of test fish, the respective ranges, and approximate loadings of fish to test volume (g/L)
- . Routine water quality characteristics of dilution water and selected test concentrations
- . Any unforeseen circumstances that may have affected the quality or integrity of the study
- . Signature of the project coordinator, program manager, and quality control officer as authorization of the report.
- . Location of all archived data and the original copy of the final report at EA

Items of data to be included in the report consist of: experimental design and test performance; effects on general appearance of test organisms; morbidity and mortality; tabular presentation and appropriate

statistical evaluation of water quality characteristics; and survival data where necessary.

5. QUALITY ASSURANCE

5.1 Amendments to Protocol

Amendments to the authorized protocol instigated by EA or by the client will be made only after proper authorization. Such authorization is achieved by completion of the Amendment to Protocol form by EA and the client.

5.2 Standard Operating Procedures

Unless otherwise specified, all procedures mentioned in the protocol are subject to detailed Standard Operating Procedures (SOPs) which are contained in the SOP manuals of the participating departments. These SOPs and protocols follow the U.S. EPA's Good Laboratory Practice Standards (GLPs) (EPA 1983).

5.3 Reference Toxicant

A reference toxicant bioassay, utilizing sodium dodecylsulfate (SDS) or other appropriate reference toxicant, will be used as an internal quality check of the sensitivity of each batch of test organisms. Testing will be prior to or concurrent with the test or following the acclimation period for newly acquired organisms. The results of each test will be compared with historical toxicological information of reference toxicant tests performed at EA on the species, to determine if the results are within acceptable limits using the control charts outlined in Peltier and Weber (1983).

5.4 Quality Assurance Evaluation

The study described in this protocol may be subject to internal audit by the Quality Assurance Unit. A quality control officer will be responsible for monitoring each study to assure the client that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with EA's QC program and EPA's GLPs.

5.5 Inspection by Regulatory Authorities

In the event of an inspection of EA by an outside authority during the course of the study, the client will be consulted before inspectors are permitted access to any of the project records or the experimental areas.

5.6 Archives

Copies of project-specific records shall be transferred to the client promptly after the project is completed or as negotiated and budgeted with each client. Original primary data will be retained by EA for 5 years. Primary data include laboratory data sheets, records, memoranda, notes, photographs, microfilm, and computer printouts that are a result of the original observations and activities of the study and which are necessary for the reconstruction and evaluation of the study report.

5.7 Location

This study will be conducted by the Aquatic Toxicology Services Department of the Scientific Operations Division of EA Engineering, Science, and Technology, Inc. at the Mid-Atlantic Regional Offices, Sparks, Maryland.

6. SPECIFICATIONS FOR FLOW-THROUGH ACUTE TOXICITY TEST WITH FISH

6.1 Basic References

American Public Health Association et al. 1985. Standard Methods for Examination of Water and Wastewater, 16th edition. APHA, Washington, D.C. 1,268 pp.

American Society for Testing and Materials (ASTM). 1980. Standard Practice for Conducting Acute Tests with Fishes, Macroinvertebrates, and Amphibians. ASTM Designation: E729-80, Philadelphia, Pa. pp. 285-309.

EA Manual T-101. 1981. Aquatic Toxicology Studies, Quality Control and Standard Operating Procedures Manual. Revised, August 1986. (Internal document prepared by Aquatic Toxicology Services, EA Engineering, Science, and Technology, Inc.)

Peltier, W.H. and C.I. Weber. 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. EPA-600/4-85-013. U.S. Environmental Protection Agency, Cincinnati, Ohio.

U.S. EPA. 1983. Toxic Substances Control; Good Laboratory Practice Standards, Title 40 CFR Part 792. Fed. Regist. 28(230):53922-53944.

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6.2 Test Specifications

Test organism:	A fish species to be specified in final report
Fish age:	All fish for any one test will be selected from the same age-class
Fish length:	The largest fish will not be more than 50 percent longer than the smallest fish
Temperature:	To be specified
Light quality:	Wide-spectrum fluorescent lights
Light intensity:	50-100 f.c.
Photoperiod:	16-hour light, 8-hour dark
Diluter and test chamber flow rate:	Continuous flow--a minimum of five volume replacements per test chamber during a 24-hour period
Aeration:	None, unless dissolved oxygen falls below 60 percent saturation in first 48 hours of the test or below 40 percent saturation thereafter
Loading ratio:	In general, test chambers may not contain greater than 0.8 g/L biomass
Dilution water:	Dechlorinated tap water, reconstituted fresh water, synthetic seawater, or appropriate receiving water
Test container:	19- or 38-L all glass aquaria
No. of concentrations:	Minimum of five concentrations and a control in duplicate series
Test animals per container:	10 (if loading is exceeded, more replicates or larger aquaria may be used)

PROTOCOL ATS-FAF-GT-01

Feeding regime:

Animals are not fed during the test unless fish are too small to survive 96 hours without feeding. Feeding is terminated 48 hours before test initiation in the lot from which organisms are obtained

Test type and duration:

Rangefinding--24-96 hours
Definitive--96 hours
Reference toxicant--24 hours

End point:

Lethality--defined as cessation of opercular movement and inability to respond to external stimuli (gentle prodding with glass rod)

ATTACHMENT II

DATA SHEETS

Aquatic Organism Acute Toxicity Test Data Sheet

Project No.: 7000310
 Test Article: Fire Fighting Foam 6%
 Client: Naval Research
 QC Test No.: FT-020491-094
 Sample No.: ATI-018
 Dilution No.: 20% salt water

TEST: Static Flow through Renewal

TEST ORGANISM

Test Container: 2.5 gal aquariums

Common Name: Mus musculus

Test Volume: 5 L

Scientific Name: E. heliophila

Test Duration: 96 hrs

Lot No.: FU-004A

Source: Hampton Roads

Acclimation: 14 days or Age 96 days

Beginning Date: 2/1/71 Time: 13:40

Ending Date: _____ Time: _____

Sample _____ Diluent _____

Alkalinity: _____

Hardness: _____

Conductivity: _____

Conc. or %	Test Container No.	All Tests															Salt or Brackish Water Tests									
		Number of Live Organisms					Temperature (C)					Dissolved Oxygen (mg/liter)					pH					Salinity (ppt)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
control	A						20.0		19.9	20.3		6.7		7.7	7.6		8.3		8.3	8.3		20.5		20.1	20.5	
	B							20.3		20.1			7.8		7.6		8.3		8.4			20.3		20.5		20.5
420	A						19.9		20.2	20.3		3.5		6.9	5.8		8.0		1.9	7.8		20.3		20.1	20.5	
	B							20.8		20.6			7.0		5.0			8.0		7.7			20.6		20.6	
750	A						19.9		20.6	20.6		4.0		6.5	5.3		7.6		1.8	7.8		20.5		20.2	20.6	
	B							20.3		20.1			6.0		4.8			7.8		7.7			20.4		20.5	
1350	A						19.6		20.5	20.2		4.0		5.4	5.5		7.2		7.7	7.8		20.2		20.3	20.5	
	B							20.6		20.4			6.5		5.6			7.8		7.9			20.5		20.5	
2400	A						19.4		20.5	20.3		4.0		4.5	3.8		7.6		1.6	7.6		20.4		20.6	20.5	
	B							20.6		20.4			5.5		4.2			7.7		7.7			20.4		20.5	
4200	A						19.5		20.5	20.2		4.0		5.0	4.2		7.7		7.6	7.6		20.2		20.6	20.5	
	B							20.9		20.6			4.2		3.4			7.6		7.6			20.4		20.5	
Instrument Number							90	90	90	90		2	2	2	2		90	90	90	90		1	1	1	3	
Correction Factor							NA	NA	NA	NA		8.5	8.6	8.7	8.6		7.0	7.1	7.1	7.1		0.0	0.0	0.0	5.6	
Time							133	142	143	146		134	142		140		135	142	143	146		134	142	143	146	
Investigators							LS	LS	LS	LS		LS	LS	LS	LS		LS	LS	LS	LS		LS	LS	LS	LS	

ACUTE TOXICITY TEST OBSERVATION DATA SHEET

Project No. 70003 10
Client Naval Research
QC Test No. FT-020191-094
Test Article Fire Fighting Foam 6%
Sample No. AT1-018
Diluent 20% salt water
Diluent No. N4

Test Organism: Funneliformis heteroclites
Lot No. FH-004 A
Acclimation Period 14 days
Acclimation Temp _____
Range _____ Mean _____
EC50 Test _____ Ref Tox. _____

Beginning Date 2/4/91 Time 13:40
Ending Date _____ Time _____
Test: Static, flow through, Renewal _____
Test Container 25-gal carboys
Test Volume 50
Test Duration 9/6 hrs
Test Temp: Range _____
QC Completed _____

[illegible]

AQUATIC TOXICOLOGY BENCH SHEET

Project No.: 71003 10

Client: Naval Research

QC Test No.: ET-020491-C94

Date/Time	Activity	Investigator
2/5/91 1400	Test was aerated. Dissolved oxygen dropped to below 1.0 in all concentrations except 4200 ppm and control.	L
1.4	In 4200 ppm is 4.2 (57%) Fish in 4200 ppm are at the surface of the water, rolling on their side, ventilating rapidly.	L
2/7/91 1515	Fish in 4200 ppm remain floating at surface, not swimming. Fish in 2400 ppm are all alive but also remain at the surface of the water, swimming slowly.	LS
2/7/91 1.45	DO in the 4200 ppm was 3.7 and 5.1 for reps A & B respectively RS	RS

AQUATIC TOXICOLOGY LABORATORY BENCH SHEET

PROJECT NO: 70003.10
 CLIENT: Naval Research
 TEST NO: FT-020491-094

TEST SET-UP

TEST INITIATION

Date/Time	Initials	Activity
2/4/91 1030	RSH	Dilutions made
1100	LS, RSH	Test Vessels filled
1340	LS	Transferred organisms
1356	RSH	Head Counts

COMMENTS:

TEST CONCENTRATION SERIES

Test Conc. (% or mg/l)	Volume Test Material (ml)	Final Volume
Control	0	60 l
420 ppm	25.2	↓
750	45.0	
1350	81.0	
2400	144.0	
4200	252.0	

INTERMEDIATE DILUTION PREPARATION AND FEEDING

Dilution Preparation

Food: _____

Feeding

Day	Time	Initials	Sample/Diluent	Day	Time	Initials	Time	Initials	Amt	Amt	Amt
0	1230	RSH	ATI-018 / 20% salt	0							
1	1200	LS	ATI-018 / 20% salt	1							
2	1305	LS	ATI-065 / 20% salt	2							
3	1200	LS	ATI-065 / 20% salt	3							
4				4							
5				5							
6				6							

COMMENTS:

Aquatic Organism Acute Toxicity Test Data Sheet

Project No.: 1000310

Test Article: 1.5 mg/Lyngbya Feam 6%

Client: Alvin Research

QC Test No.: FT-020991-094

Sample No.: 171-198

Dilution No.: 20% salt water

TEST: Static, Flow through, Renewal

Test Container: 2.5 gal aquaria

Test Volume: 5 L

Test Duration: 72 hrs

TEST ORGANISM

Common Name: Mummichog

Scientific Name: Fundulus heteroclitus

Lot No.: FU-001A

Source: Hampton Roads

Acclimation: 14 d or Age 96

Beginning Date: 3/1/91 Time: 1340

Ending Date: 3/8/91 Time: 1430

Sample Diluent

Alkalinity

Hardness

Conductivity

Conc. or %	Test Container No.	All Tests																				Salt or Brackish Water Tests									
		Number of Live Organisms					Temperature (C)					Dissolved Oxygen (mg/liter)					pH					Salinity (ppt)									
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A	10	10	10	10	10	19.5	20.2	20.9	19.8		7.3	7.8	8.0	8.1		8.4	8.4	8.4	8.4		19.5	19.5	19.6	19.9						
	B	10	10	10	10	10																									
1/20	A	10	10	10	10	10	19.4	20.2	20.9	19.7		7.4	7.8	7.9	8.1		8.4	8.4	8.4	8.3		19.5	19.5	19.5	19.5						
	B	10	10	10	10	10																									
750	A	10	10	10	10	10	19.3	20.2	20.8	19.6		7.4	7.7	7.9	8.0		8.4	8.4	8.4	8.3		19.5	19.5	19.4	19.8						
	B	10	10	10	10	10																									
1350	A	10	10	10	10	10	19.2	20.3	20.9	19.6		7.4	7.8	7.9	8.0		8.4	8.4	8.4	8.3		19.2	19.5	19.7	19.9						
	B	10	10	10	10	10																									
2400	A	10	10	10	10	10	19.2	20.2	20.9	19.6		7.3	7.7	8.0	8.0		8.4	8.4	8.4	8.3		19.5	19.6	19.9	19.8						
	B	10	10	10	10	10																									
4200	A	10	10	10	8	3	19.2	20.2	20.9	19.6		7.3	7.7	7.9	8.0		8.3	8.4	8.4	8.3		19.2	19.5	19.9	19.8						
	B	10	10	10	6	2																									
Instrument Number		90	90	90	90		2	2	2	2		90	90	90	90		1	1	1	1											
Correction Factor		7/10	7/10	7/10	7/10		8.5	8.5	8.6	8.7		7/10	7/10	7/10	7/10		0.0	0.0	0.0	0.0											
Time		1356	1358	1342	1400	1110	1322	1348	1431	1440		1110	1337	1426	1412		1113	1347	1420	1428											
Investigators		LS	LS	LS	LS	LS	LS	LS	LS	LS		LS	LS	LS	LS		LS	LS	LS	LS											

Aquatic Organism Acute Toxicity Test Data Sheet

Project No.: 7000310 TEST: Static (Flow through, Renewal) TEST ORGANISM: Mummichog Beginning Date: 1/5/11 Time: 1345
 Test Article: Fine Fighting Fertilizer Test Container: 25g 11. Aquarium Common Name: Mummichog Ending Date: 2/1/11 Time: 1345
 Client: Naval Research Test Volume: 52 L Scientific Name: F. heteroclitus Sample Diluent:
 QC Test No.: FT 012891076 Test Duration: 96 hrs. Lot No.: F0-004 Alkalinity:
 Sample No.: AF1-017 Source: Hampton Roads Hardness:
 Dilution No.: 20% salt water Acclimation: 18 days or Age 92 days Conductivity:

Conc. or %	Test Container No.	All Tests															Salt or Brackish Water Tests									
		Number of Live Organisms					Temperature (C)					Dissolved Oxygen (mg/liter)					pH					Salinity (ppt)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Control	A	10	10	10	10	10	18.7	17.3	17.3	20.2		8.2	8.0	8.3	8.0		8.5	8.5	8.4	8.4		18.7	17.5	19.5	19.0	
	B	10	10	10	10	10																				
115 ppm	A	10	10	10	10	10	18.7	17.2	17.2	20.2		8.1	8.1	8.3	8.0		8.5	8.5	8.4	8.4		18.7	17.5	19.5	19.3	
	B	10	10	10	10	10																				
240 ppm	A	10	10	10	10	10	18.6	17.1	17.2	20.0		8.1	8.1	8.2	8.0		8.4	8.7	8.4	8.4		18.6	17.0	19.5	19.3	
	B	10	10	10	10	10																				
490 ppm	A	10	10	10	10	10	18.6	17.0	17.1	20.0		8.1	8.2	8.3	8.0		8.4	8.7	8.4	8.4		18.5	17.0	19.5	19.4	
	B	10	10	10	10	10																				
1000 ppm	A	10	10	10	10	10	18.5	17.0	17.1	20.2		8.3	8.2	8.3	7.9		8.4	8.4	8.4	8.4		18.5	17.5	19.5	19.5	
	B	10	10	10	10	10																				
1100 ppm	A	10	10	0	-	-	18.7	17.0	-	-		8.2	8.2	-	-		8.4	8.4	-	-		18.5	17.0	-	-	
	B	10	10	0	-	-																				
Instrument Number							011	90	90	90		2	2	2	2		011	90	90	90		3	3	3	3	
Correction Factor							NA	NA	NA	NA		8.7	8.4	8.6	8.8		7/10	7/10	7/10	7/10		-0.5	-0.5	-0.5	-0.5	
Time							1355	1345	1320	1345	1345	1210	1141	1138	1131		1210	1141	1137	1126		1200	1141	1132	1129	
Investigators							LS	LS	LS	LS	ASH	ASH	ASH	ASH	ASH		ASH	ASH	ASH	ASH		ASH	ASH	ASH	ASH	

Aquatic Organism Acute Toxicity Test Data Sheet

Project No.: 7000 2.10

Test Article: First Fighting Form 3%

Client: Naval Research

QC Test No.: FT 0128 91076

Sample No.: AF1-017

Dilution No.: 20% salt H₂O

TEST: Static (Flow through) Renewal

Test Container: 250 mL Aquaria

Test Volume: 5 L. line

Test Duration: 72 hrs

TEST ORGANISM:

Common Name: Mummichog

Scientific Name: Fundulus heteroclitus

Lot No.: 1017

Source: Hampton Roads SD

Acclimation: 10 days or Age 92 days

Beginning Date: 1.23.77 Time: 1:54

Ending Date: Time:

Sample Diluent

Alkalinity:

Hardness:

Conductivity:

TOLD

		All Tests																				Salt or Brackish Water Tests										
Conc. or %	Test Container No.	Number of Live Organisms					Temperature (C)					Dissolved Oxygen (mg/liter)					pH					Salinity (ppt)										
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	
Control	A							11.2	19.4	19.3	18.9		7.2	7.9	7.7	8.2		8.4	8.3	8.4		19.0		19.6	18.5							
	B								19.3		16.7			7.9		8.3		8.4		8.4			19.5		18.6							
115 ppm	A							11.2		19.3	18.8		5.4		7.4	7.6		8.2		8.2	8.2		19.1		19.5	18.7						
	B								19.5		19.1			6.7		7.2			8.2		8.2			20.0		19.0						
240 ppm	A							19.3		19.4	19.0		4.8		6.3	6.2		8.2		8.0	8.0		19.2		19.7	19.3						
	B								19.2		18.6			6.7		5.8			8.0		7.4			20.0		19.2						
490 ppm	A							17.6		19.4	18.9		5.0		7.1	7.3		8.2		8.0	8.0		18.5		19.7	19.5						
	B								19.4		18.7			7.3		7.5			8.0		8.0			20.2		19.5						
1000 ppm	A							18.1		17.4	19.0		4.4		6.5	6.4		8.0		7.9	7.4		18.5		19.7	19.3						
	B								19.3		19.1			6.0		5.4			7.8		7.8			20.3		19.5						
2100 ppm	A							17.1	19.5				4.5	2.0 (24%)				7.6				18.5	20.5									
	B								19.7					3.7 (45%)				7.7					20.8									
Instrument Number								90	90	90	90		2	2	2	2		90	90	90	90		3	3	3	3						
Correction Factor								NA	NA	NA	NA		8.4	8.6	8.8	8.8		7.0	7.0	7.0	7.10		0.5	0.5	0.5	0.5						
Time								11:51	11:28	11:27	13:20		11:41	11:40	11:35	13:30		11:28	11:28	11:27	13:20		11:28	11:34	11:30	13:40						
Investigators								RSN	RSN	RSN	RSN		RSN	RSN	RSN	RSN		RSN	RSN	RSN	RSN		RSN	RSN	RSN	RSN						

ACUTE TOXICITY TEST OBSERVATION DATA SHEET

Project No. 7000-210
Client Naval Research
QC Test No. ET 012 191 076
Test Article Two Highpurity Iron 50%
Sample No. A11207
Diluent 20% nitric acid
Diluent No. NA

Test Organism E. coli
Lot No. 100-100
Acclimation Period 12 days
Acclimation Temp. _____
Range _____ Mean _____
ICB: Test _____ Ref. Test _____

Beginning Date 1/28/77 Time 1300
Ending Date _____ Time _____
Test: Static, Flow through, Renewal _____
Test Container 2 5 gal _____
Test Volume 5.1 _____
Test Duration 76 hrs _____
Test Temp: Range _____
QC Completed _____

Fig. 1. *Phragmites* and *Lythrum*[illegible]

AQUATIC TOXICOLOGY LABORATORY BENCH SHEET

PROJECT NO: 7000110

CLIENT: Naval Research

TEST NO: FT 012371026

TEST SET-UP

TEST INITIATION

Date/Time	Initials	Activity
1/28 1145	RSH WLM	Dilutions made
1/28 1200	WLM LS	Test Vessels filled
1/28 1345	RSH	Transferred organisms
1/28 1355	LS	Head Counts

COMMENTS:

TEST CONCENTRATION SERIES

Test Conc. (% or mg/l)	Volume Test Material(ml)	Final Volume
Control	0	60 L
115 ppm	6.7 ml	
240 ppm	14.4 ml	
440 ppm	24.4 ml	
1000 ppm	60 ml	
2100 ppm	126 ml	

INTERMEDIATE DILUTION PREPARATION AND FEEDING

Dilution Preparation

Food: _____

Feeding

Day	Time	Initials	Sample/Diluent	Day	Time	Initials	Time	Initials	Amt	Amt	Amt
0	1145	WLM RSH	ATI-017/20% SW	0							
1	1030	RSH, LS	ATI-017/20% SW	1							
2	1025	LS, RSH	ATI-017/20% SW	2							
3	1035	LS, RSH	ATI-017/20% SW	3							
4				4							
5				5							
6				6							

COMMENTS:

AQUATIC TOXICOLOGY BENCH SHEET

Project No.: 70003.10

Client: Naval Research

EC Test No.: FT 012891076

Date/Time	Activity	Investigator
1/28/91 1700	DO in 100% was 89% saturation 7.35 Fish were showing abnormal behavior. At the surface on their sides very sluggish some are rolling very inactive	RS14

Flow rates not started 1/28/91 1744 RCH JLB

Flow	mL/min
Control	42
115	42
240	36
490	39
1000	39
2100	39

1/29/91 1345 Activity of fish in 1000 ppm is observed; LS
fish swimming at surface of water.
All fish in 2100 ppm are alone, but
must remain at the surface rolled
onto their side.

1635 DO of 2100 ppm is 28.5% (2.62) LS
2100 ppm is 39.0% (3.19)
1000 ppm is 52.0% (4.79) - test to
1000 ppm is 49.6% (4.55)

1/29/91 1700 Test was started LS RS14

1/30/91 1650 DO of 1000 ppm is 5.6 (69%) LS
6.0 (74.5%)

2/1/91 1345 Organisms in the 1000 ppm concentration were
swimming at the surface somewhat lethargic RS14

Length-Weight Data Sheet

Naval Research

Project No(s): 70003.10Investigator: SCG GM 1100QC Test No(s): FT-012891-076Time, Date: 2-5-91Test Species: Fundulus heteroclitusCalibrated balance 1g 100g 1000gLot No.: Fu-004

Individual No.	Weight (g)	Standard Length (mm)
1	0.17	21.09
2	0.27	24.36
3	0.25	23.46
4	0.23	22.64
5	0.28	24.67
6	0.18	20.08
7	0.16	20.09
8	0.26	23.93
9	0.24	23.10
10	0.18	21.28
11	0.29	23.49
12	0.28	24.49
13	0.23	22.60
14	0.27	23.26
15	0.27	24.50
16	0.19	21.72
17	0.29	25.06
18	0.16	20.36
19	0.23	21.65
20	0.17	21.10
21	0.20	21.49
22	0.24	23.29
23	0.21	22.42
24	0.15	20.90
25	0.17	21.07
26	0.24	23.04
27	0.32	24.76
28	0.25	23.51
29	0.15	20.84
30	0.22	23.11
Range	0.15 - 0.32	20.08 - 25.16
Mean	0.225	22.582
S.D.	0.0483343	1.5169193

Calculations checked:
2-7-91 KZ

Length-Weight Data Sheet

NAVAL RESEARCH

Project No(s): 70003 10Investigator: LSQC Test No(s): FT-094Time, Date: 3/6/91Test Species: F. heteroclitusLot No.: ES-00002

Individual No.	Weight (g)	Standard Length (mm)
1	0.15	20.02
2	0.25	23.51
3	0.39	26.4
4	0.35	25.44
5	0.25	23.97
6	0.21	21.91
7	0.18	21.07
8	0.23	23.77
9	0.21	22.70
10	0.31	23.90
11	0.25	23.77
12	0.20	23.16
13	0.26	23.39
14	0.20	21.34
15	0.19	21.77
16	0.38	26.4
17	0.31	24.92
18	0.43	28.00
19	0.25	23.51
20	0.17	19.77
21	0.45	22.95
22	0.26	24.22
23	0.37	25.88
24	0.27	23.43
25	0.30	24.91
26	0.28	24.20
27	0.21	22.04
28	0.28	24.28
29	0.31	25.23
30	0.21	23.17
Range	0.15 - 0.45	19.77 - 26.41
Mean	0.27	23.49
S.D.	0.077	1.67

QC
3/14/91
mal

Aquatic Toxicology Laboratory
15 Loveton Circle
Sparks (Baltimore), Maryland 21152

Client: Naval Research Project No.: 70003-10

FOR OFFICE USE ONLY

Species to be tested:

_____ <i>D. magnus</i>	_____ <i>Manduca</i> sp.
_____ <i>D. pulex</i>	_____ <i>P. pupio</i>
_____ <i>Cardenebris</i> sp.	_____ <i>C. variegatus</i>
_____ <i>P. promelas</i>	_____ <i>M. harris</i>
_____ Other	_____ Other

A=Acute C=Chronic B=Bioaccumulation

[illegible]

Sampled By:	Date/Time	Received By:	Date/Time
Relinquished By:	Date/Time	Received By:	Date/Time
Relinquished By:	Date/Time	Received By: Laboratory: <i>L. Smith</i>	Date/Time: <i>2/5/91 1045</i>

Client Purchase Order Number: _____

Comments:

Sample Shipped By: (circle)

Fed. Ex.

Puro.

UPS

Airborne

Other:

Air Bill Number:

Sample Temperature at Receipt: _____ C

ATTACHMENT 1111

STATISTICS

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*****
[190,192]STEFZZ.BAC      14-Feb-91      09:31 AM
TEST NAME: NAVAL RESEARCH F. HETEROCLITUS FT-02-04-91-094 96-HR LC50
*****
CONC.      NUMBER      NUMBER      PERCENT      BINOMIAL
           EXPOSED      DEAD        DEAD        PROB.(PERCENT)
4200       20           15          75          2.06947
2400       20           0            0          LESS THAN 0.001

```

THE BINOMIAL TEST SHOWS THAT 2400 AND 4200 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 OF 3613.01 IS OBTAINED BY NONLINEAR INTERPOLATION BETWEEN 2400 AND 4200

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE METHOD NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE REASONABLE.

[190,192]STEPZZ.BAC 14-Feb-91 09:30 AM
 TEST NAME: NAVAL RESEARCH F. HETEROCLITUS FT-01-28-91-076 96-HR LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
2100	20	20	100	LESS THAN 0.001
1000	20	0	0	LESS THAN 0.001

THE BINOMIAL TEST SHOWS THAT 1000 AND 2100 CAN BE
 USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
 CONFIDENCE LIMITS BECAUSE THE ACTUAL CONFIDENCE LEVEL
 ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 OF 1449.14 IS OBTAINED BY
 NONLINEAR INTERPOLATION BETWEEN 1000 AND 2100

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
 PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING
 AVERAGE METHOD NOR THE PROBIT METHOD CAN GIVE ANY
 STATISTICALLY SOUND RESULTS.

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE REASONABLE.

ATTACHMENT IV
REPORT/ANALYSIS REVIEW RECORD

CLIENT THE ROYAL CANADIAN MOUNTED POLICE DATE SUBMITTED _____
PROJECT NUMBER 7063 DATE DUE TO CLIENT APR 89
PROGRAM Alternative Toxicology QC TEST NO. _____
AUTHOR W. McCallum / K. L. Lee TYPE ANALYSIS _____

QA/QC ITEM	REVIEWER	DATE
1. Samples/data collected, transported, and received according to study plan requirements.	<u>W. H. H. H.</u>	<u>5 Mar 91</u>
2. Samples prepared and processed according to study plan requirements.	<u>W. H. H. H.</u>	<u>5 Mar 91</u>
3. Samples and data collected and analyzed using calibrated equipment.	<u>W. H. H. H.</u>	<u>5 Mar 91</u>
4. Quality control activities completed.	<u>W. H. H. H.</u>	<u>5 Mar 91</u>
5. Data checked for correctness, source identification, and data confirmation.	<u>W. H. H. H.</u>	<u>5 Mar 91</u>
6. Calculations checked:		
- Hand calculations checked by reviewer	<u>W. H. H. H.</u>	<u>5 Mar 91</u>
- Documented and verified computer programs used.	<u>W. H. H. H.</u>	<u>5 Mar 91</u>
7. Results reviewed for compliance with study plan requirements.	<u>W. H. H. H.</u>	<u>5 Mar 91</u>
8. Reported results and facts checked against original sources.	<u>W. H. H. H.</u>	<u>5 Mar 91</u>
9. Figures and tables checked for correct presentation of data.	<u>W. H. H. H.</u>	<u>5 Mar 91</u>

EA-0A-07