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REPORT OF TEST

Aquatic Toxicity Tests versus
Oncorhynchus mykiss,
Daphnia pulex, and
Selenastrum capricornutum

COLDFIRE 302

Conducted for:

North American Environmental Oil and
Chemical Cleaning Supply Company
270A Route 46
Rockaway, New Jersey 07866

March 11, 1993

To the best of our knowledge, this study was conducted in compliance with the Good Laboratory Practice Standards of: United States EPA 40 CFR, Part 792. Data have been archived at the above laboratory address.

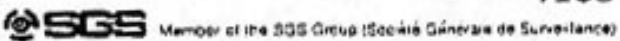
Daniel Cooke 3/11/93
Project Director Date

TEST REPORT NO. 065316

SIGNED FOR THE COMPANY

BY
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AQUATIC TOXICITY REPORT

Client: North American Environmental Oil
& Chemical Cleaning Supply Company
270A Route 46
Rockaway, New Jersey 07866

Manufacturer:

Fire-Freeze Worldwide, Inc.
270A Route 46
Rockaway, New Jersey 07866

Testing Facility: United States Testing Company
Biological Services Division
1415 Park Avenue
Hoboken, New Jersey 07030

Sample Description,

Handling & Stability: Identified by client as ColdFire 302, fire
suppressor: Class A/B Fire Suppressing Agent.
Straw colored, mobile liquid, with a mild lemon
odor, water soluble. Sample considered stable.
Sample received 2/ 1/93.

Project: 96 hour Acute LC50 versus Oncorhynchus mykiss (rainbow trout)
48 hour Acute LC50 versus Daphnia pulex (water flea)
96 hour Acute EC50 versus Selenastrum capricornutum (alga)

Test Dates: 2/7 - 3/2/93

Summary of Results:

The acute toxicity of ColdFire 302 to the rainbow trout, Oncorhynchus mykiss, was found to be:

96 hour LC50 = 105.1 ppm
The No Observed Effect Concentration (NOEC) was 62.5 ppm.

The acute toxicity of ColdFire 302 to the water flea, Daphnia pulex, was found to be:

48 hour LC50 = 159.3 ppm
The No Observed Effect Concentration (NOEC) was 62.5 ppm.

The acute toxicity of ColdFire 302 to the freshwater alga, Selenastrum capricornutum, was found to be:

96 hour EC50 = 153.9 ppm
The No Observed Effect Concentration (NOEC) was <93.75 ppm.

INTRODUCTION:

This is a report of aquatic toxicity testing versus North American Environmental Oil & Chemical Cleaning Supply Company and Fire-Freeze International's product ColdFire 302 fire suppressant.

Testing was performed versus rainbow trout (O. mykiss), water fleas (D. pulex), and algae (S. capricornutum). Test solutions were diluted with media suitable for survival and growth of each of the organisms. Observations for possible adverse effects were made initially and daily for the duration of the tests.

Stock solutions of ColdFire 302 were prepared by adding measured amounts of product to test water. The solutions were thoroughly mixed, and added directly to the test chambers.

No chemical confirmation of concentration was performed for these tests. All concentrations were prepared volumetrically (from the saturated solution), and were judged by the investigators to be satisfactory.

QA REPORT

Sponsor: North American Environmental Oil
& Chemical Cleaning Supply Company

Study: Aquatic Toxicity versus trout, daphnia and algae

Report: 065318-1

Project Start: February 10, 1993

Project Finish: March 11, 1993

To the best of our knowledge, this study was conducted in compliance with the Good Laboratory Practice Standards of US EPA 40 CFR, Part 792.

The studies were conducted at the Biological Services Division of USTC, in a setting which involved frequent repetition of similar or identical procedures. At or about the time the studies were conducted, inspections were made by the QA auditor of the critical procedures relevant to this study type.

The findings of these inspections were reported promptly to the study director and management.

To the best of our knowledge and belief, the final report accurately reflect the conduct of the study, the data obtained and the conclusions that can be shown, within the limits of the procedures used.


James Siniscalchi, PhD
Quality Assurance Auditor

SUMMARY OF PROCEDURES: Acute Toxicity versus Oncorhynchus mykiss
(rainbow trout), 96 hour LC50

Reference : 40 CFR Part 797.1400 "Fish acute toxicity test"
USEPA 1989.
USTC Procedure PRO/FT FISH 224-7.

Sample storage : Room temp (21°C) original, sealed container.

Test type : Static, renewal

Organism source : Aquatic Research Organisms, Hampton, NH

Organism history: Hatch: 12/23/93 - 1/3/93

Organism age : 52 - 64 days

Organism size : ≤ 35 mm, uniform size

Temperature(°C) : $12 \pm 2^\circ\text{C}$

Illumination : 16:8 hour light/dark cycle, fluorescent,
50 to 100 ft-candles (lab ambient)

Test vessels : 4L polypropylene vessels

Exposure volume : 3L

Replication : Minimum 10 fish per replicate
2 replicates per treatment

Feeding regime : None during test

Aeration : Aerate by mixing test solutions to saturation
prior to test; if dissolved oxygen falls below
80 percent saturation in any replicate during
the test, supply oil free air at 100 ± 10
bubbles per minute.

Concentrations : Minimum 5

Dilution Factor : approximately 0.5

Dilution Water : US EPA hard reconstituted water

Solvent : None necessary

Controls : Diluent only

SUMMARY OF PROCEDURES: Acute Toxicity versus Oncorhynchus mykiss
(rainbow trout), 96 hour LC50 (continued).

Controls : Diluent only

Test duration : 96 hours

Response(s) : Mortality, reflex loss, erratic swim daily

Physical data : Temperature, D.O., pH, conductivity initially
and daily thereafter

Chemical data : Alkalinity and hardness of control, initially
TOC and TSS of dilution water prior to test
initiation

Acceptability : $\geq 90\%$ survival in controls after 96 hours

Data analysis : Probit Analysis, Spearman - Karber Method,
or graphical interpolation for lethality

Special comments: None

Deviations from
Test Method : None

Acute Toxicity versus Oncorhynchus mykiss

Sample: ColdFire 302

Screening Tests (2/7 - 8/93)

The following mortality data is from initial range finding screens. The screens were performed on a wide range of test product concentrations; from this data, an approximate range of toxicity was determined. The range of toxicity determined in the screen was then bracketed in the definitive assay in order to determine the LC50.

		24 hour Mortality vs. Concentration						
		10,000	1,000	100	10	1.0	0.1	0
ColdFire 302		5/5	5/5	5/5	0/5	0/5	0/5	0/5

Acute Toxicity versus Oncorhynchus mykiss

Sample: ColdFire 302

Test Dates: 2/24 - 28/93

Conc. ppm	No Org.	Cumulative Mortality				% Mortality
		24hr	48hr	72hr	96hr	
0 (control)	20	0	0	0	0	0
31.25	20	0	0	0	0	0
62.5	20	0	0	0	0	0
125	20	13	13	15	15	75
250	20	20	20	20	20	100
500	20	20	20	20	20	100

Data Summary:

24hr LC50 = 112.7 ppm (95% C.L. 97.2 - 130.6)

48hr LC50 = 112.7 ppm (95% C.L. 97.2 - 130.6)

72hr LC50 = 105.1 ppm (95% C.L. 91.9 - 120.2)

96hr LC50 = 105.1 ppm (95% C.L. 91.9 - 120.2)

Statistical Method: Spearman-Karber Trim

The No Observed Effect Concentration (NOEC) was determined to be 62.5 ppm.

Comments:

A stock solution of ColdFire 302 was prepared by diluting 6.0 ml of product to 6 liters with EPA hard reconstituted water. Test solutions were diluted with fresh EPA hard water to the above concentrations.

Though there was no mortality observed in the 62.5 ppm concentration, the exposed fish appeared discolored (dark) after 24 hours. After 96 hours, the fish in the 62.5 ppm concentration appeared to be in a state of torpor. The fish exposed to 31.25 ppm ColdFire 302 did not appear to suffer any adverse effects.

Juvenile fish were used for this assay (<8 weeks old). Factors such as age and small size generally maximize toxic effect, numerically expressed as the LC50.

Acute Toxicity versus Oncorhynchus mykiss

Sample: Coldfire 302

Test Dates: 2/24 - 28/93

Physical / Chemical Data

Test Conc	Control	31.25 ppm	62.5 ppm	125 ppm	250 ppm	500 ppm
Temp (°C)						
mean (N=5)	11.1	11.1	11.1	11.1	11.1	11.1
range	11.0-11.5	11.0-11.5	11.0-11.5	11.0-11.5	11.0-11.5	11.0-11.5
D.O. (mg/L)						
mean (N=8)	9.6	9.6	9.6	9.6	9.6	9.6
range	9.4 -9.8	9.4 -9.8	9.4 -9.8	9.4 -9.8	9.4 -9.8	9.4 -9.8
pH (std)						
mean (N=8)	7.9	7.9	7.9	7.9	8.0	8.0
range	7.8 -8.1	7.8 -8.1	7.8 -8.1	7.8 -8.1	7.8 -8.1	7.8 -8.1
Cond (µmhos)						
mean (N=5)	366	366	366	366	350	350
range	350 - 380	350 - 380	350 - 380	350 - 380	350	350
Alk (mg/L)						
mean (N=5)	150					
range	140 - 160					
Hard (mg/L)						
mean (N=3)	136					
range	130 - 150					
TOC (mg/L)						
mean (N=1)	<2.0					
range	<2.0					
TSS (mg/L)						
mean (N=1)	0					
range	0					

N = number of determinations used in calculation of mean and range.
 Conc = concentration, Temp = temperature, D.O. = dissolved oxygen, pH given
 in standard units, Cond = conductivity, Alk = Alkalinity (mg/L CaCO₃), Hard
 = Hardness (mg/L CaCO₃), TOC = total organic carbon, TSS = total suspended
 solids.

SUMMARY OF PROCEDURES: Acute Toxicity versus Daphnia pulex
(water flea) 48 hour LC50.

Reference : 40 CFR Part 797.1300, 9/27/85,
"Daphnid acute toxicity test"
USTC Procedure PRO/ST DAPHNIA 231-1.

Sample storage : Room temperature (21°C) original, sealed container.

Test type : Static, non-renewal

Organism source : USTC stock cultures

Organism history: Hatch: 2/27 - 28/93

Organism age : ≤ 24 hours

Temperature(°C) : 22 ± 1°C

Illumination : 16:8 hour light/dark cycle, fluorescent,
50 to 100 ft-candles (lab ambient)

Test vessels : 25 x 150 mm glass test tubes, capped

Exposure volume : 40 ml

Replication : Minimum 5 daphnia per replicate
4 replicates per treatment

Feeding regime : None during test

Aeration : Aerate by mixing test solutions to saturation prior
to test; no aeration during test.

Concentrations : Minimum 5

Dilution Factor : Approximately 0.5

Dilution Water : US EPA hard reconstituted water

Solvent : None

Controls : Diluent only

SUMMARY OF PROCEDURES: Acute Toxicity versus Daphnia pulex
(water flea) 48 hour LC50 (continued).

Controls : Diluent only

Test duration : 48 hours

Response(s) : Mortality, morbidity, and appearance

Physical data : Temperature, D.O., pH, conductivity initially
and at test termination

Chemical data : Alkalinity and hardness of control, initially
TOC and TSS of dilution water prior to test
initiation

Data analysis : Probit Analysis or Spearman - Karber Method when
possible, otherwise, graphical interpolation

Special comments: None

Deviations from
Test Method : None

Acute Toxicity versus Daphnia pulex

Sample: ColdFire 302

Screening Tests (2/7 - 8/93)

The following mortality data is from initial range finding screens. The screens were performed on a wide range of test product concentrations; from this data, an approximate range of toxicity was determined. The range of toxicity determined in the screen was then bracketed in the definitive assay in order to determine the LC50.

	24 hour Mortality vs. Concentration						
	10,000	1,000	100	10	1.0	0.1	0
ColdFire 302	0/5	0/5	5/5	0/5	0/5	0/5	0/5

Acute Toxicity versus Daphnia pulex

Sample: ColdFire 302

Screening Tests (2/7 - 8/93)

The following mortality data is from initial range finding screens. The screens were performed on a wide range of test product concentrations; from this data, an approximate range of toxicity was determined. The range of toxicity determined in the screen was then bracketed in the definitive assay in order to determine the LC50.

	24 hour Mortality vs. Concentration						
	10,000	1,000	100	10	1.0	0.1	0
ColdFire 302	0/5	0/5	5/5	0/5	0/5	0/5	0/5

Acute Toxicity versus Daphnia pulex

Sample: ColdFire 302

Test Dates: 2/28 - 3/2/93

Physical / Chemical Data

Test Conc.	Control	31.25ppm	62.5 ppm	125 ppm	250 ppm	500 ppm
Temp (°C)						
mean (N=3)	21.0	21.0	21.0	21.0	21.0	21.0
range	21.0	21.0	21.0	21.0	21.0	21.0
D.O. (mg/L)						
mean (N=2)	9.0	9.0	9.0	9.0	9.0	9.0
range	8.4 -9.6	8.4 -9.6	8.4 -9.6	8.4 -9.6	8.4 -9.6	8.4 -9.6
pH (std)						
mean (N=2)	8.2	8.2	8.2	8.2	8.2	8.2
range	8.1 -8.2	8.1 -8.2	8.1 -8.2	8.1 -8.2	8.1 -8.2	8.1 -8.2
Cond (µmhos)						
mean (N=2)	415	415	415	415	415	415
range	370 -460	370 -460	370 -460	370 -460	370 -460	370 -460
Alk (mg/L)						
mean (N=1)	140					
range	140					
Hard (mg/L)						
mean (N=1)	130					
range	130					
TOC (mg/L)						
mean (N=1)	<2.0					
range	<2.0					
TSS (mg/L)						
mean (N=1)	0					
range	0					

N = number of determinations used in calculation of mean and range.
 Conc = concentration, Temp = temperature, D.O. = dissolved oxygen, pH given in standard units, Cond = conductivity, Alk = Alkalinity (mg/L CaCO₃), Hard = Hardness (mg/L CaCO₃), TOC = total organic carbon, TSS = total suspended solids.

SUMMARY OF PROCEDURES: Acute Toxicity versus Selenastrum capricornutum
(freshwater alga) 96 hour EC50.

Reference : 40 CFR Part 797.1050 "Algal acute toxicity test"
USEPA 1987.
USTC Procedure ALGAE 224-7.

Sample storage : Room temp (21°C) original, sealed container.

Test type : Static, non-renewal

Organism source : USTC stock cultures, originally from UTEX

Temperature(°C) : 24 ± 2°C

Illumination : 16:8 hour light/dark cycle, fluorescent,
400 to 450 ft-candles

Test vessels : 125 ml erlenmeyer flasks, capped

Exposure volume : 50 ml

Replication : 3 replicates per treatment

Inoculum density: Initially 10,000 cells per ml

Agitation : Shaken by hand four to five times daily

Aeration : Aerate by mixing test solutions to saturation
prior to test; no aeration during test.

Concentrations : Minimum 5
Dilution factor 0.5

Dilution Water : EPA formulation
(media)

Solvent : None

Controls : Diluent only

SUMMARY OF PROCEDURES: Acute Toxicity versus Selenastrum capricornutum
(freshwater alga) 96 hour EC50 (continued).

Controls : Diluent only

Test duration : 96 hours; inhibited replicates inoculated into fresh control media and subcultured up to 9 days for algicidal/algistatic determination

Response(s) : Cell counts by hemocytometer, irregular cell shapes or decrease in cell size noted, cell mortality characterized

Physical data : Temperature and illumination initially and daily, pH initially and at test termination

Chemical data : Initial alkalinity, hardness, and TOC of control

Data analysis : Probit Analysis, Spearman - Karber Method, or graphical interpolation for EC50 (% inhibition); Dunnett's Test or Steel's Many-One Rank Test for NOEC and LOEC

Special comments: Stock solutions to be pH adjusted to 7.5 prior to use in testing.

Deviations from Test Method : None

Acute Toxicity versus Selenastrum capricornutum

Sample: ColdFire 302

Test Dates: 2/25 - 28/93

Algal Growth Inhibition

Conc	Rep	24 hr			48 hr		
		Cells/ml	Mean	% I	Cells/ml	Mean	% I
Ctrl	A	no counts			26.8	23.1	0
	B				19.4		
	C				23.2		
93.75ppm	A	no counts			19.6	18.6	19.5
	B				18.9		
	C				17.3		
187.5ppm	A	no counts			10.0	10.1	56.4
	B				11.4		
	C				8.8		
375 ppm	A	no counts			3.5	2.8	87.9
	B				3.2		
	C				1.7		
750 ppm	A	no counts			2.1	1.8	92.1
	B				2.6		
	C				0.8		
1500ppm	A	no counts			1.2	1.6	93.1
	B				2.4		
	C				0.9		

Notes: Cells/ml = cells/ml x 10⁴; %I = % inhibition; Mean = pooled meansData Summary:

24hr EC50 = not obtainable

48hr EC50 = 168.9 ppm (95% C.L. 149.5 - 190.9)

Statistical Method: Spearman-Kärber Trim

The 48hr No Observed Effect Concentration (NOEC) was determined <93.75.
No algicidal effects were observed.

Acute Toxicity versus Selenastrum capricornutum

Sample: ColdFire 302

Test Dates: 2/25 - 28/93

Algal Growth Inhibition

Conc	Rep	72 hr			96 hr		
		Cells/ml	Mean	% I	Cells/ml	Mean	% I
Ctrl	A	66.8	68.1	0	76.9	76.9	0
	B	68.7			70.6		
	C	68.7			83.3		
93.75ppmA	A	36.1	36.2	46.8	86.2	81.0	0
	B	36.8			70.9		
	C	35.7			85.8		
187.5ppmA	A	13.6	12.4	81.8	73.8	80.3	0
	B	12.4			80.1		
	C	11.1			87.0		
375 ppm	A	0.5	0.4	99.4	64.2	54.8	28.7
	B	0.4			49.2		
	C	0.3			51.0		
750 ppm	A	0.3	0.3	99.6	52.6	47.5	38.2
	B	0.3			42.0		
	C	0.3			48.0		
1500ppn	A	0.0	0.0	100	42.8	43.5	43.4
	B	0.0			44.1		
	C	0.0			43.5		

Notes: Cells/ml = cells/ml x 10⁴; %I = % inhibition; Mean = pooled means

Data Summary:

72hr EC50 = 99.5 ppm (95% C.L. 73.2 - 135.3)

96hr EC50 = 153.9 ppm (95% C.L. 139.1 - 170.3)

Statistical Method: Spearman-Kärber Trim

The 72hr No Observed Effect Concentration (NOEC) was determined <93.75.

The 96hr NOEC was determined <93.75%.

Algicidal effects were noted in the 750ppm and 1500ppm concentrations.

Acute Toxicity versus Selenastrum capricornutum

Sample: ColdFire 302

Test Dates: 2/25 - 28/93

Comments:

A stock solution of ColdFire 302 was prepared by diluting 10 ml of product to 1.0 liter with algal media, and mixing thoroughly. The resultant solution was diluted with fresh EPA algal media (no product) to the above concentrations.

The initial inoculum was 10,000 cells/ml. Cell growth was insufficient at 24 hours to establish concentration-effect relationships for all concentrations. 24 hour counts were not recorded.

At test termination, each replicate from each test concentration was subcultured into fresh EPA algal media. After six days, the subcultures were examined for the presence of algal growth. Subcultures taken from concentrations below 750 ppm were observed to have a healthy algal population. Subcultures taken from concentrations of 750 ppm and greater did not have any algal growth.

ColdFire 302 appeared to be algicidal (killed algal cells) at concentrations of 750 ppm or greater, and algistatic (slowed or stopped growth, but did not kill algal cells) at concentrations between 93.75 ppm and 750 ppm.

Acute Toxicity versus Selenastrum capricornutum

Sample: ColdFire 302

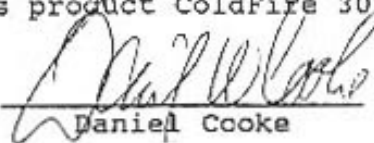
Test Dates: 2/25 - 28/93


Physical / Chemical Data

Test Conc.	Ctrl	93.75 ppm	187.5 ppm	375 ppm	750 ppm	1500 ppm
Temp (°C)						
mean (N=4)	24.5	24.5	24.5	24.5	24.5	24.5
range	24.5	24.5	24.5	24.5	24.5	24.5
pH (std)						
initial	7.5	7.5	7.5	7.5	7.5	7.5
final	9.8	9.7	9.6	8.0	7.5	7.5
Cond (µmhos)						
mean (N=1)	95					
range	95					
Alk (mg/L)						
mean (N=1)	30					
range	30					
Hard (mg/L)						
mean (N=3)	20					
range	20					
TOC (mg/L)						
mean (N=1)	<2.0					
range	<2.0					


N = number of determinations used in calculation of mean and range.
 Conc = concentration, Temp = temperature, D.O. = dissolved oxygen, pH given in standard units, Cond = conductivity, Alk = Alkalinity (mg/L CaCO₃), Hard = Hardness (mg/L CaCO₃), TOC = total organic carbon, TSS = total suspended solids.

Investigators and analysts for the aquatic toxicity study of North American Environmental Oil & Chemical Cleaning Supply Company and Fire-Freeze International's product ColdFire 302:


Daniel Cooke (Study Director)


James Siniscalchi (Quality Assurance)


Dennis Profaca (Analyst)


Rey Rolon (Analyst)


Michael Bernardine (Analyst)

APPENDIX #1
DATA ANALYSIS

BURLINGTON RESEARCH, INC.
 TRIMMED SPEARMAN-KARBER METHOD FOR CALCULATION OF
 EC50 AND LC50 VALUES IN BIOASSAYS

FOR REFERENCE, CITE

M.A. HAMILTON, R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7) 714-719
 CORRECTION 12(4) 417 (1978).

DATE _____ 2/25 - 28/93
 TEST # _____ 065318-1
 CHEMICAL _____ ColdFire 302
 SPECIES _____ *Selenastrum capricornutum*
 DURATION _____ 96 hours

RAW DATA

CONCENTRATION(ppm)	93.75	187.50	375.00	750.00	1500.00
NUMBER EXPOSED	100	100	100	100	100
MORTALITIES	18	64	92	100	100
SPEARMAN-KARBER TRIM			18.00		
SPEARMAN-KARBER ESTIMATES	EC50		153.8969116		
	95% LOWER CONFIDENCE		139.05		
	95% UPPER CONFIDENCE		170.33		

DATE _____ 2/25 - 28/93
 TEST # _____ 065318-1
 SPECIES _____ *Selenastrum capricornutum*
 DURATION _____ 72 hours

RAW DATA

CONCENTRATION(ppm)	93.75	187.50	375.00	750.00	1500.00
NUMBER EXPOSED	100	100	100	100	100
MORTALITIES	47	82	99	100	100
SPEARMAN-KARBER TRIM			47.00		
SPEARMAN-KARBER ESTIMATES	EC50		99.4887009		
	95% LOWER CONFIDENCE		73.16		
	95% UPPER CONFIDENCE		125.87		

DATE _____ 2/25 - 28/93
 TEST # _____ 065318-1
 CHEMICAL _____ ColdFire 302
 SPECIES _____ *Selenastrum capricornutum*
 DURATION _____ 48 hours

RAW DATA

CONCENTRATION(ppm)	93.75	187.50	375.00	750.00	1500.00
NUMBER EXPOSED	100	100	100	100	100
MORTALITIES	20	56	88	92	93
SPEARMAN-KARBER TRIM			20.00		
SPEARMAN-KARBER ESTIMATES	EC50		168.9844666		
	95% LOWER CONFIDENCE		149.55		
	95% UPPER CONFIDENCE		190.94		

BURLINGTON RESEARCH, INC
TRIMMED SPEARMAN-KARBER METHOD FOR CALCULATION OF
EC50 AND LC50 VALUES IN BIOASSAYS

FOR REFERENCE, CITE

N.A. HAMILTON, R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7) 714-717
CORRECTION 12(4) 417 (1978).

DATE _____ 2/24 - 28/93
TEST # _____ 065318-1
CHEMICAL _____ ColdFire 302
SPECIES _____ Oncorhynchus mykiss
DURATION _____ 96 hours

RAW DATA

CONCENTRATION(ppm)	62.50	125.00	250.00
NUMBER EXPOSED	20	20	20
MORTALITIES	0	15	20
SPEARMAN-KARBER TRIM			0.00
SPEARMAN-KARBER ESTIMATES		LC50	105.1120377
		95% LOWER CONFIDENCE	71.91
		95% UPPER CONFIDENCE	120.21

DATE _____ 2/24 - 28/93
TEST # _____ 065318-1
CHEMICAL _____ ColdFire 302
SPECIES _____ Oncorhynchus mykiss
DURATION _____ 72 hours

RAW DATA

CONCENTRATION(ppm)	62.50	125.00	250.00
NUMBER EXPOSED	20	20	20
MORTALITIES	0	15	20
SPEARMAN-KARBER TRIM			0.00
SPEARMAN-KARBER ESTIMATES		LC50	105.1120377
		95% LOWER CONFIDENCE	71.91
		95% UPPER CONFIDENCE	120.21

BURLINGTON RESEARCH, INC.
TRIMMED SPEARMAN-KARBER METHOD FOR CALCULATION OF
EC50 AND LC50 VALUES IN BIOASSAYS

FOR REFERENCE, CITE
M.A. HAMILTON, R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-717
CORRECTION 12(6) 417 (1978).

DATE ----- 2/24 - 28/93
TEST # ----- 065318-1
CHEMICAL ----- ColdFire 302
SPECIES ----- Oncorhynchus mykiss
DURATION ----- 96 hours

RAW DATA

CONCENTRATION(ppm)	62.50	125.00	250.00
NUMBER EXPOSED	20	20	20
MORTALITIES	0	15	20
SPEARMAN-KARBER TRIM			0.00
SPEARMAN-KARBER ESTIMATES		LC50	105.1120377
		95% LOWER CONFIDENCE	91.91
		95% UPPER CONFIDENCE	120.21

DATE ----- 2/24 - 26/93
TEST # ----- 065318-1
CHEMICAL ----- ColdFire 302
SPECIES ----- Oncorhynchus mykiss
DURATION ----- 72 hours

RAW DATA

CONCENTRATION(ppm)	62.50	125.00	250.00
NUMBER EXPOSED	20	20	20
MORTALITIES	0	15	20
SPEARMAN-KARBER TRIM			0.00
SPEARMAN-KARBER ESTIMATES		LC50	105.1120377
		95% LOWER CONFIDENCE	91.91
		95% UPPER CONFIDENCE	120.21

APPENDIX #2
CHEMICAL CONFIRMATION

Acute Toxicity versus ColdFire 302

Appendix #2 - Chemical Confirmation

Regarding aquatic toxicity tests performed according to United States Environmental Protection Agency's "Good Laboratory Practice Standards" (40 CFR, Parts 797-1300 and 797-1400), guidelines recommend confirmation of nominal test concentrations by chemical analysis.

Analytical methodology is generally supplied by the Client, and should be sensitive enough to detect the test compound at environmental levels (levels at which the compound is likely to enter the environment).

Since test concentrations were prepared volumetrically, no confirmation of concentration was performed. The stated concentrations were judged by investigators to be correct.

APPENDIX #3
TOXICITY TEST PLANS AND PROCEDURES

United States Testing Company, Inc.

Test Plan (GLP)

Client: North American Environmental Oil
& Chemical Cleaning Supply Company
270A Route 46
Rockaway, New Jersey 07866

Manufacturer:

Fire-Freeze Worldwide, Inc.
207A Route 46
Rockaway, New Jersey 07866

Testing Laboratory: United States Testing Company, Inc.
Biological Services
1415 Park Avenue
Hoboken, New Jersey 07030

Study Director: Daniel Cooke

Test Material: ColdFire 302, fire suppressor: Class A/B Fire
Suppressing Agent. Straw colored, mobile liquid,
with a mild lemon smell, water soluble. Sample
considered stable, received 2/ 1/93.

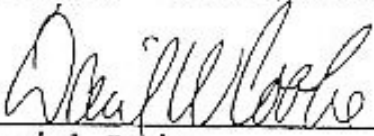
Storage/
Handling Conditions: Room temperature, in original, sealed container,
as per MSDS dated 7/26/92.

Procedures: 96 Hour Acute LC50 versus Oncorhynchus mykiss
96 Hour Acute EC50 versus Selenastrum capricornutum
48 Hour Acute LC50 versus Daphnia pulex

Amendments/
Specifications: See attached protocols

Test Dates: February - March, 1993

Submitted by:


Daniel Cooke
Manager, Ecotoxicology

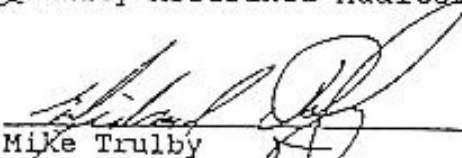
2/3/93
Date

Reviewed by:


James Siniscalchi, Ph.D.
Quality Assurance Auditor

2/18/93
Date

Approved by:
(Client)


Mike Trulby
North American Environmental Oil
& Chemical Cleaning Supply Company

2/10/93
Date

United States Testing Company, Inc.

SUMMARY OF PROCEDURES: Acute Toxicity versus Oncorhynchus mykiss
(rainbow trout), 96 hour LC50

Reference : 40 CFR Part 797.1400 "Fish acute toxicity test"
USEPA 1989.
OECD Guidelines for Testing of Chemicals,
Method 203 "Fish Acute Toxicity Test" 1984.
FDA Environmental Assessment Technical Assistance
Handbook, Method 4.11, "Freshwater Acute Toxicity".
USTC Procedure PRO/FT FISH 224-7.

Sample storage : Room temp (21°C) original, sealed container,
or as specified by MSDS

Test type : Static, renewal

Organism source : Commercial supplier (to be specified)

Organism history: Hatch date and pertinent information

Organism age : in days

Organism size : ≤ 40 mm, uniform size

Temperature(°C) : $12 \pm 2^\circ\text{C}$

Illumination : 16:8 hour light/dark cycle, fluorescent,
50 to 100 ft-candles (lab ambient)

Test vessels : 4L polypropylene vessels

Exposure volume : 3L

Replication : Minimum 10 fish per replicate
2 replicates per treatment

Feeding regime : None during test

Aeration : Aerate by mixing test solutions to saturation
prior to test; if dissolved oxygen falls below
80 percent saturation in any replicate during
the test, supply oil free air at 100 ± 10
bubbles per minute.

Concentrations : Minimum 5

Dilution Factor : approximately 0.5

Dilution Water : US EPA hard reconstituted water

Solvent : As necessary

Controls : Diluent only and solvent control (if necessary)

United States Testing Company, Inc.

SUMMARY OF PROCEDURES: Acute Toxicity versus Selenastrum capricornutum
(freshwater alga) 96 hour EC50 (continued).

- Controls : Diluent only, solvent control (if necessary)
- Test duration : 96 hours; inhibited replicates inoculated into fresh control media and subcultured up to 9 days for algicidal/algistatic determination
- Response(s) : Cell counts by hemocytometer, irregular cell shapes or decrease in cell size noted, cell mortality characterized
- Physical data : Temperature and illumination initially and daily, pH initially and at test termination
- Chemical data : Initial alkalinity, hardness, TSS and TOC of control media
- Data analysis : Probit Analysis, Spearman - Karber Method, or graphical interpolation for EC50 (% inhibition); Dunnett's Test or Steel's Many-One Rank Test for NOEC and LOEC
- Special comments: Nominal concentrations prepared volumetrically (no confirmation).
- Stock solution of product to be adjusted to pH 7.5 prior to test initiation.
- Deviations from Test Method : 40 CFR 797.1050 specifies a light/dark cycle of 14 hrs light /10 hrs dark. An illumination cycle of 16/8 will be used to more closely match lab culture conditions.
- Other deviations to be specified.

United States Testing Company, Inc.

SUMMARY OF PROCEDURES: Acute Toxicity versus Daphnia pulex
(water flea) 48 hour EC50.

Reference : 40 CFR Part 797.1300 "Daphnid acute toxicity test"
USEPA 1987.
OECD Guideline for Testing of Chemicals,
Method 202, "Daphnia sp., Acute Immobilisation
Test" 1984.
FDA Environmental Assessment Technical Assistance
Handbook, Method 4.08 "Daphnia Acute Toxicity".
USTC Procedure PRO/ST DAPHNIA 231-1.

Sample storage : Room temp (21°C) original, sealed container,
or as specified by MSDS.

Test type : Static, non-renewal

Organism source : USTC stock cultures

Organism history: Hatch date and pertinent information

Organism age : \leq 24 hours

Temperature(°C) : $21 \pm 1^\circ\text{C}$

Illumination : 16:8 hour light/dark cycle, fluorescent,
50 to 100 ft-candles (lab ambient)

Test vessels : 25 x 150 mm glass test tubes, capped

Exposure volume : 40 ml

Replication : Minimum 5 daphnia per replicate
4 replicates per treatment

Feeding regime : None during test

Aeration : Aerate by mixing test solutions to saturation
prior to test; no aeration during test.

Concentrations : Minimum 5

Dilution Factor : approximately 0.5

Dilution Water : US EPA hard reconstituted water

Solvent : As necessary

Controls : Diluent only and solvent control (if necessary)

United States Testing Company, Inc.

SUMMARY OF PROCEDURES: Acute Toxicity versus Daphnia pulex
(water flea) 48 hour EC50 (continued).

Controls : Diluent only and solvent control (if necessary)

Test duration : 48 hours

Response(s) : Mortality (immobilization), morbidity, and appearance

Physical data : Temperature, D.O., pH, conductivity initially
and at test termination

Chemical data : Alkalinity, hardness, TSS, and TOC of control
initially.

Data analysis : Probit Analysis, Spearman - Karber Method,
or graphical interpolation

Special comments: Nominal concentrations prepared volumetrically
(no confirmation).

Stock solution of product to be adjusted to pH of
dilution water.

Deviations from
Test Method : To be specified.

HAZARD RANKING OF CHEMICALS

In the Office of Pollution Prevention and Toxics, the established criteria for ranking the acute toxicity of chemicals to the aquatic environment are:

HIGH CONCERN.....	< 1 mg/L
MODERATE CONCERN.....	1 to 100 mg/L
LOW CONCERN.....	> 100 mg/L