LR: 8/52.3

ENVIRONMENTAL LABORATORY
DATA SUMMARY REPORT ON THE TESTS FOR
ACUTE & CHRONIC TOXICITY OF FLUOROCHEMICALS
TO <u>DAPHNIA</u> <u>MAGNA</u> (WATER FLEA)
(9972512600)

TEST SUBSTANCE

Description: FC-143 LOT 264

Additional Identification:

STATIC ACUTE TEST

Begin: 8/2/84 End: 8/4/84 Duration: 48 HOURS

Dose Range: 25, 40, 63, 100, 160, 250, 400, 630 mg/l

Temperature: 22°C

Results:

- 24-Hr. EC_{50} (95% Confidence Limits): 416 (366-472) mg/l
- 48-Hr. EC_{50} (95% C.L.): 266 (231-307) mg/l

RENEWAL CHRONIC TEST

Begin: 8/7/84 End: 8/28/84 Duration: 21 DAYS

Dose Range: 5, 8, 13, 22, 36, 60 mg/l

Temperature: 22±1°C

Results:

	Young Reproduction Impairment	Adults Mortality	
- Approximate NOEC	13		mg/l
- MATC (Limits) GM-MATC	17 (13-22)		mg/l
- 14-Day EC_{50} (95% C.L.)	33 (26-45)	>60	mg/l
- 21-Day EC_{50} (95% C.L.)	38 (35-42)	40 (31-52)	mg/l
- Chronic/Acute Ratios			
- Application Factors (MATC/Acute EC_{50})			
- Env. Safety Factor (MATC/EEC)			

* A statistical significance test was performed using P=0.05.

ACCEPTABILITY OF CHRONIC TEST

The <u>D. magna</u> control population have met the following
quality criteria:

- Adults Mortality: *NONE* (max. 20%)
- First Brood Release: *7 DAYS* (max. 9 days)
- Cumulative No. Young per Adult (14-Day): *72*(min. 20)
- Cumulative No. Young per Adult (21-Day): *165*(min. 40-60)
- Ephippia: *NONE*(none)
- D.O. Saturation: *82%*(min. 60%)
- pH Level: *8.0 to 0.2*(max. ±0.5
                                                                  from initial)

COMMENTS

Reported and Approved by: *[signature]*

Date: *8/31/84*

TABLE I. SUMMARY OF ACUTE TOXICITY OF <u>FC-143, LOT 264</u>

TO <u>DAPHNIA</u> <u>MAGNA</u> UNDER STATIC EXPOSURE CONDITIONS[1]

CHEMICAL TREATMENT (mg/l)	24-HR. EXPOSURE MORTALITY (%)	48-HR. EXPOSURE MORTALITY (%)
CONTROL	0	0
25	0	0
40	0	0
63	0	0
100	0	0
160	0	15
250	5	30
400	40	90
630	95	100
EC_{50} (95% CONFIDENCE LIMITS)[2] mg/l	416 (366-472)	266 (231-307)

(1) Data are averages of two replications for each treatment with ten (10) daphnids per replicate. A total of twenty daphnids were used per treatment.

(2) EC_{50} values were calculated using logistic regression methods (Probit Analysis).

TABLE II.   SUMMARY OF CHRONIC TOXICITY OF <u>FC-143, LOT 2164</u>

TO <u>DAPHNIA</u> <u>MAGNA</u> UNDER RENEWED STATIC EXPOSURE CONDITIONS[1]

CHEMICAL TREATMENT (mg/l)	14-DAY EXPOSURE		21-DAY EXPOSURE	
	YOUNG REPRODUCTION IMPAIRMENT (%)	ADULT MORTALITY (%)	YOUNG REPRODUCTION IMPAIRMENT (%)	ADULT MORTALITY (%)
CONTROL	0	0	0	0
5	14	0	12	5
8	26	0	16	20
13	36	0	21	20
22	38	0	21	20
36	47	0	35	25
60	68	5	78	80
EC_{50} (95% CONFIDENCE LIMITS)[2] mg/l	32 (26-42)	>60 highest test concent.	40	42 (34-56)
	33 (26-45)[3]		38 (35-42)[3]	40 (31-52)[3]

(1)   Data are averages of four replications for each treatment with five (5) daphnids per replicate. A total of twenty daphnids were used per treatment.

(2) EC_{50} values were calculated using logistic regression methods (Probit Analysis).

(3) EC_{50} values were calculated using Moving Average Angle Method.

YOUNG REPRODUCTION DATA
CUMULATIVE NUMBER

TEST SUBSTANCE FC-143 LOT 264   TEST DATE 8/7/84 — 8/28/84

Sample Description	14 Day	Organism	21 Day	
	Per Test Unit	Per Adult	Per Test Unit	Per Adult
Control	322	64.4	753	150.6
	347	69.4	840	168
	390	78	908	181.6
	378	75.6	802	160.4
$\overline{X} \pm S$	359 ± 31	72 ± 6	826 ± 65	165 ± 13
5 mg/l	260	52	528	105.6
	353	70.6	867	173.4
	285	57	718	143.6
	347	69.4	777	155.4
$\overline{X} \pm S$	311 ± 46	62 ± 9	723 ± 143	145 ± 29
P =	0.1430	0.1430	0.2603	0.2603
8 mg/l	187	37.4	534	120.15
	308	61.6	772	173.68
	167	33.4	370	74
	397	79.4	915	185.55
$\overline{X} \pm S$	265 ± 108	53 ± 22	648 ± 243	138 ± 52
P =	0.1908	0.1908	0.2514	0.3871
13 mg/l	151	30.2	406	92.6
	233	46.6	649	134.25
	146	29.2	556	112.95
	384	76.8	911	182.2
$\overline{X} \pm S$	229 ± 111	46 ± 22	631 ± 212	131 ± 38
P =	0.1080	0.1080	0.17108	0.18164

YOUNG REPRODUCTION DATA
CUMULATIVE NUMBER

TEST SUBSTANCE FC-143 LOT 264   TEST DATE 8/7/84 - 8/28/84

Sample Description	14 Day		21 Day	
	Per Test Unit	Per Adult	Per Test Unit	Per Adult
CONTROL $\bar{X} \pm S$				
22 mg/l	247	49.4	564	127.8
	228	45.6	657	133.4
	266	53.2	704	143
	156	31.2	577	117.55
$\bar{X} \pm S$	224 ± 48	45 ± 10	626 ± 67	130 ± 11
P =	0.0052*	0.0052*	0.0078*	0.0092*
36 mg/l	142	28.4	334	74.05
	243	48.6	596	119.2
	196	39.2	543	110.4
	184	36.8	619	127.5
$\bar{X} \pm S$	191 ± 42	38 ± 8	523 ± 130	108 ± 24
P =	0.0013*	0.0013*	0.0141*	0.0131*
60 mg/l	105	21	115	24.33
	155	31	202	52.4
	100	20	150	31.35
	101	20.2	154	34.27
$\bar{X} \pm S$	115 ± 27	23 ± 5	155 ± 36	36 ± 12
P =	0.0001*	0.0001*	0.0001*	0.0000*

ENVIRONMENTAL LABORATORY
SUMMARY OF EXPERIMENTAL DESIGN AND CONDITIONS FOR CONDUCTING
RENEWAL CHRONIC EFFECTS TESTS WITH DAPHNIDS

Project: Fate of Fluorochemicals - Phase II (9972512600)

Exposure: Type - renewal static
        Renewal frequency - every 2 days
        Duration - 14-21 days

Test Species: Daphnia magna or Daphnia pulex (use of D. magna is preferred)
        - Age of test animals - 12+12 hrs instars (neonates)
        - No. animals/beaker - 5
        - No. of replicate test beakers per concentration - 4
        - Total No. animals per concentration - 20

Test Vessel: 250 ml glass beaker (Pyrex) covered with a watch glass

Test Solution Volume: 200 ml

Loading Ratio: One animal per 40 ml

Dilution Water Supply: Aerated carbon-filtered well water (Bldg. 2) of
                      consistent chemical quality. Water hardness
                      classification: hard (hardness $\sim$ 250 mg/L as $CaCO_3$).

Untreated Controls: 100% well water

Toxicant Dose Range: Minimum of five logarithmic concentrations (by weight)

Test Conditions:

        Temperature   - 22+2°C (68-75°F)
        Light Quality - Type - ambient laboratory light
                      (cool-white fluorescent) .
                 - Intensity - ambient lab levels (50-100 fc)
                 - Photoperiod - 16 hrs light; 20 minutes
                     transition period
        Aeration During Test - None

Food and Feeding Regime: A suspension of fish food (trout chow) and yeast (Fleischmann's) containing 5 mg dry solids per one ml of mixture, on a daily basis.

Measurements and Observations:

Effects (noted daily and recorded on renewal days)
- No. young, live and dead; brood size, and no. broods
- No. dead adults
- Immobility, presence of unhatched eggs or ephippia, and other signs of stress

Water Quality (of dilution water and of replicates of old test solutions made on renewal days)
- Physical: temperature, D.O., and pH
- Chemical: analytical measurements of toxicant concentrations in initial stock and in old test solutions (optional)

Validity of Test: *

The control population of D. magna should meet the following requirements:
- Mortality of adults - maximum 20%
- First brood release - maximum 9 days
- No. young per adult (14 days) - minimum 20
- No. young per adult after 21 days - minimum 60
- Ephippia - none
- D.O. saturation - minimum 60% throughout the test
- pH - maximum ± 0.5 units from initial

Treatments of Results:

48-Hr Acute Test
- 24-hr EC_{50}
- 48-hr EC_{50} with 95% confidence limits

14-21 Day Chronic Test
- Approximate no observed effects concentration "NOEC"
- 14- and/or 21-day EC_{50} with 95% confidence limits
- Maximum acceptable toxicant concentration "MATC"
- Chronic/acute ratios
- Dose/response curve based on cumulative mortality of adults
- Safety factor

References: - USEPA-1982. Env. Effects Test Guidelines. EPA560/6-82-002
           - OECD-1981. Guidelines for Testing of Chemicals
           - ASTM-(latest drafts, 1983). Proposed Standard Practice for
             Conducting Renewal Chronic Effects Tests with Daphnids

*Final results are calculated from mean values of <u>cumulative no. young per adult</u>, or <u>cumulative mortality of adults</u>.

Definitions:

<u>Daphnia Chronic Toxicity Test</u> - A 14-21 day experimental study of the survival, growth and reproduction of asexually reproducing <u>Daphnia</u> beginning with 12±12 hr old organism.

<u>Significant</u> - "Statistically significantly different" refers to $p < 0.05$ with respect to the control.

<u>Approximate NOEC</u> - The highest test concentration that will produce no significant observed effect on reproduction of young or adult survival.

<u>Chronic EC$_{50}$(s)</u> - The values of toxicant concentrations which impair reproduction of young or induce adult mortality by 50%.

<u>MATC</u> - The highest concentration of toxicant that has no adverse effect on survival, growth, and reproduction of a species.

GM-MATC is the calculated geometric mean between the highest test concentration having no significant effect and the lowest test concentration having a significant effect.

<u>Application Factor (AF)</u> - The quotient of the chronic MATC divided by the acute 48-hr EC$_{50}$ (MATC/Acute EC$_{50}$). "AF" is a number used to estimate concentration of toxicant that will not cause significant effect to a population during chronic exposure.

<u>Environmental Safety Factor</u> - The ratio of the chronic MATC with the expected environmental concentration (MATC/EEC). Safety factors >20 at the predicted exposure level are desirable to have sufficient confidence in the environmental safety assessment.

ENVIRONMENTAL LABORATORY FINAL REPORT

LAB REQUEST NO. B2055

REQUESTER NAME: RRR
DEPT: 0222
PROJECT NO: 06
DATE RECEIVED: 8-16-84
DESC: JUNE CARBON-FILTERED WELL WATER

CONTRACT LAB:
CONTRACT LAB COST: 0
3M E-L HOURS: 3
EST CMPLT DATE: 8-24-84
DATE COMPLETED: 8-24-84

SAMPLE	DATE	CODE	DESCRIPTION	TEST	RESULT
1	7-06-84	RM #4		PH	7.8
				RES-CL	<0.02 MG/L
				COND	340 UMHOS/CM
				TOT-HARD	240 MG/L
				TOT-ALK	230 MG/L
				TS	270 MG/L
				NH3-N	<0.4 MG/L
				SOL-P	0.2 MG/L
				COD-LOW	<0.4 MG/L
				TOT-COLIF	NEGATIVE
				SPC	54 PER ML
2		DI	WATER	PH	6.6
				RES-CL	<0.02 MG/L
				COND	1.8 UMHOS/CM
				SPC	14 PER ML

* = CONTRACT LAB

APPROVED AND SUBMITTED BY _____ DATED __8-24-84__

AScI

AScI Corporation

August 10, 1998

Ms. Susan Beach
3M Environmental Technology and Safety Services
935 Bush Avenue
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331

Dear Sue:

FC-143 *Daphnia magna* reproduction data for concentrations above the survival NOEC were not included during hypothesis testing for the reproduction NOEC and LOEC. EPA guidance stipulates that concentrations which significantly effect survival should not be included in hypothesis testing for reproduction. The exclusion of such concentrations usually results in a more powerful and appropriate statistical analysis.

Sincerely,

Joe Dierkes
Biologist, AScI

Title: FC-143 DAPHNIA MAGNA 21 DAY REPRODUCTION
File: fc21d   Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	4	115876.3000	28969.0750	1.0871
Within (Error)	15	399732.5000	26648.8333	
Total	19	515608.8000		

(p-value = 0.3980)

Critical F = 4.8932 (alpha = 0.01, df = 4,15)
           = 3.0556 (alpha = 0.05, df = 4,15)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Press any key to continue or ESC to return to menu...

Title: FC-143 DAPHNIA MAGNA 21 DAY REPRODUCTION
File: fc21d   Transform: NO TRANSFORMATION

Dunnett's Test   -   TABLE 1 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	CONTROL	825.7500	825.7500		---
2	5 MG/L	722.5000	722.5000		
3	8 MG/L	647.7500	647.7500	0.8945	
4	13 MG/L	630.5000	630.5000	1.5420	
5	22 MG/L	625.5000	625.5000	1.6915	
				1.7348	

Dunnett critical value = 2.3600   (1 Tailed, alpha = 0.05, df = 4,15)

Press any key to continue...

Title: FC-143 DAPHNIA MAGNA 21 DAY REPRODUCTION
File: fc21d   Transform: NO TRANSFORMATION

Dunnett's Test   -   TABLE 2 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	4			
2	5 MG/L	4	272.4182	33.0	103.2500
3	8 MG/L	4	272.4182	33.0	178.0000
4	13 MG/L	4	272.4182	33.0	195.2500
5	22 MG/L	4	272.4182	33.0	200.2500

Press any key to continue or ESC to return to menu...

```
Title:  FC-143 DAPHNIA MAGNA 21 DAY REPRODUCTION
File:            fc21d           Transform:        NO TRANSFORMATION
                 Shapiro - Wilk's Test for Normality
-------------------------------------------------------------------------------

        D = 399732.5000
        W =   0.9767

        Critical W   = 0.8680 (alpha = 0.01 , N = 20)
                 W   = 0.9050 (alpha = 0.05 , N = 20)

-------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.
```

21d repro NOEC

```
            Press any key to continue or ESC to return to menu...

Title:  FC-143 DAPHNIA MAGNA 21 DAY REPRODUCTION
File:            fc21d           Transform:        NO TRANSFORMATION
                 Bartlett's Test for Homogeneity of Variance
-------------------------------------------------------------------------------

Calculated B1 statistic =  6.7904                (p-value = 0.1474)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

-------------------------------------------------------------------------------

Critical B = 13.2767   (alpha = 0.01, df =   4)
           =  9.4877   (alpha = 0.05, df =   4)




            Press any key to continue or ESC to return to menu...
```

Title: FC-143 DAPHNIA MAGNA 14 DAY REPRODUCTION
File: FC14D   Transform: NO TRANSFORMATION

Dunnett's Test   -   TABLE 1 OF 2   Ho:Control<Treatment

                                                                    SIG
                              TRANSFORMED   MEAN CALCULATED IN   0.05
GROUP   IDENTIFICATION   MEAN   ORIGINAL UNITS   T STAT
----- -------------------   -----------   ---------------- ------ ---
  1   CONTROL   359.2500   359.2500
  2   5 MG/L   311.2500   311.2500
  3   8 MG/L   264.7500   264.7500   1.0073
  4   13 MG/L   228.5000   228.5000   1.9830
  5   22 MG/L   224.2500   224.2500   2.7437   *
  6   36 MG/L   191.2500   191.2500   2.8329   *
  7   60 MG/L   115.2500   115.2500   3.5254   *
-- 5.1203 *
Dunnett critical value = 2.4600   (1 Tailed, alpha = 0.05, df [used] = 6,20)
                                                  (Actual df = 6,21)

                       —

                    Press any key to continue...

Title: FC-143 DAPHNIA MAGNA 14 DAY REPRODUCTION
File: FC14D   Transform: NO TRANSFORMATION

Dunnett's Test   -   TABLE 2 OF 2   Ho:Control<Treatment

                      NUM OF   MIN SIG DIFF   % OF   DIFFERENCE
GROUP   IDENTIFICATION REPS   (IN ORIG. UNITS) CONTROL   FROM CONTROL
----- ------------- -------   ---------------- -------   ------------
  1   CONTROL   4
  2   5 MG/L   4   117.2286   32.6   48.0000
  3   8 MG/L   4   117.2286   32.6   94.5000
  4   13 MG/L   4   117.2286   32.6   130.7500
  5   22 MG/L   4   117.2286   32.6   135.0000
  6   36 MG/L   4   117.2286   32.6   168.0000
  7   60 MG/L   4   117.2286   32.6   244.0000

                 Press any key to continue or ESC to retu

1312.0015

Title: FC-143 DAPHNIA MAGNA 14 DAY REPRODUCTION
File: FC14D   Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	152796.3571	25466.0595	5.6071
Within (Error)	21	95377.5000	4541.7857	
Total	27	248173.8571		

(p-value = 0.0013)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
           = 2.5727 (alpha = 0.05, df = 6,21)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Press any key to continue or ESC to return to menu...

1312.0016

Title: FC-143 DAPHNIA MAGNA 14 DAY REPRODUCTION
File: FC14D   Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

    D = 95377.5000
    W =   0.9434

    Critical W = 0.8960 (alpha = 0.01 , N = 28)
             W = 0.9240 (alpha = 0.05 , N = 28)

Data PASS normality test (alpha = 0.01). Continue analysis.

14d
repro
NOEC

Press any key to continue or ESC to return to menu...

Title: FC-143 DAPHNIA MAGNA 14 DAY REPRODUCTION
File: FC14D   Transform: NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 10.3740   (p-value = 0.1098)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

Critical B = 16.8119   (alpha = 0.01, df = 6)
           = 12.5916   (alpha = 0.05, df = 6)

Press any key to continue or ESC to return to menu...

FC-143   *21 day Survival*
                 NOEC, LOEC

Summary of Fisher's Exact Tests

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG 0.05
	CONTROL	20	0	
1	5 MG/L	20	1	
2	8 MG/L	20	4	
3	13 MG/L	20	4	
4	22 MG/L	20	4	
5	36 MG/L	20	5	*
6	60 MG/L	20	16	*

Press any key to continue...

*21d
Survival
LOEC*

FC 143 14d survival
NOEC, LOEC

Summary of Fisher's Exact Tests

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG 0.05
	CONTROL	20	0	
1	5 mg/l	20	0	
2	8 mg/l	20	0	
3	13 mg/l	20	0	
4	22 mg/l	20	0	
5	36 mg/l	20	0	
6	60 mg/l	20	1	

Press any key to continue...

ENTER THE NUMBER OF MORTALITIES AT EACH CONCENTRATION:
      5 20 20 20 25 80
WOULD YOU LIKE THE AUTOMATIC TRIM CALCULATION(Y/N)? Y

DATE: 8/7/1984   TEST NUMBER: 1

                                                DURATION: 21 DAYS

CHEMICAL: FC-143

                                                SPECIES: D.MAGNA

RAW DATA:
   CONCENTRATION(MG/L)   5.00   8.00   13.00   22.00   36.00   60.00
   NUMBER EXPOSED: 100   100   100   100   100   100
   MORTALITIES: 5   20   20   20   25   80
   SPEARMAN-KARBER TRIM: 20.00%

   SPEARMAN-KARBER ESTIMATES: EC50:   44.57
                95% LOWER CONFIDENCE: 40.88
                95% UPPER CONFIDENCE: 48.60

WOULD YOU LIKE TO HAVE A COPY SENT TO THE PRINTER(Y/N)?

21d
survival
EC50

1312.0020

Toxicant/Effluent: FC-143
Test Start Date: 8/7/1984 Test Ending Date: 8/21/198
Test Species: D.MAGNA
Test Duration: 14 DAY
DATA FILE:

Conc. ID	Number Replicates	Concentration MG/L	Response Means	Std. Dev.	Pooled Response Means
1	4	0.000	359.250	30.739	359.250
2	4	5.000	311.250	45.959	311.250
3	4	8.000	264.750	107.952	264.750
4	4	13.000	228.500	111.075	228.500
5	4	22.000	224.250	48.072	224.250
6	4	36.000	191.250	41.548	191.250
7	4	60.000	115.250	26.588	115.250

The Linear Interpolation Estimate: 39.6711   Entered P Value: 50

Number of Resamplings: 80   80 Resamples Generated
The Bootstrap Estimates Mean: 39.1710 Standard Deviation: 6.7021
Original Confidence Limits: Lower: 27.6757 Upper: 48.1897
Expanded Confidence Limits: Lower: 20.4784 Upper: 53.3009
Resampling time in Seconds: 1.32   Random_Seed: -309057854
            Press Any Key to Continue

IC p
IC₅₀
14d
21d

Toxicant/Effluent: FC-143
Test Start Date: 8/7/1984 Test Ending Date: 8/28/84
Test Species: D.MAGNA
Test Duration: 21 DAY
DATA FILE:

Conc. ID	Number Replicates	Concentration MG/L	Response Means	Std. Dev.	Pooled Response Means
1	4	0.000	825.750	65.383	825.750
2	4	5.000	722.500	143.412	722.500
3	4	8.000	647.750	242.861	647.750
4	4	13.000	630.500	212.111	630.500
5	4	22.000	625.500	66.556	625.500
6	4	36.000	523.000	129.956	523.000
7	4	60.000	155.250	35.753	155.250

The Linear Interpolation Estimate: 43.1869   Entered P Value: 50

Number of Resamplings: 80   80 Resamples Generated
The Bootstrap Estimates Mean: 42.7226 Standard Deviation: 2.9224
Original Confidence Limits: Lower: 35.0020 Upper: 46.3442
Expanded Confidence Limits: Lower: 30.0910 Upper: 48.2386
Resampling time in Seconds: 1.26   Random_Seed: -523848694
            Press Any Key to Continue

1312.0021