

ACUTE TOXICITY TO FISH (MUMMICHOG)

TEST SUBSTANCE

Identity: A mixture containing perfluorooctanesulfonate; may also be referred to as PFOS, FC-95, or as a component of FC-203C and L-5440. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: The 3M production lot number was not noted. The test sample is FC-203C and is referred to by the testing laboratory as "81-1/8". Current information indicates it is a mixture of 1.47% test substance, 60% water, 30% diethylene glycol butyl ether, 3% sodium octyl sulfate, 3% hydroxy foamer, 10% polyoxyethylene monooctylphenyl ether, 0.12% sodium lauryl sulfate, and 0.05% benzotriazol.

The following summary applies to the test sample as a mixture of the test substance in water solution with incompletely characterized concentration of impurities. Data may not accurately relate toxicity of the test sample with that of the fluorochemical component.

METHOD:

Method: EG&G, Bionomics protocol entitled "Protocol for Flow-through Toxicity Tests with Fishes," January, 1980.

Test type: Flow-through acute

GLP: No

Year Completed: 1981

Species: *Fundulus heteroclitus*

Supplier: Commercial supplier

Analytical monitoring: Temperature, pH, DO, and salinity

Exposure period: 96-hours

Statistical methods: LC₅₀ values calculated using the computer program (created by Stephan, 1978) based on the moving average angle analysis (24-, 48-, and 72-hour LC₅₀'s), or binomial probability (96-hour LC₅₀).

Test fish age: Adult.

Length and weight: Length = 31 – 37 mm with a mean of 35 mm
Weight = 0.43 – 0.80 g with a mean of 0.63 g

Loading: 0.13 g/L

Pretreatment: Not noted.

Test conditions:

Dilution water: Natural, filtered seawater from Big Lagoon, a Gulf of Mexico estuary, Florida.

Dilution water chemistry:

Salinity: 28 – 29 parts per thousand

Lighting: Fluorescent lights provided a daily photoperiod of 12 hours light and 12 hours dark with an intensity of 1,100 lux incident to the water surface.

Stock and test solution preparation: A primary stock solution of 523,000 mg/L was prepared by adding 523 grams of test sample to a 1-L volumetric flask and bringing to volume with deionized water. All test solutions were prepared by a diluter delivering five nominal concentrations of the test sample and control water.

Flow-through rate: Approximately 3 aquarium volume replacements per 24 hour period.

Stability of the test chemical solution: Not noted.

Exposure vessels: 30 x 30 x 91 cm glass tanks containing ~ 50 liters of solution volume.

Number of replicates: two

Number of fish per replicate: 10

Number of concentrations: 5 plus a blank control

Water chemistry during the study:

pH range (0-96 hours):

7.8 – 8.0 (control)

7.6 – 8.0 (2000 mg/L)

Temperature range (0-96 hours):

22 – 24°C (for all aquaria)

Dissolved oxygen range (0-96 hours):

6.3 – 7.1 mg/L (control)

3.8 – 7.1 mg/L (2000 mg/L)

Salinity range (0-96 hours):

28 – 29 parts per thousand (for all aquaria)

RESULTS

Nominal concentrations: Bk control, 125, 250, 500, 1000, and 2000 mg/L

Percent mortality (20 fish per concentration)

Nominal Concentration (mg/L)	Percentage mortality			
	24-hours	48-hours	72-hours	96-hours
Control	0	0	0	0
125	0	0	0	0
250	0	0	0	0
500	0	0	0	0
1000	0	0	0	0
2000	90	95	95	100

Element value: 24-hour LC₅₀ = 1,500 (1,400 – 1,600) mg/L
48-hour LC₅₀ = 1,500 (1,400 – 1,600) mg/L
72-hour LC₅₀ = 1,500 (1,400 – 1,600) mg/L
96-hour LC₅₀ = 1,400 (1,000 – 2,000) mg/L

Element values based on nominal concentrations.

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The value reported applies to that mixture and not the fluorochemical proportion alone.

CONCLUSIONS

The FC-203C 96-hour LC₅₀ for *Fundulus heteroclitus* was determined to be 1,400 mg/L with a 95% confidence interval of 1,000 to 2,000 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 2. The study meets all criteria for quality testing. However, sample purity was not properly characterized and the study lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

REFERENCES

This study was conducted by EG&G Bionomics Marine Research Laboratory of Pensacola, FL at the request of the 3M Company, 3M Lab Request number 7038, 1981.

OTHER

Last changed: 6/26/00

EAI # 81215

LR No. 7038

Acute toxicity of a surfactant
solution (81-1/8) to the mummichog
Fundulus heteroclitus in a flow-
through system

FC-203C

Toxicity Test Report

Submitted to

Minnesota Mining & Manufacturing Company

Environmental Engineering and Pollution Control

Environmental Laboratory

St. Paul, Minnesota 55133

Project Number R36

Report Number BP-81-10-163

EG&G Bionomics
Marine Research Laboratory
10307 Gulf Beach Highway
Pensacola, Florida 32507
October 1981

EG&G Bionomics
Marine Research Laboratory
10307 Gulf Beach Highway
Pensacola, Florida 32507

TOXICITY TEST SUMMARY SHEET

Client: Minnesota Mining and Manufacturing Company

Client Contact or Principal Investigator: Mr. Eric Reiner

Report date & number: October 1981; BP-81-10-163

Bionomics project number: R36 Study Director: Mr. G. Scott Ward

Test material: Surfactant solution 81-1/8

Description: Deep orange-yellow liquid

Date material received: 12 August 1981

Date of definitive test: 5-9 October 1981

Test condition: Flow-through, 96-hour duration, 23±1 degrees Celcius

Test procedure: BMRL test protocol, "Protocol for Flow-through Toxicity Tests with Fishes," dated 29 January 1980.

Test animal: Mummichog; *Fundulus heteroclitus*

Source and age: Purchased from a commercial supplier, adults

Seawater: Natural, filtered, from Big Lagoon, 28-29 parts per thousand salinity

Solvent/carrier: none

Nominal test concentrations: 125, 250, 500, 1,000 and 2,000 milligrams per liter (mg/l)

Effect criterion: Mortality

96-hour LC50: 1,400 mg/l

95% confidence limits: 1,000 - 2,000 mg/l

A flow-through marine toxicity test was conducted at EG&G Bionomics Marine Research Laboratory (BMRL), Pensacola, Florida, to determine the acute effect of a surfactant solution (81-1/8) on the mummichog *Fundulus heteroclitus*. The criterion for effect was death. Results of the test are reported as 24-, 48-, 72-, and 96-hour LC50 values (the concentrations of test material estimated to be lethal to 50% of the test animals at the specified times of exposure).

All data related to this study are stored at BMRL.

MATERIALS AND METHODS

Test material

The test material was received at BMRL on 12 August 1981 in one 9.5 liter (ℓ) cubetainer labeled "3M SAMPLE 81-1/8." The test material, a deep orange-yellow liquid, was tested as received.

All test concentrations are reported here as milligrams (mg) of surfactant solution per ℓ of seawater or as parts per million (ppm).

Test animals

Mummichogs were purchased from a commercial supplier and maintained at BMRL in flowing, natural seawater for 5 days. During the 96-hour period immediately before the test, the salinity of the holding water was 28-29 parts per thousand (‰) and the temperature was 19-21 degrees Celsius (°C). No mortality was observed during the entire holding period.

The control fish were 31-37 millimeters (mm) standard length and 0.43-0.80 grams (g) wet weight; mean length and weight were 35 (S.D.±2) mm and 0.63 (S.D.±0.10) g, respectively. The control fish appeared to be representative of the test population.

Test water

Water used to maintain and test the fish was natural seawater which was pumped from Big Lagoon, a Gulf of Mexico estuary adjacent to BMRL. The pump intake was located about 80 meters (m) offshore at a depth of approximately 3 m. Water was pumped by a 316 stainless steel pump through hard polyvinylchloride (PVC) pipes, through sand-filled fiberglass filters, through charcoal-filled fiberglass filters, and through 10-micrometers (μm) pore size polypropylene core filters into an elevated fiberglass reservoir. Water was chilled by Teflon® heat exchangers and flowed by gravity into the laboratory.

Test conditions

Methods for the 96-hour flow-through test were those described in BMRL's "Protocol for flow-through toxicity tests with fishes" dated 2 January 1980. The test was conducted in an intermittent-flow system by using a proportional diluter (Mount and Brungs, 1967) constructed to deliver 1 ℓ /cycle/test aquarium at a dilution rate of 50%. The average number of cycles was approximately 6.4/hour. This cycling rate provided approximately 3.0 volume additions in 24 hours.

The surfactant stock solution (523,000 mg/ ℓ) was prepared by weighing 523 g of surfactant solution in a 1- ℓ volumetric flask and bringing to volume with deionized water. The stock solution was placed in a 4- ℓ glass reservoir attached to a "dipping bird" (Mount and Brungs, 1967). A 14.9 milliliter volume of stock solution was delivered into the mixing cell via the dipping bird during each diluter cycle. A Teflon solenoid valve, controlled by float switches, regulated seawater flow into the water cells. Mixing of various volumes of uncontaminated dilution seawater from the water cells with

various volumes of stock solution in the test material cells produced the five concentrations of test material which were distributed to the appropriate test chambers. Test chambers were 30 centimeters (cm) x 30-cm x 91-cm glass aquaria which contained approximately 50% of test solution or control seawater.

Light was provided by two 3.7-m fluorescent bulbs suspended 46 cm above the test containers, providing approximately 1,100 lux incident to the water surface. Photoperiod was 12 hours of light and 12 hours of darkness.

Five concentrations of surfactant solution and a control were maintained. Selection of surfactant solution concentrations were based on the results of a 96-hour static test and a maximum test concentration established by verbal authorization from Mr. Eric Reiner of the 3M Company. The nominal concentrations were 125, 250, 500, 1,000, and 2,000 ppm.

To begin the test, the diluter cycled until all test chambers were filled with test solutions or control seawater. Ten fish were then placed into each duplicate test container.

Survival of fish in all treatments was recorded at 24-hour intervals. The dissolved oxygen concentration and pH were measured in each test container at 0 and 96 hours and in both controls, low, middle, and high test solutions after 24, 48, and 72 hours of exposure. The temperature and salinity were measured in both controls daily throughout the test.

The test was conducted 5-9 October 1981.

Statistical analyses

Based on the results of the test with the surfactant solution, 24-, 48-, 72-, and 96-hour LC50's and 95% confidence limits were calculated. The computer program estimated LC50 values by three statistical methods; moving average angle analysis, probit analysis, or binomial probability. The method selected was determined by the characteristics of the data, that is, the presence or absence of 0% and 100% mortality and the number of concentrations in which $>0\% < 100\%$ mortality occurred (Stephan, 1977). The moving average angle method was used to calculate the 24-, 48-, and 72-hour LC50's. The 96-hour LC50 was determined by the binomial probability method.

RESULTS AND DISCUSSION

After 24 hours of exposure to the surfactant solution, mortality ranged from 0% in surfactant concentrations $\leq 1,000$ ppm to 90% in the 2,000 ppm test concentration. The 24 hour LC50 was 1,500 ppm with 95% confidence limits of 1,400-1,600 ppm (Tables 1 and 2).

After 96 hours of exposure, mortality of fish ranged from 0% in test concentrations $\leq 1,000$ ppm to 100% in the 2,000 ppm test concentration (Table 1). The 96-hour LC50 was 1,400 ppm with 95% confidence limits of 1,000-2,000 ppm (Table 2). Physical or behavioral abnormalities were observed only in the 2,000 ppm test concentration. After 24 hours of exposure, the 2 surviving fish in 2,000 ppm were observed to be lethargic and remained so until their death.

Salinity during the test ranged from 28-29 ‰. The temperature ranged from 22-24°C; mean was $23 \pm 1^\circ\text{C}$. The dissolved oxygen concentration remained $\geq 30\%$ of saturation in all test containers throughout

the exposure. The pH was 7.5-8.0 in all test containers throughout the exposure (Appendix A).

REFERENCES

EG&G Bionomics Marine Research Laboratory. 1980. Protocol for Flow-Through Toxicity Test with Fishes. 2 January 1981.

5 pp.

Mount, D.I. and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. Water Research, 1:21-29.

Stephan, C.E. 1977. Methods for calculating an LC50. ASTM, Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. F.L. Mayer and J.L. Hamelink, eds. pp. 65-84.

TABLE 1. Mortality of mummichogs (*Fundulus heteroclitus*) exposed to a surfactant solution (81-1/8) in flowing natural seawater. Test concentrations are given as nominal concentrations of whole material.

Nominal Concentration (mg/l;ppm)	Mortality (%)			
	24 hour	48 hour	72 hour	96 hour
Control	0	0	0	0
125	0	0	0	0
250	0	0	0	0
500	0	0	0	0
1,000	0	0	0	0
2,000	90	95	95	100

TABLE 2. Calculated LC50's for mummichogs (*Fundulus heteroclitus*) exposed to a surfactant solution (81-1/8) in flowing natural seawater. Test concentrations are given as nominal concentrations of whole material.

Hour	LC50 (mg/l; ppm)	95% confidence limits (mg/l; ppm)
24	1,500	1,400-1,600
48	1,500	1,400-1,600
72	1,500	1,400-1,600
96	1,400	1,000-2,000

PREPARED BY:

G. Scott Ward

G. Scott Ward 20 October 1981
Study Director Date

AUDITED BY:

Alan G. Miller

Alan G. Miller 19 October 1981
Quality Assurance Unit Date
Raw data audit: 16 October 1981
Preliminary report audit: 16 October 1981
Final report audit: 19 October 1981

REVIEWED AND APPROVED BY:

Peter J. Shuba, Ph.D.

Peter J. Shuba 22 October 1981
Technical Coordinator Date

PAGE: 8
NO.: BP-QAF-001
EFFECTIVE: December 1980

SUBJECT: ACUTE TOXICITY DATA SHEET

CLIENT: 3M Company
PROJECT NO: R36

SPECIES: Fundulus heteroclitus
TEST MATERIAL: Surfactant Solution (81-1/8)

ANIMAL HISTORY

LOT NO: 9-30-81-A-58

DATE MAINTENANCE/ACCLIMATION BEGAN: 30 September 1981

CONDITION OF ANIMALS: excellent

LIFE STAGE: juvenile

SEE PAGE NO: 3 OF 1981 ANIMAL

MORTALITY(%) IN TANK 48 HOURS PRIOR TO TESTING: 0 %

COLLECTION/RECEIPT LOG FOR RAW DATA

SEE PAGE NO. 37 OF 1981 FISH holding log FOR RAW DATA

TEST CONDITIONS

☐ PRELIMINARY	☐ STATIC	TIME ADDED CHEMICAL/ANIMALS	TEMPERATURE (°C)	TEST SYSTEM
☒ DEFINITIVE	☒ FLOW-THRU	0900 / 1330	20, (22) 30 -	☒ OPEN ☐ CLOSED

NUMBER OF ANIMALS/CONTAINER	NUMBER OF TEST CONTAINERS/CONC	TEST CHAMBER VOLUME (l)	DILUENT VOLUME (l)	SALINITY (‰)
3, 5, (10) 20, -	1, (2) 4, -	1, 3.8, 19 (50l)	0.9, 3, 15 (81l)	AMBIENT ✓

PROTOCOL FOLLOWED: "Protocol for flow-through toxicity tests
with fisher" dated 2 January 1980

CIRCLE ONE: TEST MATERIAL AS ACTIVE INGREDIENT/WHOLE MATERIAL										SOLVENT/CARRIER
%	TEST CONCENTRATIONS mg/l	µg/l	µl/l	CON- TROL	SOLV CONT					
AMOUNT OF CHEMICAL/STOCK ADDED ()	N.A.	N.A.		N.A.	N.A.	NA				S/C CONCENTRATION
STOCK SOLUTION USED (1°, 2°, 3°)	N.A.	N.A.								
AMOUNT OF SOLVENT ADDED (ml)	N.A.			N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	

ADDITIONAL COMMENTS: (DOCUMENT ANY DEVIATIONS FROM PROTOCOL)

7th October 1981

GSW

Six extra fish were removed from the tank
at 500 ppm A and five from the tank at
1000 ppm B. Carol mistakenly added too many
fish - she had been adding five at a time
to each tank. GSW

		0-HOUR				24-HOUR				48-HOUR				72-HOUR				96-HOUR									
DATE		5 OCT 81				6 Oct. 1981				7 Oct. 1981				8 Oct. 1981				9 October 1981									
CONTROL TEMP °C/SAL.		22 °C 29 ‰				22 °C 29 ‰				23 °C 29 ‰				22 °C 28 ‰				24 °C 29 ‰									
TIME		1315				1345				1330				1330				1330									
DATA BY		CAL				GSW				GSW				GSW				GSW									
NOMINAL CONCENTRATION (mg/l) µg/l % µl/l	REP	OBS	PH	DO (mg/l)	OBS	NO. DEAD	CUM DEAD	PH	DO	OBS	NO. DEAD	CUM DEAD	PH	DO	OBS	NO. DEAD	CUM DEAD	PH	DO	OBS	NO. DEAD	CUM DEAD	PH	DO			
CONTROL	A	NONE	8.0	7.1	NONE	0	0	7.9	6.7	NONE	0	0	7.9	6.7	NONE	0	0	7.9	6.9	NONE	0	0	7.8	6.3			
	B	NONE	8.0	7.1	NONE	0	0	7.9	6.7	NONE	0	0	7.9	6.8	NONE	0	0	7.9	6.9	NONE	0	0	7.8	6.4			
SOLVENT CONTROL	A																										
	B																										
125	A	SB	8.0	6.9	SB	0	0	7.9	5.8	SB	0	0	7.9	6.0	SB	0	0	7.8	5.9	SB	0	0	7.7	4.9			
	B	SB	8.0	7.1	SB	0	0	7.9	5.9	SB	0	0	7.9	5.8	SB	0	0	7.8	5.8	SB	0	0	7.7	4.9			
250	A	SB	8.0	7.0	SB	0	0	—	—	SB	0	0	—	—	SB	0	0	—	—	SB	0	0	7.7	4.8			
	B	SB	8.0	7.1	SB	0	0	—	—	SB	0	0	—	—	SB	0	0	—	—	SB	0	0	7.7	5.0			
500	A	SB	8.0	6.9	SB	0	0	7.9	4.9	SB	0	0	7.8	4.9	SB	0	0	7.8	5.4	SB	0	0	7.7	4.2			
	B	SB	8.0	7.1	SB	0	0	7.9	5.4	SB	0	0	7.9	5.4	SB	0	0	7.8	5.5	SB	0	0	7.7	4.2			
1,000	A	SB	8.0	7.0	SB	0	0	—	—	SB	0	0	—	—	SB	0	0	—	—	SB	0	0	7.6	3.5			
	B	SB	8.0	7.0	SB	0	0	—	—	SB	0	0	—	—	SB	0	0	—	—	SB	0	0	7.5	2.1			
2,000	A	SB	8.0	7.0	SB LETH	9	9	7.9	5.4	SB LETH	0	9	7.8	5.2	SB LETH	0	9	7.8	5.6	SB	1	10	7.6	3.8			
	B	SB	8.0	7.1	SB LETH	9	9	7.9	4.6	SB	1	10	7.9	5.5	SB	0	10	—	—	—	0	10	—	—			
	A																										
	B																										
DO METER USED		#1				#1				#1				#1				#1									
PH METER USED		#4				#4				#4				#4				#4									
SALINITY MEASUREMENT		SCT #1				SCT #1				SCT #1				SCT #1				SCT #1									
TEMPERATURE MEASUREMENT		SCT #1				SCT #1				SCT #1				SCT #1				SCT #1									
OBSERVATION KEY																								TEST KEY			
NONE - OBSERVATION WAS MADE AND NOTHING OUT OF THE ORDINARY WAS OBSERVED AS - AT THE SURFACE MSP - MUSCLE SPASM CLE - COMPLETE LOSS EQUILIBRIUM PLE - PARTIAL LOSS EQUILIBRIUM LETH - LETHARGIC HYP - HYPERACTIVE ERR - ERRATIC GY - GYRATING DRK - DARK PIGMENTATION HEM - HEMORRHAGIC RA - RAPID RESPIRATION G - GULPING SB - SOAP-LIKE BUBBLES AT THE SURFACE CLDY - CLOUDY SOLUTION PRE - PRECIPITATE FOS - FILM ON SURFACE UC - UNDISSOLVED CHEMICAL																											