

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-206C and 81-2/8. (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-206C and is referred to by the testing laboratory as "81-2/8". Current information indicates it is a mixture of 0.73% PFOS, 76.09% water, 15% diethylene glycol butyl ether, 4% urea, 1.55% sodium octyl sulfate, 1.47% hydroxy foamer, 1.05% polyoxyethylene monooctylphenyl ether, 0.06% sodium lauryl sulfate, and 0.05% benzotriazole.

The following summary applies to a mixture with incompletely characterized concentration of impurities. Data may not accurately reflect the toxicity of the fluorochemical component of the test sample.

METHOD:

Method: EG&G, Bionomics protocol entitled "Protocol for Flow-through Toxicity Tests with Fishes," January 2, 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 could not be calculated.

Test fish age: Juvenile.

Length and weight: Length = 27 – 35 mm with a mean of 31 mm
Weight = 0.28 – 0.63 g with a mean of 0.46 g

Loading: 0.092 g/L

Pretreatment: Not noted.

Test conditions:

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

Dilution water chemistry:

Salinity: 27 –28 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 502,000 mg/L was prepared by adding 502 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 1.5 aquarium volume replacements per 24 hours.

Stability of the test chemical solution: Not noted.

Exposure vessels: 30 x 30 x 91 cm glass tanks containing ~50 L of test 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 – 7.9 (control exposure)

7.4 – 8.0 (2000 mg/L exposure)

Temperature range (0-96 hours):

22 – 27°C (for all aquaria)

Dissolved oxygen range (0-96 hours):

5.7 – 6.9 mg/L (control exposure)

0.6 – 6.8 mg/L (2000 mg/L exposure)

Salinity range (0-96 hours):

27 – 28 parts per thousand (for all aquaria)

Remarks: During the third day of exposure, the diluter stopped cycling and resulted in only 6 cycles (1 L solution / cycle) for the 24 hour period. Since the test concentration did not change, it was concluded that this did not affect the validity of the test.

RESULTS

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

Element value:	24-hour LC ₅₀ :	>2,000 mg/L
	48-hour LC ₅₀ :	>2,000 mg/L
	72-hour LC ₅₀ :	>2,000 mg/L
	96-hour LC ₅₀ :	>2,000 mg/L

Element values based on nominal concentrations.

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

CONCLUSIONS

The FC-206C 96-hour LC₅₀ for *Fundulus heteroclitus* was determined to be >2,000 mg/L, the highest concentration tested. 10% mortality was observed at this concentration.

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. Additionally, D.O. concentrations fell significantly below acceptable levels.

REFERENCES

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

OTHER

Last changed: 6/26/00

EAI# 81218

LR# 7038

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

81-2/8 FC-206C

Toxicity Test Report

Submitted to

Minnesota Mining & Manufacturing Co.

Environmental Engineering and Pollution Control

Environmental Laboratory

St. Paul, Minnesota 55133

Project Number R36

Report Number BP-81-9-151

EG&G Bionomics
Marine Research Laboratory
10307 Gulf Beach Highway
Pensacola, Florida 32507
September 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: Dr. Eric Reiner
Report date & number: September 1981; BP-81-9-151
Bionomics project number: R36 Study Director: Mr. Scott Ward
Test material: Surfactant solution, 20% butyl carbitol
Description: Deep orange-yellow liquid
Date material received: 12 August 1981
Date of definitive test: 10-14 September 1981
Test condition: Flow-through, 96-hour duration
Test procedure: BMRL's Test Protocol: "Protocol for Flow-Through
Toxicity Tests with Fishes"

Test animal: Mummichog, Fundulus heteroclitus
Source and age: Commercial supplier, juveniles
Seawater: Natural, filtered, 27-28 parts per thousand salinity

Solvent/carrier: None
Nominal test concentrations: 125, 250, 500, 1,000, and 2,000 milligrams
(mg)/liter (ℓ)
Effect criterion: Mortality

96-hour LC50: Could not be calculated; maximum mortality was 10% in
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 on the mummichog (Fundulus heteroclitus). The criterion for effect was death. Results of the test are reported as a 96-hour LC50 value (the concentration of test material estimated to be lethal to 50% of the test animals after 96 hours 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 81-2/8." 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 15 days. During the 96-hour period immediately before the test, the salinity of the holding water was 27-28 parts per thousand (‰) and the temperature was 21-22 degrees Celsius (°C). No mortality was observed during the entire holding period.

The control fish were 27-35 millimeters (mm) standard length and 0.28-0.63 grams (g) wet weight; mean length and weight were 31 (S.D.±2) mm and 0.46 (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 number of cycles was approximately 3.2/hour, except during day 3 when the diluter cycled only 6 times in 24 hours because of a malfunction. The cycling rate of 3.2 cycles/hour provided approximately 1.5 volume additions in 24 hours.

The surfactant stock solution (502,000 mg/ ℓ) was prepared by weighing 502 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 15.5 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 l of test solution or control seawater.

Light was provided by two 3.7-m fluorescent blubs suspended 46 cm above the test containers, providing approximately 1100 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.

Diluter function was checked daily by observation. Only one problem occurred. During the third day of exposure the diluter stopped cycling shortly after the test was checked on a weekend. This problem,

however, did not result in any change in the test concentrations and therefore did not affect the validity of the test.

The test was conducted 10-14 September 1981.

RESULTS AND DISCUSSION

After 96 hours of exposure to surfactant solutions, mortality of fish ranged from 0% in test concentrations $\leq 1,000$ ppm to 10% in the 2,000 ppm test concentration (Table 1). A 96-hour LC50 could not be calculated because mortality in the maximum test concentration was $< 50\%$. Physical or behavioral abnormalities were observed only in the 2,000 ppm test concentration. After 24 hours of exposure, 4 fish were observed to be darker than normal, however, this was observed again only after 96 hours when 1 fish was apparently darker than normal. After 72 hours of exposure, 11 fish were observed at the surface of the water in the 2,000 ppm test concentration. This probably occurred because of the diluter malfunction and correspondingly low dissolved oxygen concentrations.

Mean salinity during the test ranged from 27-28 ‰. The temperature ranged from 22-27°C; mean was $25 \pm 2^\circ\text{C}$. The dissolved oxygen concentration remained $> 9\%$ of saturation in all test containers throughout the exposure. Dissolved oxygen concentrations were low after 48 and 72 hours of exposure, especially in the 2,000 ppm test concentration. The low DO may have been caused by a high oxygen demand after 48 hours and the lack of changeover during day 3. The pH was 7.4-8.0 in all test containers throughout the exposure (Appendix A).

REFERENCES

- EG&G Bionomics Marine Research Laboratory. 1980. Protocol for Flow-Through Toxicity Tests with Fishes. 2 January 1981. 5 pp.
- Mount, D.I. and W.A. Brings. 1967. A simplified dosing apparatus for fish toxicological studies. Water Research, 1:21-20.

TABLE 1. Mortality of mummichogs (Fundulus heteroclitus) exposed to a surfactant solution 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	0	0	0	10

PREPARED BY:

G. Scott Ward

G. Scott Ward 28 Sept. 1981
Study Director Date

AUDITED BY:

Alan G. Miller

Alan G. Miller 28 September 1981
Quality Assurance Unit Date

Raw data audit: 18 September 1981Preliminary report audit: 18 September 1981Final report audit: 24 September 1981

REVIEWED AND APPROVED BY:

Peter J. Shuba, Ph.D.

Peter J. Shuba 28 Sept. 1981
Technical Coordinator Date



Marine Research Laboratory
Pensacola, Florida

APPENDIX A

A-1

PAGE: 7A
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

ANIMAL HISTORY

LOT NO: 8-26-81-A-54

DATE MAINTENANCE/ACCLIMATION BEGAN: 26 AUG 81

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. 34 OF ^{1981 FISH} HOLDING LOG FOR RAW DATA

TEST CONDITIONS

☐ PRELIMINARY	☐ STATIC	TIME ADDED CHEMICAL/ANIMALS	TEMPERATURE (°C)	TEST SYSTEM
☒ DEFINITIVE	☒ FLOW-THRU	/ 1530	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 (81)	0.9, 3, 15 (50)	AMBIENT ✓

PROTOCOL FOLLOWED: BMRL'S "Protocol for flow-through toxicity tests with fisher"

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

ADDITIONAL COMMENTS: (DOCUMENT ANY DEVIATIONS FROM PROTOCOL)

		0-HOUR			24-HOUR				48-HOUR				72-HOUR				96-HOUR								
DATE		10 Sept. 1981			11 SEP 81				12 Sept '81				13 Sept 81				14 SEP 81								
CONTROL TEMP °C/SAL.		23 22 °C 28 ‰/100			25 25 °C 28 ‰/100				26 26 °C 27 ‰/100				27 27 °C 27 ‰/100				24 25 °C 27 ‰/100								
TIME		1500			1525				1530				1515				1500								
DATA BY		GEM + CAL			AGM + CAL				AGM				AGM				CAL								
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					
CONTROL		A	NONE	7.9	6.9	NONE	0	0	7.9	6.1	NONE	0	0	7.8	5.8	NONE	0	0	7.8	5.7	NONE	0	0	7.9	6.6
		B	NONE	7.9	6.9	NONE	0	0	7.9	6.2	NONE	0	0	7.9	5.7	NONE	0	0	7.8	5.7	NONE	0	0	7.9	6.5
SOLVENT CONTROL		A																							
		B																							
125		A	SB	7.9	6.7	SB	0	0	7.8	5.1	SB	0	0	7.7	5.2	SB	0	0	7.5	2.8	CLOY	0	0	7.8	5.2
		B	SB	7.9	6.8	SB	0	0	7.8	5.2	SB	0	0	7.7	5.3	SB	0	0	7.6	3.3	CLOY	0	0	7.9	5.4
250		A	SB	7.9	6.8	SB	0	0	-	-	SB	0	0	-	-	SB	0	0	-	-	CLOY	0	0	7.8	4.6
		B	SB	8.0	6.8	SB	0	0	-	-	SB	0	0	-	-	SB	0	0	-	-	CLOY	0	0	7.8	3.9
500		A	SB	7.9	6.7	SB	0	0	7.8	4.5	SB	0	0	7.4	2.9	SB	0	0	7.4	1.1	CLOY	0	0	7.8	3.3
		B	SB	8.0	6.7	SB	0	0	7.8	4.9	SB	0	0	7.6	3.5	SB	0	0	7.5	1.7	CLOY	0	0	7.8	5.1
1,000		A	SB	7.9	6.7	SB	0	0	-	-	SB	0	0	-	-	SB	0	0	-	-	CLOY	0	0	7.8	3.8
		B	SB	8.0	6.8	SB	0	0	-	-	SB	0	0	-	-	SB	0	0	-	-	CLOY	0	0	7.8	3.8
2,000		A	SB	7.9	6.8	SB	0	0	7.8	4.1	SB	0	0	7.5	1.4	SB	0	0	7.4	0.6	CLOY	0	0	7.8	4.1
		B	SB	8.0	6.8	SB	0	0	7.8	4.8	SB	0	0	7.5	1.3	SB	0	0	7.4	0.8	CLOY	2	2	7.9	4.7
		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

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

CLOY - CLOUDY SOLUTION

PRE - PRECIPITATE

FOS - FILM ON SURFACE

UC - UNDISSOLVED CHEMICAL

SB - Soap-like bubbles at surface

TEST KEY

⊕ One missing.