

CHRONIC TOXICITY TO THE SALTWATER MYSID

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (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 test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.1350

Type: Flow-through chronic

GLP: Yes

Year completed: Study completed 1999. Report completed 2000

Species: *Mysidopsis bahia*

Supplier: In-house cultures, Wildlife International, Ltd., Easton, MD

Analytical monitoring: PFOS measured on days 0, 7, 14, 21, 28, and 35

Exposure period: 35 days

Statistical methods: Survival data was evaluated (prior to pairing and after pairing) using 2 X 2 contingency tables to identify treatment groups that showed a statistically significant difference ($p < 0.05$) from the negative control group. All continuous-variable data (reproduction and growth) were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. Analysis of variance and Dunnett's test were used to evaluate differences between treatment and control means. All statistical tests were performed using a personal computer with SPSS/PC Version 2.0 or "TOXSTAT Release 3.5" statistical software.

Test mysids age: < 24-hours old at test initiation

Pretreatment: None

Test conditions: Natural seawater diluted to 20‰ with well water, 0.45µm filtered.

Dilution water chemistry (during the 4-week period immediately preceding the test):

Salinity: 20 (20-20) ‰

TOC: < 1.0 mg/L

Stock and test solution preparation: Primary stock prepared at 0.0895 mg/L and mixed for approximately 24 hours prior to use. After mixing, the primary stock solution was proportionally diluted with dilution water to prepare five additional stock solutions at concentrations of 0.0447, 0.0224, 0.0112, 0.00559, and 0.00280 mg/L. The six stocks were injected into the diluter mixing chambers (at a rate of 4.60 mL/minute) where they were mixed with dilution water (at a rate of 150 mL/minute) to achieve the desired test concentrations.

Flow through rate: Approximately eleven volume additions of test water every 24- hours

Stability of the test chemical solutions: Extremely stable

Exposure vessels: Prior to pairing, mysids placed in glass beakers with nylon mesh screen attached to two holes on opposite sides. After reaching sexual maturity, pairs placed in glass petri dishes with sides of nylon mesh screen attached with silicone adhesive. Both pre-pairing and post-pairing exposure vessels were placed in 9L glass aquaria filled with approximately 5 L of test solution. The depth was approximately 6.2 cm prior to pairing and 5.5 cm after pairing.

The test chambers for the second generation exposure were 2L beakers with 1L of test solution which was dipped out of a test chamber from the appropriate treatment group.

Number of replicates: four

Number of concentrations: six plus a negative control

Number of fish per replicate: Fifteen juveniles before pairing, 5 pairs (10 adults) when possible after pairing.

Feeding: Fed live brine shrimp nauplii 3 or four times per day. Not fed the last day of the test.

Water chemistry during the study:

Dissolved oxygen range (0 – 35 days):

6.0 – 6.4 mg/L (control exposure)

5.9 – 6.3 mg/L (1.3 mg/L exposure)

pH range (0 – 35 days)

8.2 – 8.4 (control exposure)

8.3 – 8.4 (1.3 mg/L exposure)

Test temperature range (0 – 35 days)

24.5 – 25.2°C (control exposure)

24.4 – 25.1°C (1.3 mg/L exposure)

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Bk control, 0.086, 0.17, 0.34, 0.69, 1.4, 2.7 mg/L

Measured concentrations: <LOQ, 0.057, 0.12, 0.25, 0.55, 1.3, 2.6 mg/L

Element value: 20-day survival (pre-pairing) NOEC = 0.55 mg/L

35-day (post-pairing) survival NOEC = 0.55 mg/L

35-day reproduction NOEC = 0.25 mg/L

35-day growth NOEC = 0.25 mg/L

35-day reprod & growth LOEC = 0.55 mg/L

2nd generation acute survival NOEC = 0.55 mg/L (highest concentration tested)

All element values based on mean measured concentrations

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.0458 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 92.8. Samples collected at pre-test ranged from 57.4 to 99.3% of nominal. Samples at test initiation had measured values from 67.1 to 103% of nominal. Measured values for samples taken at test termination ranged from 59.8 to 90.0% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Duplicate Values at 0, 7, 14, 21, 28, 35, Days, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	-	-
0.086	0.0694, 0.0578, 0.0478, 0.0619, 0.0606, 0.0614, 0.0554, 0.0509, 0.0515, 0.0569, 0.0580, 0.0514	0.057	66
0.17	0.125, 0.114, 0.0778, 0.125, 0.124, 0.127, 0.0970, 0.112, 0.122, 0.128, 0.124, 0.119	0.12	71
0.34	0.289, 0.286, 0.231, 0.197, 0.276, 0.253, 0.227, 0.212, 0.262, 0.271, 0.278, 0.251	0.25	74
0.69	0.562, 0.659, 0.581, 0.450, 0.543, 0.542, 0.516, 0.528, 0.529, 0.544, 0.556, 0.583	0.55	80
1.4	1.23, 1.32, 1.13, 1.20, 1.35, 1.27, 1.23, 1.15, 1.39, 1.39, 1.26, 1.20	1.3	93
2.7	2.56, 2.79, 2.58, 2.30, 2.54, 2.69, all mysids dead after 14-days exposure	2.6	96

Biological observations

Survival: All surviving mysids appeared normal. Survival in the 1.3 and 2.6 mg/L treatments were statistically significantly different from the negative control group.

Reproduction: The day of first brood release in this study was Day 22. Dunnett's test showed that reproduction was significantly reduced in the 0.55 mg/L treatment group when compared to the negative control ($p \leq 0.05$). The 1.3 and 2.6 mg/L treatment groups were not included in the statistical analysis of the reproduction data due to a statistically significant difference in survival.

Growth: Mysids exposed to PFOS at concentrations ≤ 0.25 mg/L showed no statistically significant reductions in length or dry weight ($p \leq 0.05$).

Second Generation Acute Exposure: Survival in all PFOS treatment groups was $\geq 95\%$ and was not statistically different from the controls. All surviving mysids in the second generation exposure appeared normal with no overt signs of toxicity.

Percent Survival

Mean Measured Concentration, mg/L	Juvenile Pre-Pairing Survival, Day 20	Adult Post-Pairing Survival, Day 35
Negative Control	78	92
0.057	92	96
0.12	75	90
0.25	82	97
0.55	83	95
1.3	32	57
2.6	0	-

Second Generation Survival

Mean Measured Concentrations, mg/L	Total Number Exposed	Number Alive after 96-hours	Percent Survival
Negative Control	71	68	96
0.057	65	63	97
0.12	83	79	95
0.25	62	59	95
0.55	13	13	100

Adult Mysid Growth

Mean Measured Concentration, mg/L	Number of Surviving Mysids/Number Exposed	Total Length, Mean $\pm$ SD, mm	Dry Weight, Mean $\pm$ SD, mg
Negative Control	36/39	6.43 $\pm$ 0.0634	0.634 $\pm$ 0.0510
0.057	44/46	6.43 $\pm$ 0.0729	0.599 $\pm$ 0.0276
0.12	36/40	6.56 $\pm$ 0.105	0.641 $\pm$ 0.0241
0.25	36/37	6.40 $\pm$ 0.0548	0.622 $\pm$ 0.0227
0.55	35/37	6.14 $\pm$ 0.0794	0.562 $\pm$ 0.00624
1.3	8/14	5.85 $\pm$ 0.178	0.436 $\pm$ 0.0441

Reproduction

Mean Measured Concentration, mg/L	Replicate	Number of Reproductive Days	Number of Young	Mean Number of Young/Reproductive Day	Overall Mean $\pm$ SD
Negative Control	A	70	18	0.257	0.315 $\pm$ 0.0925
	B	53	14	0.264	
	C	70	20	0.286	
	D	42	19	0.452	
0.057	A	60	17	0.283	0.261 $\pm$ 0.0873
	B	70	14	0.200	
	C	70	13	0.186	
	D	56	21	0.375	
0.12	A	70	21	0.300	0.361 $\pm$ 0.101
	B	46	22	0.478	
	C	54	22	0.407	
	D	70	18	0.257	
0.25	A	70	19	0.271	0.252 $\pm$ 0.0723
	B	56	12	0.214	
	C	61	21	0.344	
	D	56	10	0.179	
0.55	A	54	3	0.0556	0.0559 $\pm$ 0.0376
	B	56	6	0.107	
	C	70	3	0.0429	
	D	56	1	0.0179	
1.3	A	22	0	-	-
	B	14	0	-	
	C	0	0	-	
	D	11	0	-	

CONCLUSIONS

There were no statistically significant effects on survival, reproduction or growth of mysid shrimp exposed to potassium perfluorooctanesulfonate at concentrations ≤ 0.25 mg/L for 35 days. Reproduction, length and dry weight were the most sensitive biological endpoints in this study. Second generation mysids exposed to PFOS during a static 96-hour exposure showed no adverse effects.

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DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company.

OTHER

Last changed: 5/3/00