BIOCONCENTRATION

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-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: Sample from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ^{1}H-HMR, ^{19}F-NMR and elemental analyses techniques.

METHOD:

Method/guideline followed: US EPA OPPTS 850.1730 and OECD 305
Type: Flow-through exposure with flow-through depuration phase.
GLP (Y/N): Yes
Year: 2001
Species: Bluegill (*Lepomis macrochirus*)
Supplier: Osage Catfisheries, Inc., Osage Beach, Missouri
Length and weight at test termination:
Mean length = 62 mm, range 56-66 mm
Mean weight = 2.70 g, range 2.03 – 3.32 g
Loading: 0.48 g fish/L/day (based on initial loading of 90 fish per tank, using mean fish weight at the end of the study and volume of water that passed through test chamber in 24-hours).
Fish age: Approximately 7 months at test initiation
Analytical monitoring: Concentration of PFOS in water and fish.
Pretreatment: None
Number of concentrations: Two plus a negative control
Test concentrations (mean measured): Negative control, 0.086 and 0.87 mg/L
Uptake period: 62-days (0.086 mg/L exposure)
35-days (0.87 mg/L exposure – this exposure ended after 35-days due to fish mortality)
Depuration period: 56-days (0.086 mg/L exposure)
None (0.087 mg/L exposure)
Test conditions:
Dilution water: Moderately–hard well water
Dilution water chemistry:
Specific conductance: 313 (310 – 315 umhos/cm)
Hardness: 130 (128 – 132 mg/L)
Alkalinity: 178 (176 – 178)
pH: 8.1 (8.0 – 8.2)

Measured during the 4-week period immediately preceding the test.

Stock and test solution preparation: Two stock solutions were prepared at 10 and 100 mg a.i./L. Stock solutions stirred with an electric top-down mixer to aid in the solubilization of the test substance. After mixing, the stocks appeared clear and colorless. Stocks were prepared at approximately weekly intervals during the uptake phase. Stocks injected into the diluter mixing chambers at a rate of 3.5 mL/minute where they were mixed with dilution water at a rate of 350 mL/minute to achieve the desired test concentrations. All final test solutions appeared clear and colorless.

Diluter flow rate: Approx. 6.3 volume additions per 24-hours

Exposure vessels: 104 L stainless steel aquaria filled with approximately 80 L solution.

Number of replicates: None – one vessel per concentration

Number of fish per vessel: 90

Diet: Flake food, Ziegler Brothers, Inc., Gardners, PA

Water chemistry ranges during the study:

	Neg. Control	0.086 mg/L	0.87 mg/L
Dissolved oxygen, mg/L:	6.8 – 8.6	6.8 – 8.6	6.4 – 8.2
Temperature, °C:	21.8 – 22.0	21.7 – 22.0	21.7 – 21.9
pH:	7.9 – 8.2	7.9 – 8.2	7.9 – 8.2

Photoperiod: 16 hours light and 8 hours dark with a 30 minute transition period.

Light intensity: 278 lux at surface of the negative control vessel at test initiation

Collection of tissue samples: Fish were collected from test chambers by random selection at 12 time points during the 62-day uptake phase. They were euthanized, blotted dry, weighed and measured. Fish then rinsed with dilution water, blotted dry again and dissected into edible and nonedible tissue fractions. The fractions were individually weighed. The head, fins and viscera were considered to be nonedible tissue. The remaining tissue, including skin was considered to be edible tissue.

Statistical methods: Whole fish concentrations were calculated based on the sum of the edible and nonedible parts. Steady-state bioconcentration BCF values calculated from the tissue concentrations at apparent steady-state divided by the mean water concentration. Tissue concentrations were considered to be at apparent steady-state if 3 or more consecutive sets of tissue concentrations were not significantly different ($p > 0.05$). Tissue concentrations were evaluated for normality and homogeneity of variance suing the Shapiro-Wilk's test and Bartlett's test, respectively. If the data did not meet the assumptions, data was transformed in an attempt to correct the data. Mean tissue concentrations were then compared using ANOVA and Dunnett's test.

The kinetic bioconcentration factor (BCFK), uptake rate (k_1) and depuration rate (k_2) were calculated for the edible, nonedible and whole fish exposed to 0.086 mg/L PFOS using BIOFAC computer software. BIOFAC is a nonlinear parameter estimate routine which estimates rate constants from a set of sequential time-concentration data. These rate constants were then used to calculate a BCFK ($BCFK = K_1/K_2$).

RESULTS

Nominal concentrations: Negative control, 0.1 and 1.0 mg/L
Mean measured concentrations: < 0.05, 0.086 and 0.87 mg/L

Bioconcentration factors (BCF):

0.086 mg/L apparent steady-state BCF

Edible	Nonedible	Whole Fish
484	1124	856

0.87 mg/L (study ended prior to achieving steady-state) BCF:

Edible	Nonedible	Whole Fish
136	386	278

BIOFAC Estimates (using 0.086 mg/L exposure)

	Edible	Nonedible	Whole Fish
BCFK:	1866	4312	3614
Time to reach 50% clearance:	146 days	133 days	152 days

PFOS Concentrations in Tissues of Bluegill Exposed to 0.086 mg/L

Values are from 4 individual fish at each sample period.

Uptake Day	Edible Tissue, mg/kg	Nonedible Tissue, mg/kg	Whole Fish Conc., mg/kg
0 (4-hours)	0.167, 0.155, 0.144, 0.182	0.415, 0.519, 0.417, 0.497	0.293, 0.351, 0.286, 0.363
1	0.734, 0.726, 0.631, 0.806	1.68, 1.85, 1.72, 2.07	1.26, 1.34, 1.29, 1.53
3	1.73, 2.07, 2.03, 2.11	4.59, 5.50, 5.47, 5.97	3.21, 4.04, 4.18, 4.38
7	3.73, 4.25, 4.73, 6.25	10.2, 10.6, 11.9, 15.2	7.33, 7.66, 8.73, 11.4
14	11.4, 9.07, 13.7, 12.6	27.3, 23.2, 35.3, 32.6	20.2, 16.9, 26.0, 24.6
21	11.7, 12.0, 12.9, 10.6	33.3, 22.7, 24.6, 24.4	23.3, 18.4, 19.8, 18.5
28	18.3, 13.7, 23.9, 23.1	49.4, 40.7, 65.3, 57.9	35.3, 29.2, 45.4, 44.1
35	22.6, 27.7, 23.8, 20.6	67.1, 73.3, 62.0, 59.1	46.3, 53.8, 46.6, 40.9
42	27.6, 25.3, 21.2, 27.6	64.0, 68.1, 54.4, 79.6	50.1, 49.4, 40.9, 56.3
49	33.3, 36.2, 39.0, 30.6	85.0, 95.1, 93.1, 77.7	62.8, 69.6, 70.8, 57.4
56	48.3, 38.9, 44.1, 38.3	122, 94.2, 73.2, 106	90.6, 71.6, 63.3, 74.8
62	42.4, 66.2, 42.2, 39.2	101, 112, 105, 96.4	77.0, 92.7, 79.6, 73.1
Depuration Day			
14	48.5, 31.8, 31.6, 42.0	124, 79.4, 81.8, 113	90.3, 60.4, 61.6, 85.3
28	26.0, 33.3, 38.7, 55.8	85.7, 95.1, 85.7, 94.8	58.2, 70.1, 68.1, 81.1
42	24.1, 31.2, 30.0, 33.0	71.7, 80.6, 78.3, 82.1	51.4, 61.4, 61.0, 62.2
56	21.1, 37.6, 32.9, 31.2	57.7, 80.3, 85.4, 84.4	41.6, 66.5, 65.8, 62.1

PFOS Concentrations in Tissues of Bluegill Exposed to 0.87 mg/L

Values are from 4 individual fish at each sample period.

Uptake Day	Edible Tissue, mg/kg	Nonedible Tissue, mg/kg	Whole Fish Conc., mg/kg
0 (4-hours)	1.46, 1.48, 1.19, 1.39	3.52, 4.37, 4.22, 4.06	2.71, 3.08, 2.84, 2.89
1	4.68, 6.59, 5.56, 5.64	11.1, 14.2, 13.3, 12.1	8.00, 10.9, 10.2, 9.47
3	17.3, 15.8, 19.0, 20.8	39.3, 42.0, 43.8, 51.8	30.5, 30.7, 34.5, 39.1
7	42.0, 44.0, 57.7, 46.8	100, 102, 102, 120	74.9, 77.0, 85.3, 89.8
14	87.1, 81.6, 90.7, 73.3	177, 207, 245, 214	141, 157, 180, 158
21	79.4, 117, 104, 102	201, 278, 246, 229	146, 210, 185, 172
28[(1)]	102, 131, 107, 133	289, 372, 320, 361	205, 267, 232, 263

[(1)] Sampling of fish stopped after Uptake Day 28 due to mortality.

Test organism mortality:
Negative control: None during the uptake phase (62 days) or depuration phase (35 days)
0.086 mg/L exposure: One fish died after 49 days and one after 59 days of exposure in the uptake phase, none during the depuration phase.
0.87 mg/L exposure: Mortality first noted on Day 9 and continued through Day 35 of the uptake phase at which time all of the fish had either died or had been sampled

Analytical methodology: Analyses of test solutions and fish tissues were performed at Wildlife International, Ltd. Water samples were diluted and analyzed by HPLC with single quadrupole mass spectrometric detection. Tissue samples were homogenized, extracted, diluted and analyzed by HPLC with triple quadrupole mass spectrometric detection. When determining the concentration of the test substance in the samples, 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 was 0.05 mg/L for water in this study. For tissue samples, the LOQ was calculated on an individual basis for each sample since each entire submitted sample, of differing weight, was extracted without an adjustment to constant weight.

Recovery was excellent in both water and fish tissues, ranging from 84.9 to 122% of fortification levels. Analytical results were not corrected for procedural recovery.

CONCLUSIONS

PFOS bioconcentrated in the tissues of bluegill sunfish during this study. Apparent steady-state was attained on Day 49 for the fish exposed to 0.086 mg a.i./L. Although Day 49, 56 and 62 tissue residues were not statistically significantly different, PFOS concentrations appeared to be still increasing during this time. Apparent steady-state BCF values for edible, nonedible and whole fish tissues were calculated to be 484, 1124, and 859, respectively.

PFOS depurated slowly. The BIOFAC estimates for the time to reach 50% clearance for edible, nonedible and whole fish tissues were 146, 133 and 152 days, respectively.

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

This study was conducted at Wildlife International, Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

OTHER

Last changed: 6/26/01