

BIOCONCENTRATION - REVISED

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: Sample from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ¹H-NMR, ¹⁹F-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, Report amended 2002

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.

Test conditions (cont.):

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 were originally calculated from the tissue concentrations at apparent steady-state using the BIOFAC model. Upon further investigation, this model was deemed inappropriate for use with this data set. The tissue concentrations had reached a point where there were not statistically significant differences between the last three sample days. However, a plot of the data clearly shows a trend of increasing concentrations in the tissues. In addition, the BIOFAC program is not accepted as appropriate for surfactants. An amended report was issued with BCFK values calculated as outlined in the draft OPPTS 850.1730 Guidance Document. 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. These rate constants were then

used to calculate a BCFK ($BCFK = K_1/K_2$) and half-lives for clearance for each tissue type. The results from this data reanalysis are presented below.

RESULTS

Nominal concentrations: Negative control, 0.1 and 1.0 mg/L

Mean measured concentrations: < 0.05, 0.086 and 0.87 mg/L

Kinetic Bioconcentration factors (BCFK):

0.086 mg/L exposure

	<u>Edible</u>	<u>Nonedible</u>	<u>Whole Fish</u>
BCFK:	1124	4013	2796
Time to reach 50% clearance:	86 days	116 days	112 days

0.87 mg/L exposure

Although BCF values were calculated using the BIOFAC software, the results are not reported here. All of the fish had died or been sampled prior to achieving steady-state. As a result, the BCF values were underestimated and are not relevant.

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 (total of 2.2% mortality during the study).

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. The BCFK values calculated for the edible, nonedible and whole fish tissues from the 0.086 mg/L exposure were calculated to be 1124, 4013, and 2796, respectively.

PFOS depurated slowly. The BIOFAC estimates for the time to reach 50% clearance for edible, nonedible and whole fish tissues from the 0.086 mg/L exposure were 86, 116 and 112 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. The report was amended and reissued June 6, 2002.

OTHER

Last changed: 6/11/02