# FETAX – FROG EMBRYO TERATOGENESIS ASSAY – *XENOPUS*

## TEST SUBSTANCE

**Identity:** Perfluorooctanesulfonate; may also be referred to as PFOS, U2723 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 obtained 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:** ASTM E1439-91
**Test type:** Static renewal
**GLP:** In-life phase – no; stock solution preparation and measurement of test concentrations - yes
**Year completed:** 2001
**Number of studies:** 3

| | Study 1 | Study 2 | Study 3 |
|---|---|---|---|
| **Start date:** | 5/15/00 | 5/22/00 | 5/22/00 |
| **End date:** | 5/19/00 | 5/26/00 | 5/26/00 |

(Study 2 and 3 set up concurrently with common stock solutions)
**Analytical monitoring**: PFOS measured at 0 and 96-hours
**Species:** *Xenopus laevis*
**Source:** Breeding colonies at the University of Maryland Wye Research and Education Center (UMD/WREC), Queenstown, Maryland.
**Test organisms laboratory culture**: Mating pairs were bred in the dark in 23.5 $\pm$ 0.5ºC UMD/WREC non-chlorinated well water at ~ 70 day intervals by injecting 400 and 800 I. U. of human chorionic gonadotropin (HCG) in the dorsal lymph sac of the males and females, respectively. Amplexus occurred 4-6 hours after injecting HCG; egg deposition occurred 9-12 hours following HCG injection.
**Age at test initiation:** Embryos; normal stage 8 blastula to normal stage 11 gastrula
**Loading:** 25 embryos/10 mL
**Pretreatment:** Embryos de-jelled in a 2% L-cysteine solution, then rinsed and re-suspended in FETAX solution prior to introduction to test chambers.
**Element basis:** mortality, malformations (via the atlas of Bantle et al., 1991), growth
**Exposure period:** 96-hours

**Test Conditions (all 3 studies):**

Dilution water: ASTM (1998) FETAX solution
**Test temperature:** 24.0 ± 0.2ºC
**Light levels:** 60-85 foot candle fluorescent lights
**Photoperiod:** 12-hour light:12-hour dark

**Stock and test solution preparation:** A primary stock solution was prepared in FETAX medium (supplied by UM-WREC) by Wildlife International, Ltd. at 48 mg PFOS/L. The primary stock solution was mixed by sonication and stirring. After mixing, the primary stock solution was proportionally diluted with FETAX medium to prepare the six test concentrations. The six test concenetration solutions were delivered to UM-WREC prior to the start of each study.
**Reference substance:** 6-aminonicotinamide
**Stock and reference substance solution preparation:** as outlined in the ASTM (1998) protocol
**Exposure vessels:** Covered 60 mm glass Petri dishes containing 10 mL test solution
**Number of replicates:** controls – 4, treatments – 2
**Number of embryos per replicate:** 25
**Number of concentrations**: six plus a negative control plus an abiotic control at the highest concentration tested, plus two reference substance concentrations.
**Renewal frequency**: every 24 hours
**Stability of the test chemical solutions**: Extremely stable

**Water chemistry during all 3 studies:**

**pH range** (0 – 96 hours)

7.1 – 7.7 (control exposure)
7.0 – 7.6 (24 mg/L nominal exposure)

**Dissolved oxygen range (**0-96 hours)

7.3 – 8.4 mg/L (control exposure)
7.0 – 8.5 mg/L (24 mg/L nominal exposure)

**Method of calculating mean measured concentrations**: arithmetic mean

RESULTS

**Nominal PFOS concentrations:** Negative control, 1.82, 3.07, 5.19, 8.64, 14.4 and 24.0 mg/L plus 24.0 mg/L abiotic control.

**Nominal 6-aminonicotinamide concentrations:** 5.5 and 2500 mg/L
**Mean measured PFOS concentrations:**

**Study 1:** <LOQ, 2.00, 2.83, 4.73, 7.90, 14.7, 24.6 mg/L; abiotic control = 23.7 mg/L

**Study 2:** < LOQ, 1.91, 3.04, 4.82, 7.97, 13.3, 23.1 mg/L; abiotic control = 23.9 mg/L

**Study 3:** < LOQ, 1.93, 3.27, 5.25, 8.26, 14.0, 23.9 mg/L; abiotic control = 24.1 mg/L

**PFOS element values and 95% confidence intervals, mg/L**

| Study Number | 96-Hr $LC_{50}$ | 96-Hr $EC_{50}$ | Minimum conc. to Inhibit Growth (MCIG) | Teratogenic Index (TI) |
|---|---|---|---|---|
| **1** | 13.8 (12.4 -15.3) | 12.1 (10.0 – 14.6) | Not calculable | 1.1 |
| **2** | 17.6 (15.5 – 20.0) | 17.6 (13.5 – 22.9) | 7.97 | 1.0 |
| **3** | 15.3 (13.1 – 17.8) | 16.8 (12.4 – 22.8) | 8.26 | 0.9 |

**All element values based on mean measured concentrations**

**Statistical methods:** The Trimmed Spearman-Karber statistical procedure was used to determine the 96-hour $LC_{50}$ for mortality and 96-hour $EC_{50}$ for malformations. The MCIG was determined by Bonferroni's T-Test. All statistical tests were performed using Toxstat (WEST and Gulley, 1994). A minimum probability level of 0.05 was used. The teratogenic index (TI) was calculated by dividing the $LC_{50}$ by the $EC_{50}$.

**Analytical Methodology:** Analyses of test solutions were performed at Wildlife International Ltd., Easton, MD 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 0.240 mg/L in these studies. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 97.2%. Samples collected at test initiation had measured values from 112 to 141% of nominal in the first study, and in the second and third studies, from 95.8 to 117% of nominal. Measured values for samples taken at 96-hours ranged from 54.7 to 98.6% of nominal in the first study and 80.7 to 112% of nominal in the second and third studies. The samples from the abiotic 24.0 mg/L treatment group was comparable to samples from the 24.0 mg/L treatment group with the embryos present.

## Summary of analytical chemistry data:

### Study 1

| Nominal Test Concentration, mg/L | Measured Values at 0 and 96-hours Respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|---|---|---|---|
| Negative Control | All < LOQ | <LOQ | - |
| 1.82 | 2.58, 1.42 | 2.00 | 110 |
| 3.07 | 3.94, 1.72 | 2.83 | 92.2 |
| 5.19 | 6.62, 2.84 | 4.73 | 91.1 |
| 8.64 | 10.7, 5.09 | 7.90 | 91.4 |
| 14.4 | 18.5, 10.8 | 14.7 | 102 |
| 24.0 | 26.9, 22.3 | 24.6 | 103 |
| 24.0 (abiotic) | not analyzed, 23.7 | 23.7 | 98.6 |

### Study 2

| Nominal Test Concentration, mg/L | Measured Values at 0 and 96-hours Respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|---|---|---|---|
| Negative Control | All < LOQ | <LOQ | - |
| 1.82 | 1.77, 2.04 | 1.91 | 105 |
| 3.07 | 3.59, 2.49 | 3.04 | 99.0 |
| 5.19 | 5.45, 4.18 | 4.82 | 92.9 |
| 8.64 | 8.43, 7.51 | 7.97 | 92.2 |
| 14.4 | 14.5, 12.1 | 13.3 | 92.4 |
| 24.0 | 23.0, 23.1 | 23.1 | 96.3 |
| 24.0 (abiotic) | not analyzed, 23.9 | 23.9 | 99.6 |

### Study 3

| Nominal Test Concentration, mg/L | Measured Values at 0 and 96-hours Respectively, mg/L | Mean Measured Concentration, mg/L | Percent of Nominal |
|---|---|---|---|
| Negative Control | All < LOQ | <LOQ | - |
| 1.82 | 1.77, 2.08 | 1.93 | 106 |
| 3.07 | 3.59, 2.94 | 3.27 | 107 |
| 5.19 | 5.45, 5.05 | 5.25 | 101 |
| 8.64 | 8.43, 8.09 | 8.26 | 95.6 |
| 14.4 | 14.5, 13.5 | 14.0 | 97.2 |
| 24.0 | 23.0, 24.7 | 23.9 | 99.6 |
| 24.0 (abiotic) | not analyzed, 24.1 | 24.1 | 100 |

## Biological observations after 96-hours:

### Mortality and Malformations

| | Test 1 | | Test 2 | | Test 3 | |
|---|---|---|---|---|---|---|
| Nominal Concentration*, mg/L | Percent Mortality | Percent Malformations | Percent Mortality | Percent Malformations | Percent Mortality | Percent Malformations |
| Negative Control | 1.0 | 4.0 | 1.0 | 4.0 | 0 | 2.0 |
| 1.82 | 2.0 | 8.2 | 0 | 8.0 | 0 | 6.0 |
| 3.07 | 4.0 | 15 | 10 | 4.4 | 0 | 4.0 |
| 5.19 | 10 | 22 | 8.0 | 11 | 0 | 6.0 |
| 8.64 | 12 | 25 | 10 | 20 | 14 | 14 |
| 14.4 | 38 | 65 | 30 | 37 | 44 | 39 |
| 24.0 | 100 | - | 70 | 67 | 78 | 73 |

*Nominal concentrations used for ease of comparison table

**Malformations:** The most common types of malformations noted were improper gut coiling, edema, notochord abnormalities and facial abnormalities.

### Growth – Mean length (mm) after 96-hours Exposure

| Nominal Concentration*, mg/L | **Test 1** | **Test 2** | **Test 3** |
|---|---|---|---|
| Negative Control | 8.59 | 8.88 | 9.47 |
| 1.82 | 8.29 | 8.45 | 9.10 |
| 3.07 | 8.80 | 8.57 | 9.28 |
| 5.19 | 8.51 | 8.72 | 9.28 |
| 8.64 | 8.71 | 7.93** | 8.51** |
| 14.4 | 8.08 | 7.51** | 8.11** |
| 24.0 | - (total mortality) | 7.39** | 7.80** |

*Nominal concentrations used for ease of comparison table
** Significantly different at alpha = 0.05 (Bonferroni T-Test)

**Control response**: satisfactory.
**Reference substance response**: satisfactory at low concentration (5.5 mg/L). Did not meet ASTM (1998) criteria for high concentration (2,500 mg/L). However, results obtained at high concentration were consistent and not at variance with previous experience in this testing laboratory.

**Observations:** Majority of embryo mortality appeared to be caused by the gut coiling through the body wall at the two highest test concentrations.

## CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour $LC_{50}$ range for FETAX was determined to be 13.8 – 17.6 mg/L. The 96-hour $EC_{50}$ range was 12.1 – 17.6 mg/L. The range for Maximum Concentration to Inhibit Growth (MCIG) was 7.97 to >14.7 mg/L. The Teratogenic Index (TI) was found to be 0.9 – 1.1. This TI range indicates that potassium perfluorooctanesulfonate has a low potential to be a developmental hazard.

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## DATA QUALITY

**Reliability:** Klimisch ranking = 2
Although these were well-conducted studies, the in-life phases were not conducted in accordance with Good Laboratory Practices.

## REFERENCES

These studies were conducted at the University of Maryland Wye Research and Education Center (UM-WREC) in Queenstown, Maryland and at Wildlife International Ltd., Easton, MD at the request of the 3M Company. Lab Request number U2723

## OTHER

**Last changed:** 6/12/01