

DIETARY ACUTE MALLARD DUCK STUDY **REVISED**

Tissue analysis is now included.

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: 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 850.2200, OECD 205, and FIFRA Subdivision E. Section 71-2

Type: Dietary acute

GLP: Yes

Year completed: 2000

Species: *Anas platyrhynchos*

Supplier: Whistling Wings, Inc., Hanover, IL

Analytical monitoring: PFOS measured on Day 0 for homogeneity in feed and verification, and Day 5 for stability.

Test phases: Acclimation – 9 days

Exposure - 5 days

Post-exposure observation – 3 or 17 days

Statistical methods: LC₅₀ values calculated by probit analysis using the computer software of C.E. Stephan. Body weight data were compared by Dunnett's test using TOXSTAT software. No statistical analyses were applied to feed consumption data.

Test bird age: 10 days

Pretreatment: None

Test conditions:

Housing and environmental conditions: Indoors in batteries of thermostatically controlled brooding pens. Floor space of each pen measured approximately 62 X 90 cm. Ceiling height was approximately 25.5 cm. External walls, ceilings and floors were constructed of galvanized steel wire and sheeting.

Identification: Each group of birds identified by pen number and test concentration. Individuals identified by wing bands.

Number of replicates: Six for controls, two for each treatment group

Number of ducks per replicate: five

Number of concentrations: Eight plus a negative control
Feed and water: Game bird ration formulated as below, water from the town of Easton public water supply. Both provided ad libitum during acclimation and testing.

Game Bird Ration

Ingredients	Percent
Fine Corn Meal	44.83
Soy Bean Meal, 48% Protein	30.65
Wheat Midds	6.50
Protein Base	6.00
Agway Special, 60% Protein	4.00
Alfalfa Meal, 20% Protein	3.00
Dried Whey	2.50
Ground Limestone	0.90
Eastman CalPhos	0.60
Methionine Premix + Liquid	0.35
Vitamin and Mineral Premix (see below)	0.32
GL Ferm (Fermatco) ¹	0.25
Salt Iodized	0.10

¹Fermentation by-products (source of unidentified growth factors)

Vitamin and Mineral Premix

Vitamin or Mineral	Amount Per Ton
Vitamin D ₃	2,000,000 I.C.U.
Vitamin A	7,000,000 I.U.
Riboflavin	6 grams
Niacin	40 grams
Pantothenic Acid	10 grams
Vitamin B ₁₂	8 mg
Folic Acid	600 mg
Biotin	64 mg
Pyridoxine	1.2 grams
Thiamine	1.2 grams
Vitamin E	20,000 I.U.
Vitamin K (Menadione dimethylpyrimidinol bisulfite)	5.8 grams
Manganese	102 grams
Zinc	47 grams
Copper	6.8 grams
Iodine	1.5 grams
Iron	51 grams
Selenium	182 grams

Prophylaxis: None

Brooding compartment mean temperature: 38 ± 2°C

Ambient room mean temperature: $25.2 \pm 0.7^{\circ}\text{C}$

Average relative humidity: $53 \pm 18\%$

Photoperiod: Sixteen hours light per day

Lighting: fluorescent lights which closely approximate noon-day sunlight; average approximately 207 lux.

Test diet preparation: Test substance mixed directly into the ration by means of a Hobart mixer. No carrier was used.

Diet sampling: Homogeneity of the test substance in the diet evaluated by collecting six samples from the 9.1 ppm and six from the lowest and highest concentration. Samples collected from the top, middle, and bottom of the left and right sections of the mixing vessel. These samples also served as the verification samples for these concentrations. Two verification samples from the remaining concentrations and one from the control were collected at preparation on Day 0. Stability samples were collected at the end of the exposure period (Day 5) from the control (one sample) and each treatment group (two samples each).

RESULTS

Nominal concentrations: Bk control, 9.1, 18.3, 36.6, 73.2, 146, 293, 586, and 1171 ppm

Measured concentrations: <LOQ, 9.8, 19.5, 40.2, 74.5, 174, 291, 537, and 1196 ppm

Element value: Dietary LC_{50} = 628 (448 – 958) ppm

No mortality concentration = 146 ppm

NOEC (body weight gain) = 36.6 ppm

All element values based on nominal concentrations

Analytical Methodology: Diet samples were extracted with methanol. 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 1.15 ppm in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 94.7. Samples collected for determination of homogeneity in diet ranged from 102 - 108% of nominal. Samples collected for verification in diet had

measured values from 92 to 119% of nominal. Measured values for ambient stability samples taken at Day 5 ranged from 94 – 130% of nominal.

Summary of analytical chemistry data:

Homogeneity in Avian Diet

Nominal Test Concentration, ppm	Measured Values at Day 0, ppm	Mean Measured Concentration, ppm	Percent of Nominal
9.1	9.52, 9.70, 9.79, 8.09, 10.9, 10.5	9.8	108
18.3	18.5, 23.4, 18.3, 17.3, 19.4, 19.9	19.5	107
1171	1239, 1221, 1118, 1301, 1163, 1133	1196	102

Verification in Avian Diet

Nominal Test Concentration, ppm	Measured Duplicate Concentrations at Day 0, ppm	Mean Measured Concentration, ppm	Percent of Nominal
Negative Control	< LOQ	-	-
36.6	45.7, 34.6	40.2	110
73.2	77.8, 71.2	74.5	102
146	176, 172	174	119
293	274, 307	291	99
586	550, 523	537	92

Ambient Stability in Avian Diet

Day 0 Mean Measured Concentration, ppm	Measured Duplicate Concentrations at Day 5, ppm	Mean Measured Concentration, ppm	Mean Percent of Day 0
Negative Control	< LOQ	-	-
9.8	12.3, 11.0	11.7	119
19.5	18.2, 19.7	19.0	97
40.2	47.9, 56.8	52.4	130
74.5	77.6, 77.9	77.8	104
174	167, 160	164	94.3
291	297, 293	295	101
537	552, 530	541	101
1196	1150, 1122	1136	95

Biological observations

Survival and clinical observations: No mortalities occurred in the control group, and all birds were normal in appearance and behavior throughout the test. The first deaths occurred on day 4 in the 1171 ppm treatment. Mortality occurred through Day 8 in all dose groups ≥ 293 ppm with some of the deaths being during the post-exposure period. There were no treatment-related mortalities or overt signs of toxicity at concentrations ≤ 146 ppm. Birds at all concentrations ≥ 293 ppm displayed signs of toxicity including reduced reaction to stimuli (sound and motion), loss of coordination, ruffled appearance, lethargy and lower limb weakness. Birds at the 1171 ppm level also displayed prostrate posture, depression and convulsions through Day 8. Recovery with normal appearance and behavior was noted from Day 9 through test termination

Body weight gain: When compared to the control group, there were no apparent treatment related effects on body weight among the birds in concentrations ≤ 36.6 ppm. During the Day 8-15 and Day 15-22 post-exposure periods, body weight gain appeared comparable among all groups. There was a statistically significant ($p < 0.05$) reduction in weight gain at the 9.1 ppm level for the Day 0-5 and Day 5-8 periods. However, differences from the control group at the 9.1 ppm level appear to be due to a lower mean Day 0 body weight for the 9.1 ppm level, and were not dose responsive. Therefore, these differences were not considered treatment related. Marked, treatment-related, concentration responsive effects on body weight was noted in concentrations > 73.2 ppm for Days 0-5; Day 5-8 post-exposure weight gain continued to be reduced at concentrations ≥ 293 ppm.

Feed Consumption: When compared to the control group, there was a marked reduction in feed consumption in the treatment groups ≥ 293 ppm throughout the study.

Gross Necropsy: All birds that died during the study, half of those surviving at Day 8 and the rest at test termination were subjected to a gross necropsy. Necropsy results for birds found dead were similar, including thin condition, loss of muscle mass, altered spleen color, empty crops, and empty gastrointestinal tracts. These necropsy findings were considered to be treatment related.

Percent Cumulative Mortality

Nominal Concentration, ppm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8*
Negative Control	0	0	0	0	0	0	0	0
9.1	0	0	0	0	0	0	0	0
18.3	0	0	0	0	0	0	0	0
36.6	0	0	0	0	0	0	0	0
73.2	0	0	0	0	0	0	0	0
146	0	0	0	0	0	0	0	0
293	0	0	0	0	0	0	10	20
586	0	0	0	0	10	20	30	30
1171	0	0	0	20	60	80	90	90

*No mortalities occurred in any of the treatment levels from Day 8 to Day 22

Summary of Analytical Results for Liver Tissue¹ (ug/g wet) during Day 1 through Day 7 Test Period² - Individual Values Reported

Nominal Conc., ppm	Day 4 Liver Conc.	Day 5 Liver Conc.	Day 6 Liver Conc.	Day 7 Liver Conc.
293				148
586		216	197	180
1171	116, 122	172, 137, 147, 185	131, 121	178

¹Concentrations were not corrected for purity of PFOS standard used (lot 171). No attempt was made to correct for moisture content.

²Reported results are only for concentrations where mortality occurred. During this time period, the negative control and concentrations <293 ppm exhibited no mortality. There was also no mortality in concentrations ≥ 293 ppm during days 1 through 3.

Summary of Analytical Results for Liver Tissue (ug/g wet) and Serum (ug/mL) at Day 8 and Day 22¹ - Average Value Reported

Nominal Conc., ppm	Day 8 Liver Conc.	Day 8 Serum Conc.	Day 22 Liver Conc.	Day 22 Serum Conc.
Negative Control	<LOQ (~0.030)	<LOQ (0.005)	<LOQ (~0.030)	<LOQ (0.010)
9.1	4.67	7.38	1.03	1.49
18.3	6.65	13.8	2.04	4.32
36.6	15.3	30.5	3.36	6.82
73.2	29.7	48.1	9.21	11.3
146	38.8	53.9	12.7	18.9
293	55.6	65.8	9.2	13.4
586	59.5	107	20.2	34.0
1171	NS	NS	Not applicable	LA

¹Concentrations were not corrected for purity of PFOS standard used (lot 171). No attempt was made to correct for moisture content in relevant tissue, liver.

NS = No Sample analyzed.

LA = Lab Accident

Body Weight (grams)

	Exposure Period		Recovery Period				
Nominal Concentration, ppm	Mean Body Weight, Day 0	Mean Body Weight Change Day 0-5	Mean Body Weight Change Day 5-8	Mean Body Weight Change Day 8-15	Mean Body Weight Change Day 15-22	Mean Total Body Weight Change Day 8-22	Mean Body Weight, Day 22
Negative Control	135	144	101	230	183	413	823
9.1	119	108	91	230	198	427	773
18.3	146	131	100	241	186	427	811
36.6	147	128	100	243	208	451	828
73.2	143	117	82	216	203	418	782
146	143	100	89	232	124	356	688
293	129	32	57	256	234	490	701
586	144	-6	36	221	219	439	613
1171	147	-37	1531	198	251	449	634

Mean Average Feed Consumption

Nominal Concentration, ppm	Grams Feed/Bird/Day Days 0-5	Grams Feed/Bird/Day Days 6-8	Grams Feed/Bird/Day Days 8-15	Grams Feed/Bird/Day Days 15-22
Negative Control	92	125	171	180
9.1	73	117	172	198
18.3	91	132	186	204
36.6	94	125	165	179
73.2	77	101	148	173
146	105	159	159	164
293	44	63	109	132
586	36	55	114	143
1171	22	25	106	154

Gross Pathological Observations from Birds that Died in Study

Finding	293 ppm N = 2	586 ppm N = 3	1171 ppm N = 9
Crop empty	0	2	7
Emaciated	1	1	4
G.I. Tract, primarily empty	1	1	1
Gizzard contents bile stained	0	2	4
Gizzard, empty	0	0	1
Intestinal contents, black and tar-like	0	0	1
Keel prominent	0	0	2
Kidneys, pale	0	0	1
Loss of muscle mass	2	2	5
Spleen, grey	0	0	1
Spleen, small and pale	1	1	2
Spleen, pale	0	2	3
Thin	1	1	4

CONCLUSIONS

The dietary LC50 value for Mallard Duck exposed to PFOS was determined to be 628 ppm with a 95% confidence interval of 448 to 958 ppm. The slope of the concentration-response curve was 3.67 and the chi-square value was 2.13. The no mortality concentration was 146 ppm. Based upon reductions in body weight gain at the 73.2 ppm test concentration, the no observed effect concentration was 36.6 ppm.
Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company. **The tissue analysis was conducted by 3M Environmental Laboratory, St. Paul, MN.**

OTHER

Last changed: 5/1/00, 6/28/01