DIETARY ACUTE NORTHERN BOBWHITE 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 field: 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, ^{1}H-HMR, ^{19}F-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: 1999 (Test), 2000 (Report)
Species: *Colinus virginianus*
Supplier: Wildlife International Ltd. Production Flock, Easton, Maryland
Analytical monitoring: Test substance concentration in standards and samples were determined by reversed-phase HPLC and mass spectroscopy. PFOS measured on Day 0 for homogeneity in feed and verification, and Day 5 for stability.
Test phases: Acclimation – 10 days
Exposure - 5 days
Post-exposure observation – 3 or 17 days
Statistical methods: LC_{50} 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. Each pen's floor space measured approximately 72 X 90 cm. Ceiling height was approximately 23 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 leg bands.

Number of replicates: Six for controls, two for each treatment group
Number of bobwhite per replicate: five
Number of concentrations: seven 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

[1]Fermentation 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 mgs
Folic Acid	600 mgs
Biotin	64 mgs
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: 27.3 ± 1.2°C
Average relative humidity: 31 ± 14%
Photoperiod: Sixteen hours light per day
Lighting: fluorescent lights which closely approximate noon-day sunlight; average of approximately 139 lux of illumination

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 18.3 ppm concentration and six from the 1171 ppm 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. Verification samples of the other treatment groups (two samples from each) and the control (one sample) 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, 18.3, 36.6, 73.2, 146, 293, 586, and 1171 ppm
Measured concentrations: <LOQ, 19.5, 40.2, 74.5, 174, 291, 537, and 1196 ppm
Element value: Dietary LC_{50} = 220 (164 – 289) ppm
No mortality concentration = 73.2 ppm
NOEC (body weight gain) = 73.2 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 102-107% 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 101-122% 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
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	-	-
19.5	19.2, 19.9	19.6	101
40.2	44.4, 53.8	49.1	122
74.5	76.4, 77.9	77.2	104
174	177, 174	176	101
291	318, 315	317	109
537	560, 665	613	114
1196	1260, 1187	1224	102

Biological observations

Mortalities and clinical observations: One incidental mortality occurred in the control group as a result of a broken leg on the morning of Day 5. It was subsequently euthanized on Day 6. Two other birds in the control group were intermittently noted with foot lesions associated with cage mate aggression. Otherwise, all control birds were observed to be normal in appearance and behavior throughout the test.

The first treatment-related mortalities occurred on Day 3 in the 586 and 1171 ppm treatment groups. Mortality occurred through Day 8 in all dose groups $\geq$ 146 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 $\leq$ 73.2 ppm.

There was 11% mortality in the 146 ppm treatment group, and two additional birds displayed clinical signs of toxicity (wing droop). All other birds in this test group displayed normal appearance and behavior for the duration of the test. Recovery with normal appearance and behavior occurred on Day 9 to test termination.

There was 80% mortality (occurring on Days 5,6, and 7) for birds in the 293 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, wing droop, loss of coordination, lower limb weakness and convulsions. Recovery with normal appearance and behavior occurred on Day 9 to test termination.

There was 100% mortality (occurring from Day 3 through Day 7) for birds in the 586 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, depression, wing droop, loss of coordination, lower limb weakness, lower limb rigidity, prostrate posture, and convulsions.

There was 100% mortality (occurring from Day 2 (noted on Day 3 for Day 2 afternoon) through Day 4) for birds in the 1171 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, depression, wing droop, loss of coordination, lower limb weakness, and lower limb rigidity.

Body weight gain: When compared to the control group, there were no apparent treatment related effects on body weight among the birds in concentrations ≤ 73.2 ppm. During Days 0-5, statistically significant reductions in body weight gain or body weight loss occurred in the 146, 293, and 586 ppm treatment groups. Body weight effects could not be determined for test organisms in the 1171 ppm group due to total mortality.

Feed Consumption: No apparent treatment related effects were noted for feed consumption for birds in concentrations ≤146 ppm. Reduced feed consumption was noted for birds in treatment groups ≥ 293 ppm from Days 0-5. No treatment-related effects on feed consumption in any of the surviving treatment groups during the Day 6-8 post-exposure period were observed.

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, autolysis of tissues and pale organs. These necropsy findings were considered to be treatment related. The single bird euthanized from the 293 ppm treatment was found to have treatment related necropsy findings. Necropsy results for all other birds euthanized on Day 8 and Day 22 were unremarkable.

% 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
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	10**	0	0	0	10	10
293	0	0	0	0	20	40	80	80
586	0	0	10	20	50	80	100	100
1171	0	0	30	100	100	100	100	100

*No mortalities occurred in any of the treatment levels from Day 8 to Day 22
** Bird euthanized on day 3 after sustaining a broken leg.

Summary of Analytical Results for Liver Tissue[1] (ug/g wet) during Day 1 through Day 7 Test Period[2] - Individual Values Reported

Nominal Conc., ppm	Day 3 Liver Conc.	Day 4 Liver Conc.	Day 5 Liver Conc.	Day 6 Liver Conc.	Day 7 Liver Conc.
146					111
293			249	202	153, 124
586	NE	NE	126	235	111
1171	134	132,123,124			

[1]Concentrations were not corrected for purity of PFOS standard used (lot 171). No attempt was made to correct for moisture content.
[2]Reported results are only for concentrations where mortality occurred. During this time period, the negative control and concentrations <293 ppm exhibited no mortality. (One bird euthanized after sustaining a broken leg.) There was also no mortality in concentrations ≥ 293 ppm during days 1 and 2.
NE = Not Extracted

Summary of Analytical Results for Liver Tissue (ug/g wet) and Serum (ug/mL) at Day 8 and Day 22[1] - 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 (~0.070)	<LOQ (0.010)	0.215	0.115
18.3	18.5	<LOQ	3.25	5.89
36.6	25.8	3.04	2.92	7.27
73.2	44.0	41.2	10.0	26.2
146	70.3	41.6	25.8	40.5
293	NE	Not applicable	Not applicable	Not applicable
586	NS	NS	Not applicable	Not applicable
1171	NS	NS	Not applicable	Not applicable

[1]Concentrations 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.
NE = Not Extracted

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	20	+10	+8	+23	+22	45	82
18.3	21	+11	+9	+24	+23	47	87
36.6	20	+11	+8	+26	+24	50	89
73.2	20	+9	+7	+24	+20	44	79
146	20	+7*	+6**	+24	+21	45	79

293	20	-2**	-1**	+14	+20	34	55
586	20	-4**	--	--	--	--	--
1171	20	--	--	--	--	--	-

Note: Numbers may not add manually due to rounding. Values for 293 ppm treatment group are impacted by the fact that only one bird remained in that treatment group after Day 8.
*Statistically different from the control group at p<0.05 (Dunnett's t-test).
**Statistically different from the control group at p=<0.01 (Dunnett's t-test
(--) = No data available due to mortality.

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	9	10	9	13
18.3	9	11	10	12
36.6	8	12	14	15
73.2	10	13	13	15
146	9	10	11	14
293	5	9	8	9
586	6	19	--	--
1171	4	--	--	--

(--) = No data available due to mortality.

Gross Pathological Observations from Birds that Died in Study

Finding	Male, Female, and Undetermined (ppm)				
	Control N = 1	146 N = 2	293 N = 8	586 N = 10	1171 N = 10
Abdominal cavity, some autolysis	0	0	2	2	4
Abdominal cavity, autolysis throughout	0	0	0	1	1
Crop, empty	0	0	2	5	2
Emaciated	0	0	2	5	8
Fractured leg	1	1	0	0	0
G.I. Tract empty	0	0	1	1	0
Gizzard contents bile stained	0	0	2	5	1
Heart, anterior portion mottled white color	0	0	1	0	0
Heart, pale	0	0	0	2	1
Intestinal contents tar-like	0	0	0	2	0
Keel, prominent	0	0	1	3	10
Kidneys, pale	0	0	0	2	0
Liver, pale and mottled	0	1	0	0	0
Loss of muscle mass	0	0	4	7	9
Muscular-skeletal, pale	0	1	0	0	0
Small in stature	0	0	3	0	0
Spleen, black	0	0	0	1	0
Spleen, dark	0	0	0	0	2

Spleen, grey	0	0	0	1	0
Spleen, grey-brown	0	0	0	0	1
Spleen, pale	0	0	1	0	1
Spleen, small	0	0	0	0	1
Spleen, small and pale	0	0	0	3	0
Thin	0	0	0	4	2
Not remarkable	0	0	1	0	0

CONCLUSIONS

The dietary LC50 value for Northern Bobwhite exposed to perfluorooctanesulfonate was determined to be 220 ppm with a 95% confidence interval of 164 to 289 ppm. The slope of the concentration-response curve was 7.005 and the chi-square value was 0.023. The no mortality concentration was 73.2 ppm. Based upon treatment related mortality, signs of toxicity and effects upon body weight gain at the 146 ppm test concentration, the no observed effect concentration was 73.2 ppm.

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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. The tissue analysis was conducted by 3M Environmental Laboratory, St. Paul, MN.

OTHER

Last changed: 5/3/00, 6/28/01