

Toxicological Summary

PFOS

Dietary Chronic Definitive Reproductive Study: Mallard

- Test Substance:** Perfluorooctanesulfonate (PFOS)
- Structure:** 1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-hepta-decafluoro, potassium salt. CAS# 2795-39-3.
- Test Remarks:** The test substance is a white powder (3M Lot #217), and is also known as FC-95. The sample was stored under ambient conditions and purity was determined to be 86.9% by LC/MS, ¹H-NMR, and elemental analysis techniques.

METHODS

Method: OECD 206, FIFRA Subdivision E., Section 71-4, and ASTM Standard E1062-86.

Type: Dietary Reproduction

Year: 2001 in-life phase, 2002-2003 analytical phase, Final report in 2003

Species: Mallard (*Anas platyrhynchos*)

Experimental Design: Adult mallards were exposed to PFOS at nominal concentrations of 0, 10, 50, or 150 ppm in the diet over a period of approximately 21 weeks. Each treatment and control group contained 16 pairs of mallards, one pair per pen. In addition, 4 extra pairs of mallards were maintained per treatment and used for blood collection during the test. The mallard pairs that were used for blood collection during the test were not used to assess reproduction. Due to overt signs of toxicity noted at the 150 ppm treatment group by Week 3, the treatment level was reduced to 20 ppm. The 150/20 ppm treatment group was then terminated at Week 5. The 50 ppm treatment was terminated at Week 7, also due to overt signs of toxicity. All adult mallards were observed daily for signs of toxicity or abnormal behavior. Body weights were measured at Weeks 2, 4, 6, 8, and at study termination. Feed consumption was measured weekly. Blood was collected from the extra pair of adult mallards at five-week intervals. At the time of adult termination, blood samples were collected from all surviving mallards for chemical analysis, excluding mallards from the 150/20 ppm treatment groups. All mallards, except for those from the 50 and 150/20 ppm treatment groups, were euthanized and subjected to gross necropsy at study termination. At the time of the necropsy, tissues were collected for histopathological examination and analyses.

Adult mallards were brought into reproductive phase by photostimulation at Week 11. Once egg production was initiated, eggs were weekly placed into sets for incubation. Eggs were candled and developmental stage and abnormalities were recorded for each

set. Reproductive endpoints evaluated during the study included: egg production, embryo viability, hatchability, and hatchling health and survival. After hatching, mallard chicks were fed untreated diet for 14 days. At the end of the study, chick body weights were determined. Prior to euthanasia of the last group of offspring (Lot J), blood samples were collected from 10 chicks from both the control and 10 ppm treatment groups for chemical analysis. In addition, tissues were also collected from these chicks for histopathological examination and chemical analysis. Liver weights were recorded for all necropsied mallards, and the liver samples were collected for chemical analysis. Samples were also taken of the liver, brain, kidney, gonad, proventriculus, gall bladder, adipose tissue, and Bursa of Fabricius for histopathological examination from adult and offspring mallards.

Test Bird Age: Adult mallards were 24 weeks of age at test initiation

Number of Replicates: There was 16 pair of adult mallards per treatment with one male and female per pen. In addition, 4 pairs of mallards were maintained per treatment as part of a blood monitoring study.

Feed and Water: Food and water were provided *ad libitum* during all phases of the study to both adult mallards and offspring. Feed consumption was measured once a week on a per pen basis.

Analytical Monitoring: Concentrations of PFOS in feed, red blood cells, liver tissue sera, and egg components were determined by reverse-phase HPLC and mass spectrometry. Egg components were separated using a method characterized by Bernardt and Cook (1960) and Stifani *et al.* (1990).

Statistical Methods: Upon completion of the study, an analysis of variance (ANOVA) was performed to evaluate significant differences between treatment groups. Dunnett's multiple comparison procedure was used to compare PFOS treatment groups with the controls (Dunnett, 1955; 1964). The sample units were the individual pens within each treatment group, except for adult body weights where the sample unit was the individual bird. Statistical analyses of body weights were conducted separately for male and female bobwhite quails. Percentage data (reproduction data) were examined using Dunnett's method following arcsine square root transformation. Statistical analysis of the data was performed using Avian Reproduction Data System (ARDS) Software, a validated software package developed by Wildlife International, Ltd.

Average Daily Intake (ADI) of PFOS for each treatment group was estimated on a pen basis and did not take into account potential differences between male and females within the pen. Feed consumption and adult mallard body weight data were averaged over the exposure duration of the study and the ADI was calculated as follows

$$\text{ADI (mg/kg body weight} \cdot \text{day)} = \frac{\text{Average Food Consumption (g feed/bird} \cdot \text{day)}}{\text{Average Body weight (g/bird)}} \times \text{Food (ppm PFOS)}$$

Test Diet Preparation: The test diets were prepared by mixing PFOS into a premix that was used for weekly preparation of the final diet. Homogeneity of the test substance in the diet was evaluated throughout the study.

RESULTS:

Measured Concentrations of PFOS in the Diet: To verify the concentrations in the PFOS treatment, feed samples were collected and analyzed. The means and standard deviations of the test diets are given (Table 1). Analyses of the diet also confirmed that PFOS was stable and did not degrade during the study.

Table 1. Mean and standard deviation concentrations of PFOS in the mallard diet. ^A

Nominal PFOS Concentration (ppm)	Measured PFOS Concentration (ppm)	Coefficient of Variation (%)	Percent of the Nominal Concentration
Control (0)	< LOQ ^B		
10	10.2 ± 0.553	5.44	102
20	20.8 ± 2.14	10.3	104
50	50.9 ± 2.04	4.01	102
150	161 ± 15.6	9.66	108

^A All diet concentrations on wet weight basis.

^B LOQ indicates limit of quantitation (LOQ= 2.00 ppm)

Mortalities and Clinical Observations: No treatment related mortalities were observed in the control or 10 ppm treatment groups. No clinical signs were observed at 10 ppm, with the exception of foot lesions and occasional lameness. However, none of these observations were determined to be treatment-related. The appearance and behavior of adult mallards of both sexes throughout the test were normal.

Within the first 7 weeks of the study, three incidental and ten treatment-related mortalities occurred in the 50 ppm treatment group. Within the first 5 weeks of the study, four treatment-related mortalities occurred in the 150/20 ppm treatment group. The expiring mallards exhibited overt signs of toxicity prior to death. Due to these treatment-related effects, all remaining mallards in the 150/20 ppm treatment were euthanized at Week 5, and surviving mallards in the 50 ppm treatment were euthanized at Week 8. Signs of toxicity at these doses included reduced reaction stimuli, wing droop, loss of coordination, depression, thin appearance, lacrimation, convulsions, lower limb rigidity, ruffled appearance, lower limb weakness, lethargy, and spasms.

Based on clinical signs and mortality, the No Observable Adverse Effect Concentration (NOAEC) was determined to be 10 ppm while the Lowest Observable Adverse Effect Concentration (LOAEC) was determined 50 ppm.

Adult Body Weight: Compared to controls, there were no apparent treatment-related effects on body weight in the 10 ppm treatment group (Table 2). However, starting at Week 2, there was marked and statistically significant reduction in body weight among mallards in the 50 and 150/20 ppm treatment groups. Based on these effects on body weight, the LOAEC was determined to be 50 ppm while the NOAEC was determined to be 10 ppm.

Feed Consumption: There were no treatment-related effects on feed consumption for mallards in the 10 or 50 ppm treatments when compared to the controls (Table 2). However, starting in Weeks 1 and 2, a treatment-related reduction in feed consumption was observed in mallards from the 150/20 ppm treatment. By Week 3, some recovery in feed consumption was observed with a statistically significant increase in feed consumption being noted in the 150/20 ppm treatment group. This recovery was likely due to the reduction of PFOS concentrations in the feed (from 150 ppm to 20 ppm). As a result, the LOAEC for feed consumption was 150 ppm while the NOAEC was determined to be 50 ppm.

Liver Weight: When compared to controls, no treatment related effects on liver weight were noted in the 10 ppm treatment group for either male and female adult birds (Table 2). Based on these results, the NOAEC was determined to be 10 ppm.

Table 2. Feed consumption, body and liver weight, and average daily intake (ADI) for adult mallards at study termination. ^A

Treatment (ppm)	ADI ^B (mg/kg/day)	Average Feed Consumption (g feed/bird/day)	Sex	Body weight (g)	Liver weight (g)
Control	<LOQ ^C	205 ± 53	M	1164 ± 60.0	29.42 ± 3.86
			F	1155 ± 134	41.65 ± 10.51
10	1.48 ± 0.19	193 ± 38	M	1127 ± 105	30.60 ± 4.63
			F	1144 ± 128	42.21 ± 10.27
50	6.36 ± 1.05	112 ± 45	M	916* ± 109	NA ^D
			F	781* ± 74.0	
150	20.9 ± 7.24	194* ± 73	M	1106* ± 85	NA
			F	880* ± 76	

^A Values are given as mean and standard deviations. Body and liver weights are terminal measurements, with control and 10 ppm treatments sampled at Week 20, while the 50 and 150/20 ppm treatments were sampled at Week 7 and 5, respectively. Feed consumption (FC) data was for week 20 for control and 10 ppm groups and Week 7 and 5 for 50 and 150/20 ppm, respectively.

^B ADI = average daily intake (mg PFOS/kg body weight per day).

^C LOQ for dietary PFOS concentrations was 2.00 ppm

^D NA= Not applicable; no sample reported.

* Asterisk indicates a significant difference from the control treatment at $p < 0.01$.

Gross Pathology: Necropsy results for all surviving females indicated that there were no treatment related effects in the 10 ppm treatment group when compared to the controls. However, for males from the 10 ppm treatment, there was a marked increase in the incidence of small testes size (7 of 16 birds) when compared to controls (2 of 16 birds)

that was treatment related. All other findings were considered incidental and not related to treatment.

Histopathology: No effects in the liver, kidney, brain, proventriculus, adipose tissue, or bursa of Fabricius were considered treatment-related in either adult or offspring mallards from the 10 ppm treatment. In male mallards with small testes, one control (1 of 2) and four treated (4 of 7) birds exhibited decreased spermatogenesis characterized by a reduction in the number or absence of maturing/mature spermatozoa in the seminiferous tubules. The other control and one treated bird exhibited degenerate/atrophy of the seminiferous tubule that was characterized by an absence of mature sperm and a decrease in the number of cell layers of germinal epithelium. In other birds with reduced testis size, spermatogenesis was normal as compared to control birds. These results suggest that PFOS may have accelerated post-reproductive regression, a normal physiological process. Based on these effects, the LOAEC was determined to be 10 ppm.

Reproduction: When compared to the control group, there were no statistically significant differences in any of the reproductive parameters for mallards in the 10 ppm treatment. Based on these effects, the NOAEC was determined to be 10 ppm.

Offspring Growth: There were no statistically significant differences in the body weight of hatchlings or 14-day old offspring survivors in the 10 ppm treatment when compared to the controls. There were also no treatment-related effects on the mean liver weights of offspring from the 10 ppm treatment group. While clinical observations such as neck curl, weakened condition, foot/leg lesions, and sluggishness were observed in the 10 ppm treatment group, these observations were not considered treatment-related due to the isolated nature of the effects in both control and treatment groups. Based on these results, the NOAEC in offspring was determined to be 10 ppm.

Concentrations of PFOS in Blood and Liver of Adult Mallards: Results of the chemical analysis of blood and liver samples showed that adult mallards in the 10 ppm treatment group accumulated PFOS during the study. Furthermore, at the study termination, liver and serum PFOS concentrations in males from the 10 ppm treatment were approximately 5-times greater than the measured concentrations in females from the same treatment group (Table 3). While there was a sex-specific difference in tissue concentrations, the ratio of PFOS in blood serum to that in liver (S:L ratio) for both sexes was similar, approximately 1.5 for both sexes. Since concentrations of PFOS were measured only in mallards from the control and 10 ppm treatment, a quantitative relationship between PFOS exposure and liver or serum concentrations was not developed.

Table 3. Liver and serum PFOS concentrations in adult mallards at study termination ^A

Treatment (ppm)	Sex	Liver Conc. (µg/g)	Serum Conc. (µg/mL)
Control	M	< LOQ ^B	0.19 ± 0.39
	F	< LOQ	0.06 ± 0.04
10	M	60.9 ± 19.5	87.3 ± 12.3
	F	10.8 ± 8.45	16.6 ± 12.0

^A Only control and 10 ppm samples were analyzed from this study. All values are reported on a wet weight basis.

^B LOQ = Limit of quantitation; for liver it was 0.02 – 0.0502 µg/g while for serum it was 0.04 – 0.10 µg/L.

Measurement of PFOS concentrations in blood collected throughout the reproduction study indicated that PFOS accumulated in mallards in both a time and sex-dependent manner (Table 4).

Table 4. Serum PFOS concentrations (µg/mL) in adult mallards at various time points during the study. ^A

Treatment (ppm)	Sex	Week		
		5	10	15
Control	M	<LOQ ^B	<LOQ	<LOQ
	F	<LOQ	<LOQ	<LOQ
10	M	93.5 ± 26.5	125 ± 20.6	138 ± 18.0
	F	76.9 ± 15.5	85.1 ± 39.2	9.30 ± 3.10
50		Week 5	Week 7	
	M	388	643	
	F	305	633	

^A Concentrations are reported as means and standard deviations.

^B LOQ = Limit of quantitation; LOQ was 0.01 µg/mL.

By Week 10, sex-specific difference in serum PFOS concentration became notable. The concentrations in male mallards were significantly greater than those observed in females. PFOS concentrations in serum of females decreased by approximately 85% from Week 10 concentrations to week 15. The decrease in serum concentrations coincided with the onset of the egg-laying phase in the study and indicates that female mallards were partitioning PFOS into eggs during the reproductively active phase of the study. In contrast, male serum concentrations reached an apparent saturation concentration by Week 10 and did not statistically differ for the remainder of the study.

Concentrations of PFOS were also measured in red blood cells of blood samples collected at each sampling time (Table 5).

Table 5. Red blood cell PFOS concentrations ($\mu\text{g/mL}$) in adult mallards at various time points during the study. ^A

Treatment (ppm)	Sex	Week		
		5	10	15
Control	M	< LOQ ^B	< LOQ	< LOQ
	F	< LOQ	< LOQ	< LOQ
10	M	21.5 $\pm$ 7.88	21.0 $\pm$ 8.85	58.6 $\pm$ 44.3
	F	14.1 $\pm$ 4.90	39.6 $\pm$ 25.1	3.80 $\pm$ 3.94
		Week 5	Week 7	
50	M	60.6	274	
	F	118	412	

^A PFOS concentrations are reported as means and standard deviations.

^B LOQ = Limit of quantitation; LOQ was 0.01 mg/L.

Overall, the concentration of PFOS in serum was greater than concentrations of PFOS measured in red blood cells. However, the relative volumes of serum and red blood cells in whole blood were not given in the analytical report. As a result, a mass balance of PFOS in blood could not be conducted and the partitioning of PFOS between sera and RBC was not evaluated. Furthermore, there were sex-specific differences observed between serum and red blood cell PFOS concentrations. At Week 5, male serum PFOS concentrations were approximately 5-fold greater than red blood cell concentrations while for females this difference was approximately 1.9-fold.

The trend in PFOS concentration red blood cells throughout the study was similar to that observed for serum concentrations. Sex-specific differences in red blood cell PFOS concentrations were apparent by Week 15 with the concentrations in males being greater than those measured in female from the same treatment group. By Week 15, male red blood cell PFOS concentrations were approximately 15-fold greater than those measured in females. This trend agreed with serum PFOS concentrations measured in these mallards.

Concentrations of PFOS in Blood and Liver of Juvenile Mallards: Analysis of serum and liver samples from juvenile mallards indicated that PFOS was present in the birds 12 weeks post-hatch. The PFOS concentrations in both liver and serum increased in the 10 ppm treatment group over that measured in the control group, but were less than the concentrations measured in adult mallards from the same treatments (Table 6). Unlike adult mallards, no sex specific differences were observed in either liver or serum PFOS concentrations.

Table 6. Liver and serum PFOS concentration in 2 week old juvenile mallards ^A

Treatment (ppm)	Sex	Liver Conc. (µg/g)	Serum Conc. (µg/ml)
Control	M	< LOQ ^B	0.068 ^C ± 0.037
	F	< LOQ	< LOQ
10	M	3.17 ± 1.30	4.41 ± 0.931
	F	3.61 ± 1.26	4.97 ± 1.12

^A Concentrations as mean and standard deviations. Data given as wet weight.

^B <LOQ = less than limit of quantitation for liver (50 ng/g) and serum (100 ng/ml)

^C The male serum average (n=3) was calculated using ½ the LOQ where samples were below the LOQ (n=2).

The ratio of PFOS concentrations in serum to that in liver in both male and female juvenile mallards averaged 1.40. This value was similar to that observed in adult mallards.

Concentrations of PFOS in Egg Components: Concentrations of PFOS in egg yolk were measured in eggs collected from the control and 10 ppm treatments. Eggs were selected from Weeks 19, 20, and 21 and yolks were separated and composited by treatment group. Yolk (whole) composites were then analyzed for PFOS (Table 7). In addition, to better understand the distribution of PFOS in the egg yolk, yolk composites from the 10 ppm treatment were also fractionated into 3 components, Very Low Density Lipoprotein (VLDL), phosvitin, and lipovitellin. PFOS concentrations were then determined in each yolk fraction (Table 7).

Table 7. Egg yolk and yolk component PFOS concentrations (µg/g) in mallard eggs collected during Weeks 19-21 of the study. ^A

Treatment (ppm)	Yolk Fraction	PFOS Concentration (µg/ml)
Control	Yolk-Whole	<LOQ ^B
10	Yolk-Whole	52.8 ± 0.58
	Yolk- VLDL	42.2 ± 0.50
	Yolk-Lipoprotein	8.87 ± 0.057
	Yolk-Phosvitin	3.59 ± 0.014

^A Results given as means and standard deviations on a wet weight basis. The standard deviation expresses precision of the preparation and analysis of duplicates for the same unique sample, an egg composite.

^B The limit of quantitation (LOQ) was 10 µg/ml

Results indicated that at 10 ppm, there was a significant accumulation of PFOS into egg yolks from the adult hens as compared to controls. No PFOS was quantified in control egg yolks. The ratio of the concentration of PFOS in the egg yolk to that in blood serum in adult females at week 21 was 3.1. The ratio egg yolk to adult female liver PFOS concentrations at week 21 was approximately 4.8. The greatest concentration of PFOS in

the yolk was associated with the VLDL fraction (approximately 77%) followed by lipovitellin and phosvitin.

CONCLUSIONS:

Mallards were exposed to PFOS at dietary concentrations of 0, 10, 50 and 150 ppm for up to 21 weeks. Due to signs of overt toxicity, adult mallards in the 50 and 150/20 ppm treatments were terminated at the end of 7 and 5 weeks, respectively. When compared to controls, no PFOS-related effects on adult body weight or feed consumption were observed when adult mallards were fed 10 ppm in the feed (Table 8). In adult males, there was an increase in the incidence of small testes size and decreased spermatogenesis at 10 ppm (Table 8). Thus, while early post-reproductive phase regression is a normal physiological phenomenon in birds, PFOS may have accelerated this process when compared to untreated birds. Finally, there were no treatment-related reductions in fertility, hatchability or offspring health or survival in the 10 ppm treatment. Based on these effects, the No Observable Adverse Effect Concentration (NOAEC) for female mallards and offspring was determined to be 10 ppm while the Lowest Observable Adverse Effect Concentration (LOAEC) for male mallards was determined to be 10 ppm (Table 8). PFOS concentrations associated with the No Observable Adverse Effect Level (NOAEL) and Lowest Observable Adverse Effect Level (LOAEL) in both adult and juvenile tissues are summarized in Table 9.

Table 8. Measurement endpoints and associated dietary NOAEC and LOAEC values for PFOS in a chronic study with mallards and their offspring. ^A

Endpoint	Dietary NOAEC (ppm)	Dietary LOAEC (ppm)
ADULT		
Mortality	10	50
Body weight	10	50
Feed consumption	50	150
Liver weight	10	
Gross pathology	Males < 10 Females = 10	Males = 10 Females = 50
Histopathology	10	
Reproductive	10	
OFFSPRING		
14-day survivability	10	
Hatchling/juvenile body weight	10	
Juvenile liver weight	10	

^A LOAEC was based on increased incidence of small testes and decreased spermatogenesis in adult males from the 10 ppm treatment; both attributed to early post-reproductive phase regression. All concentrations reported on a wet weight basis.

Table 9. NOAEL and LOAEL values in various matrices in adult and offspring mallards in a chronic dietary study with PFOS.

Measures of PFOS Exposure ^A	NOAEL ^B	LOAEL ^B
ADULT MALES		
Dose (ppm)		10
ADI (mg PFOS/kg body weight•day) over 21-week period		1.49
Serum (µg PFOS/mL) at study termination (21-weeks)		87.3
Liver (µg PFOS/g) at study termination (21-weeks)		60.9
ADULT FEMALES		
Dose (ppm)	10	
ADI (mg PFOS/kg body weight•day) over 21-week period	1.49	
Serum (µg PFOS/mL) at pre-reproductive phase (5-weeks)	76.9	
Serum (µg PFOS/mL) at study termination (21-weeks)	16.6	
Liver (µg PFOS/g) at study termination (21-weeks)	10.8	
OFFSPRING		
Yolk (µg PFOS/mL)	52.7	
Liver (µg PFOS/g) ^C	3.39	
Serum (µg PFOS/mL) ^C	4.69	

^A LOAEL was based on early post-reproductive phase regression in adult males from the 10 ppm treatment. All concentrations reported on a wet weight basis.

^B No and Low effect values for diet and ADI are based on dietary concentration. Serum and liver effect values are measured tissue values.

^C Offspring liver and serum LOAEL values are averages of female and male concentrations.

DATA QUALITY:

Reliability: Klimish ranking = 1

REFERENCES:

Bernardt and Cook. 1960. BBA 44: 86-96.

Decker, E.R., Flaherty, J.M. 2003. Analysis of perfluorooctane sulfonate in mallard and quail egg yolk. Exygen Report No. 023-070.

Dunnett, C.W. 1955. A multiple comparison procedure for comparing several treatments with a control. *J.Amer. Statis. Assoc.* 50: 1096-1121.

Dunnett, C.W. 1964. New tables for multiple comparisons with a control. *Biometrics* 20: 482-491.

Gallagher, S.P., Van Hoven, R.L., Beavers, J.B., Jaber, M. 2003. PFOS: A reproduction study with mallards. Final report. Wildlife International, Ltd., Project No. 454-109.

Gallagher, S. 2003. Extraction of Potassium Perfluorooctanesulfonate from Red Blood Cells and Serum for Analysis using HPLC-Electrospray/Mass Spectrometry. Exygen Research, Exygen Study Number 023-066.

Reagen, W.K. 2002. Analysis of Perfluorooctanesulfonate in Mallard and Quail Egg Yolk. Exygen Research. Exygen Report No. 023-070.

Stevenson, L.A. 2003. Analytical phase report for PFOS: A reproduction study with the mallard. 3M Environmental Laboratory Study No. E01-1256.

Stifani, S., Nimpf, J., Schneider, W.J. 1990. Vitellogenesis in *Xenopus laevis* and chicken – cognate ligands and oocyte receptors-the binding site for vitellogenin is located on lipovitellin-I. *J. Biol. Chem.* 265: 882-888.

Wildlife International, Ltd. 1994. Avian Reproductive Data System (ARDS), Version 2.

OTHER

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