

Toxicological Summary

PFOS

Dietary Chronic Definitive Reproductive Study: Northern Bobwhite

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 No. 2795-39-3.

Test Remarks: The test substance is a white powder (3M Lot #217), 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: ASTM Standard E1062-86, OECD 206, and FIFRA Subdivision E., Section 71-4.

Type: Dietary Reproduction

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

Species: Northern bobwhite quail (*Colinus virginianus*)

Experimental Design: Adult northern bobwhite quail 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 group contained 16 pairs of quail, one pair per pen. In addition, 4 other pairs of quail were maintained per treatment and were used for blood collection during the test. The pens that were used for blood collection during the test were not used to assess reproduction. Due to overt signs of toxicity noted in the 150 ppm treatment by Week 3, the treatment level was reduced to 20 ppm. The 150/20 ppm dose was then terminated at Week 5. The 50 ppm dose was terminated at Week 7, also due to overt signs of toxicity. All remaining adult quail 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 quail at five-week intervals. At time of adult termination, blood samples were collected from all surviving quail for chemical analysis, excluding quail from the 150/20 ppm treatment. All bobwhite quail, except for those in the 50 and 150/20 ppm treatments, were euthanized and subjected to gross necropsy. At the time of necropsy, tissues were collected for histopathological examination and chemical analyses.

Adult quail were brought into their reproductive phase by photostimulation at Week 7. Once egg production was initiated, eggs were set weekly for incubation. Eggs were

candled so that the developmental stage and any abnormalities could be recorded for each egg set. Reproductive endpoints evaluated during this study included: egg production, embryo viability, hatchability, and hatchling health and survival. After hatching, quail chicks were fed an 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 collected from these chicks for histopathological examination and analysis. Liver weights were recorded for all necropsied quail, and the liver was sampled for chemical analysis. Samples were also taken of the liver, brain, kidney, gonad, proventriculus, gall bladder, adipose, and Bursa of Fabricius for histopathological examination from both adult and offspring bobwhite quails.

Test Bird Age: Adult quail were 24 weeks old at test initiation.

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

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

Analytical Monitoring: PFOS concentrations 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 quail. 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 the male and female within a pen. Food consumption and adult quail body weight data were averaged over the exposure duration of the study and the ADI was calculated as follows:

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

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

RESULTS:

Measured Diet Concentrations: To verify the concentrations in the PFOS treatments, feed samples were collected and analyzed. Means and standard deviations of the test diets are given (Table 1). Feed samples from the control treatment showed no indication of containing PFOS. Analyses of the diet confirmed that PFOS was stable and did not degrade during the study.

Table 1. Mean and standard deviations of PFOS concentrations in the diet of northern bobwhite quail ^A

Nominal Concentrations of PFOS (ppm)	Measured Concentrations of PFOS (ppm)	CV (%)	Percent Nominal
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 Diet concentrations were reported on a 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 treatments. However, starting at Week 5, clinical signs were observed among quail in the 10 ppm treatment. Signs of toxicity included reduced reaction to external stimuli, ruffled appearance, and lethargy.

Within the first 5 weeks of the study, five-treatment related mortalities occurred in the 50 ppm treatment, and three mortalities occurred in the 150/20 ppm treatment. The expiring quail exhibited overt signs of toxicity prior to death. Due to PFOS-related effects, all surviving quail in the 150/20 ppm treatment were euthanized at Week 5, and surviving quail in the 50 ppm treatment were euthanized at Week 7. Signs of toxicity at these doses included reduced reaction stimuli, wing droop, loss of coordination, loss of righting reflex, lower limb rigidity, convulsions, shallow and rapid respiration, ruffled appearance, lower limb weakness, lethargy, gaping, prostrate posture and spasms.

Based on clinical signs of toxicity, the Lowest Observable Adverse Effect Concentration (LOAEC) was determined to be 10 ppm.

Adult Body Weight: Compared to controls, there were no apparent treatment-related effects on body weight among quail in the 10 ppm treatment (Table 2). However, starting at Week 2, there was a marked and statistically significant reduction in body weights among quail in the 50 and 150/20 ppm treatments. Based on these results, the body weight Lowest Observable Adverse Effect Concentration (LOAEC) was determined to be 50 ppm, while the No Observable Adverse Effect Concentration (NOAEC) was determined to be 10 ppm.

Feed Consumption: There were no treatment-related effects on feed consumption for quail in the 10 ppm treatment group when compared to controls (Table 2). However, starting at Week 1, a treatment-related reduction in feed consumption was observed in quail from the 50 and 150/20 ppm treatments. While some recovery in feed consumption was observed in the 150/20 ppm treatment group at Week 4, this improvement was likely due to a reduction in the PFOS dose (from 150 to 20 ppm in the feed). Due to the reductions in feed consumption observed through Week 7, the LOAEC for reduction in feed consumption was determined to be 50 ppm, while the NOAEC was determined to be 10 ppm.

Liver Weight: When compared to controls, there were no treatment-related effects on liver weight among male bobwhite quail in the 10 ppm treatment (Table 2). In contrast, there was a statistically significant ($p < 0.01$) treatment-related increase in liver weight in females from the 10 ppm treatment when compared to controls. Based on these results, the LOAEC for adult female quails was determined to be 10 ppm. In males, the NOAEC based on liver weight was determined to be 10 ppm.

Table 2. Feed consumption and body and liver weights of northern bobwhite quail exposed to various concentrations of PFOS ^A

Treatment (ppm)	ADI ^B (mg/ kg/day)	Average Feed Consumption (g feed/bird/day)	Sex	Body weight (g)	Liver weight (g)
Control	NA ^C	19 ± 2	M	215 ± 16	4.43 ± 0.50
			F	247 ± 20	8.66 ± 1.11
10	0.772 ± 0.06	19 ± 3	M	208 ± 11	4.48 ± 0.55
			F	249 ± 22	10.9* ± 1.60
50	2.64 ± 0.24	9* ± 2	M	179* ± 20	NA ^C
			F	185* ± 22	
150	7.32 ± 1.26	8* ± 2	M	187 ± 14	NA ^C
			F	172 ± 29	

^A Values are given as means and standard deviations. Body and liver weights are terminal measurements, with control and 10 ppm treatments sampled at Week 21, while the 50 and 150/20 ppm treatments were sampled at Week 7 and 5, respectively. Feed consumption data was for Week 20 for control and 10 ppm groups and Week 6 and 3 for 50 and 150/20 ppm, respectively.

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

^C NA = Not applicable; no sample reported.

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

Gross Pathology: Necropsy results on all surviving females indicated that there were no PFOS related effects among quail in the 10 ppm treatment when compared to the controls. However, male quail in the 10 ppm treatment had a greater incidence of reduced testis size (6 out of 16 males) than the controls (1 out of 16 males). All other findings were considered incidental and not related to treatment.

Histopathology: There were no lesions in the liver, kidney, brain, proventriculus, adipose tissue, or bursa fabricius in either adult or juvenile bobwhite quails from the 10 ppm treatment. In adult males, an increase in the incidence of small testes size in the 10 ppm treatment was not accompanied by any morphological change in spermatogenesis. This suggested that PFOS might have accelerated post-reproductive regression, a normal physiological process. Based on these results, the LOAEC was determined to be 10 ppm, based on increased incidence of small testes size.

Reproductive Results: When compared to controls, there were no treatment-related effects on egg production in the 10 ppm treatment. However, while not statistically significant, there were slight reductions in the number of viable embryos as a percentage of egg set, hatchlings as a percentage of live 3-week embryos and in 14-day old survivors as a percentage of hatchlings. Slight reductions in fertility, hatchability and offspring survival also resulted in reductions in the number of hatchlings and 14-day old survivors as percentages of both the number of eggs set and the maximum number of eggs set. While slight, there was a statistically significant reduction in the number of 14-day old survivors as percentages of the number of eggs set. Based on this effect, the LOAEC was determined to be 10 ppm.

Adult Blood and Liver PFOS Analysis: Results of the chemical analysis of blood and liver samples showed that adult quail in the 10 ppm treatment group accumulated PFOS during the study (Table 3). At the termination of the study, liver and serum PFOS concentrations measured in males from the 10 ppm treatment group were approximately 16-18 times greater than the concentrations measured in females. While there was a sex specific difference in tissue concentrations, the serum to liver ratio for both sexes was similar with a male ratio of 1.6 and a female ratio of 1.8. Since concentrations of PFOS were measured only in quail from the 10 ppm treatment, a quantitative relationship between PFOS exposure and liver or serum concentrations was not developed.

Table 3. Mean concentrations of PFOS in liver and serum of adult quail at study termination. ^A

Treatment (ppm)	Sex	Liver Conc. (µg/g)	Serum Conc. (µg/mL)
Control	M	< LOQ ^B	< LOQ
	F	< LOQ	< LOQ
10	M	88.5 ± 28.5	141 ± 30
	F	4.9 ± 1.0	8.7 ± 2.6

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

^B LOQ = Limit of quantitation; for liver it was 0.0502 µg/g while for serum it was 400 µg /ml

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

Table 4. Concentrations of PFOS (µg/mL) in serum collected from adult bobwhite quail. ^A

Treatment (ppm)	Sex	Sample time (week)		
		5	10	15
Control	M	0.024 ± 0.008	<LOQ ^B	<LOQ
	F	0.012 ± 0.004	<LOQ	<LOQ
10	M	98 ± 51	158 ± 42	164 ± 51
	F	84 ± 28	56 ± 38	8.9 ± 1.7
50		Week 5	Week 7	
	M	557	NA	
	F	525	495	

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

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

By Week 5, serum PFOS concentrations did not differ between male and female quail. However, at Week 10, sex specific differences in serum PFOS concentrations became apparent. The concentrations of PFOS in male bobwhite quail were significantly greater than those observed in females. Moreover, this trend continued to Week 15. Female bobwhite quail serum PFOS concentrations were 18-fold less than those observed in males sampled at the same times. The decrease in female serum PFOS concentrations coincided with the onset of the egg-laying phase in the study. This would seem to indicate that female quail transferred PFOS into their eggs during reproduction. In contrast, male serum PFOS concentrations reached an apparent saturation concentration by Week 10, and they did not statistically differ for the remainder of the study.

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

Table 5. Concentrations of PFOS in red blood cells ($\mu\text{g/mL}$) from adult bobwhite quail exposed to various concentrations of PFOS ^A

Treatment (ppm)	Sex	Sample Period (week)		
		5	10	15
Control	M	< LOQ ^B	< LOQ	< LOQ
	F	< LOQ	< LOQ	< LOQ
10	M	267 $\pm$ 232	184 $\pm$ 325	35 $\pm$ 26
	F	280 $\pm$ 226	74 $\pm$ 82	2.0 $\pm$ 1.0
		Week 5	Week 7	
50	M	165	359	
	F	106	110	

^A Red blood cell concentrations are reported as means and standard deviations.

^B LOQ = Limit of quantitation; in red blood cells the LOQ was 0.01 $\mu\text{g/mL}$.

There was an apparent sex difference in red blood cell mean concentrations at weeks 10 and 15 but not at week 5, although there is substantial variability in the data. Red blood cell PFOS concentrations decreased in both male and female over time, particularly from weeks 10 to 15. The ratio of serum to red blood cell concentrations were also similar in males and females at all time points but the ratio shifted over time as concentrations in red blood cells declined more than serum levels by week 15.

While the biological significance of PFOS concentration changes in red blood cells versus serum is unclear, the blood results show that PFOS concentrations declined during the reproductive phase.

Concentrations of PFOS in Blood and Liver of Juvenile Quail: The analysis of serum and liver samples from juvenile quail indicated that PFOS was still present in these quail 12 weeks after hatching. The PFOS concentrations in both liver and serum were increased in the 10 ppm treatment group over that measured in the control group, but were less than the concentrations measured in adult quail from the same treatments (Table 6). Unlike adult quail, no sex specific differences were observed in juvenile liver or serum PFOS concentrations. The serum to liver ratio for PFOS in male and female juvenile quail averaged 1.92 ± 0.46 and 1.97 ± 0.80 , respectively. The juvenile serum to liver ratio was similar to the ratio observed in adult quail (males = 1.91; females = 1.42).

Table 6. Concentrations of PFOS in the liver and serum of 2 week old juvenile quail. ^A

Treatment (ppm)	Sex	Liver PFOS Concentration ($\mu\text{g/g}$)	Serum PFOS Concentration ($\mu\text{g/mL}$)
Control	M	< LOQ ^B	< LOQ
	F	< LOQ	< LOQ
10	M	5.76 ± 0.67	12.6 ± 3.37
	F	5.49 ± 1.14	12.4 ± 3.46

^A Concentrations are reported as means and standard deviations. Data given as wet weight.

^B LOQ = Limit of quantitation; LOQ for liver = 20 ng/g; LOQ for serum = 400 ng/ml

Egg Component PFOS Concentrations: PFOS concentrations in egg components were measured in eggs collected from the control and 10 ppm treatments. Eggs were selected from Weeks 19, 20, 21 and yolks were composited and analyzed for concentrations of PFOS. Yolk (whole) composites were then analyzed for PFOS (Table 7). In addition, to better understand the distribution of PFOS in the egg yolk, yolks composites in the 10 ppm treatment were also separated into three fractions, very low density lipoprotein (VLDL), phosvitin, and lipovitellin fractions. PFOS concentrations were then determined in each yolk fraction (Table 7).

Table 7. Concentrations of PFOS in bobwhite quail egg yolk and egg yolk components ^A

Treatment	Fraction	PFOS Concentration ($\mu\text{g/g}$)
Control	Yolk-whole	< LOQ ^B
10 ppm	Yolk-whole	62 ± 15
	Yolk-VLDL	39.8
	Yolk-Phosvitin	0.83
	Yolk-Lipovitellin	1.32

^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, a composite of several eggs.

^B LOQ = Limit of quantitation; LOQ = 0.01 $\mu\text{g/g}$.

Results indicate that at 10 ppm, there was a significant accumulation of PFOS into the egg yolks of northern bobwhite quails as compared to controls. No PFOS was detected in a quantifiable concentration in the control egg yolk. Furthermore, results showed that the greatest concentration in the yolk was associated with VLDL fraction, followed by lipovitellin.

CONCLUSIONS:

Northern bobwhite quails were exposed to PFOS at dietary concentrations of 0, 10, 50 and 150/20 ppm for up to 21 weeks. Due to signs of overt toxicity, adult quail in the 50 and 150/20 ppm treatments were terminated at the end of 7 and 5 weeks, respectively.

When compared to controls, there were no PFOS-related effects on adult body weight or feed consumption at a dose of 10 ppm in the feed (Table 8). However, minor overt signs of toxicity were observed in adults dosed at 10 ppm. At 10 ppm, there was a statistically significant increase in female liver weight. In adult males, there was an increase in the incidence of small testes size (Table 8). The increase in the number of adult males in the 10 ppm treatment group with reduced testes size was not accompanied by any morphological change in spermatogenesis. This suggests that PFOS may have accelerated early post-reproductive phase regression, a normal physiological phenomenon. Additionally, there were slight, but not statistically significant treatment-related reductions in fertility, hatchability, and a statistically significant reduction in offspring survival ($p < 0.05$). Based on these effects, the Lowest Observable Adverse Effect Concentration (LOAEC) for PFOS reproductive effects in bobwhite quail was determined to be 10 ppm in feed, based on 21 weeks of exposure. PFOS concentrations associated with the 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 northern bobwhite quails and their offspring. ^{A,B}

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

^A All concentrations reported on a wet weight basis.

^B 10 ppm group exposed for 21 weeks; 50 ppm group exposed for 7 weeks.

Table 9. LOAEL values in various matrices in adult and offspring northern bobwhite quails in a chronic dietary study with PFOS.

Measures of PFOS Exposure ^A	LOAEL ^{B,C}
ADULT MALES	
Dose (ppm)	10
ADI (mg PFOS/kg body weight/day) over 21-week period	0.772
Serum (µg PFOS/mL) at study termination (21-weeks)	141
Liver (µg PFOS/g) at study termination (21-weeks)	88.5
ADULT FEMALES	
Dose (ppm)	10
ADI (mg PFOS/kg body weight/day) over 21-week period	0.772
Serum (µg PFOS/mL), pre-reproductive phase (5-weeks)	84
Serum (µg PFOS/mL), reproductive phase, at study termination (21-weeks)	8.7
Liver (µg PFOS/g) at study termination (21-weeks)	4.9
OFFSPRING	
Yolk (µg PFOS/mL)	62
Liver (µg PFOS/g) ^C	5.5
Serum (µg PFOS/mL) ^C	12.5

^A LOAEL was based on a decrease in the 14-day old survivability of offspring, increased incidence of small sized testes in adult males, and a statistically significant increase in adult female liver weight. All concentrations reported on a wet weight basis.

^B Low effect values for diet and ADI are reported as dietary concentrations. Serum, and liver effect values are reported as measured tissue values.

^C LOAEL values for males are based on histopathology, for females on increased liver weight, and on reduced 14-day survivability for reproduction.

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

DATA QUALITY:

Reliability: Klimish ranking = 1

REFERENCES:

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OTHER

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