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Acute and Chronic Effects of Perfluorobutane Sulfonate (PFBS) on the Mallard and Northern Bobwhite Quail

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Abstract Perfluorobutane sulfonate (PFBS) can be a final degradation product of perfluorobutane sulfonyl fluoride (PBSF)-based chemicals. Surfactants based on this chemistry are potential replacements for perfluorooctane sulfonate (PFOS)-related products and have many potential applications in industrial and commercial processes and applications. To evaluate the potential hazard that PFBS may pose to avian species, acute dietary studies with juvenile mallards and northern bobwhite quail, as well as a quail dietary chronic study of reproduction were conducted. In the acute studies, 10-day-old mallards and quail were exposed to nominal dietary concentrations of 1,000, 1,780, 3,160, 5,620 or 10,000 mg PFBS/kg feed, wet weight (ww) for 5 days and the birds were then fed an untreated diet and observed for up to 17 days. No treatment-related mortalities were observed in the study up to 10,000 mg PFBS/kg, ww feed. Body weight gains of quail exposed to 5620 or 10,000 mg PFBS/kg feed were statistically less than that of unexposed controls. Weight gain of mallards exposed to 10,000 mg PFBS/kg feed was statistically less than that of controls. There were no statistically significant effects on feed consumption of either species. In the acute studies, no observed adverse effect concentration (NOAEC) for mallards and quail were 5620 and 3160 mg PFBS/kg, ww feed, respectively. In a reproduction study, adult quail were exposed to nominal dietary concentrations of 100, 300, or 900 mg PFBS/kg, ww feed for up to 21 weeks. There were no treatment-related mortalities or effects on body weight, weight gain, feed consumption, histopathology measures, or reproductive parameters evaluated in the study when compared to the control group. Concentrations of PFBS in blood serum, liver, and eggs were dose-dependent but were less than the administered dose, indicating biodiminution. Based on the results from the quail reproduction study, the dietary NOAEC was 900 mg PFBS/kg, ww feed (equivalent to an ADI of 87.8 mg PFBS/kg bw/d).

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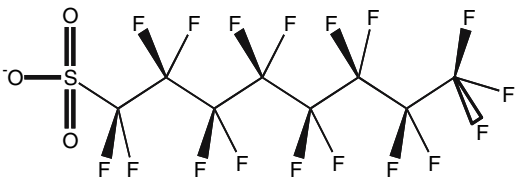
Introduction

Perfluoroalkyl acids (PFAAs) are synthetic, fully fluorinated fatty acid analogues of various chain lengths, some of which are characterized by having either a terminal sulfonate or carboxylate group. Due to their unique surface-active properties, PFAAs have been widely used in many commercial and industrial applications including uses as stain repellents on carpets, textiles, leather and

paper products, and as surfactants in fire-fighting foams, electronic applications, and plastics (Kissa 2001; Hekster *et al.* 2003). Due to their environmental persistence, many of these compounds are globally distributed and are routinely measured in various environmental matrices as well as in wildlife and humans (Giesy and Kannan 2001, 2002; Taniyasu *et al.* 2003). One of the most commonly measured PFAAs in environmental and human samples has been perfluorooctane sulfonate (PFOS) (Giesy and Kannan 2001; Martin *et al.* 2004; Olsen *et al.* 2003, 2004; Butenhoff *et al.* 2006). PFOS has a C_8 perfluorinated chain with a terminal sulfonyl functional group that imparts to it both oleophobic and hydrophobic properties (Fig. 1) (3M 2003). PFOS- and PFOS-related compounds can be released into the environment from product manufacturing processes, supply chains, product use, and disposal (3M 2003; Hekster *et al.* 2003).

In 2000, the 3M Company began a voluntary phase out of the production of PFOS and chemistries that may degrade to PFOS (Giesy and Kannan 2002). In 2003, the 3M Company began using perfluorobutanesulfonyl fluoride-based (PBSF) chemistry in some products. Perfluorobutane sulfonate (PFBS) (Fig. 1) can be one of the ultimate degradation products of PBSF-based chemistry. PFBS is a surfactant and consists of a C_4 perfluorinated chain with a terminal sulfonyl functional group. PFBS can be released directly into the environment as PFBS, or can be formed via degradation of substances based on PBSF chemistry.

While PFBS shares some of the oleophobic and hydrophobic properties of PFOS, it is not considered to be as bioaccumulative or toxic (NICNAS 2005). In a two-generation rat reproduction study, no treatment-related mortality, effects on fertility, reproduction or pup survival, size or body weight was noted in animals treated with up to 1000 mg PFBS/kg bw/day. However, some effects were noted in several liver parameters in males from the parental and F1 generation given 300 mg PFBS/kg bw/d (Butenhoff and Lieder 2006). Similarly, in a 90 day oral gavage study rats given up to 600 mg PFBS/kg bw/d exhibited no treatment-related mortality, or effects on body weight or neurological assessment endpoints (Lieder and Butenhoff 2006). Effects on several hematological parameters were observed in males exposed to 200 mg PFBS/kg bw/d and based on these subtle, nonlethal effects, the no observable effect level (NOEL) was determined to be 60 mg PFBS/kg bw/day. While studies with mammals have found that PFBS is significantly less toxic than PFOS (3M 2003), no studies have been conducted with wildlife such as birds to evaluate this finding. Here we report the results of acute dietary studies of PFBS with two avian species, the mallard (*Anas platyrhynchus*) and the northern bobwhite quail (*Colinus virginianus*) as well as a chronic dietary study of reproduction with bobwhite quail. Mallards and quail were selected for study based on the fact that they had previously been used in PFOS toxicity studies and would provide comparative data to PFBS study results. The potential for accumulation of PFBS into liver, blood serum, and eggs was also evaluated for both species exposed to dietary concentrations of PFBS. Finally, results from this study were compared to PFBS concentrations measured in environmental samples.



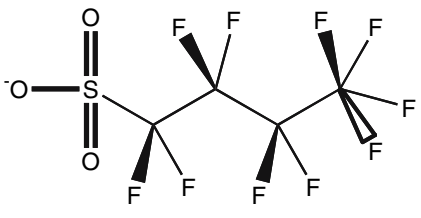


Fig. 1 Structures of perfluorooctane sulfonate (PFOS) and perfluorobutane sulfonate (PFBS)

Materials and Methods

Test Material

Two production lots of potassium perfluorobutane sulfonate (PFBS; CAS No. 29420-49-3) were obtained from 3M, Specialty Materials Manufacturing Division (St. Paul, MN) as white powders that were identified as T-7485. For the acute studies, PFBS (lot # 2) had a reported purity of 97.3% while that used in the chronic study (lot # 5), was reported to be 98.2%. Purity was determined by liquid chromatography/mass spectrometry and nuclear magnetic resonance (NMR). Nominal PFBS concentrations in the feed and standards were adjusted based on the purity of the technical material so that the values reported represent only PFBS.

Quail and Ducks

Fertilized eggs of mallards and northern bobwhite quail were obtained from Whistling Wings, Inc (Hanover, IL) and Wildlife International Ltd., (Easton, MD), respectively, to provide test organisms for the acute studies. Individuals

of each species were from the same hatch, and were phenotypically similar to wild mallards or bobwhites, respectively. Newly hatched individuals of each species were acclimated to the holding facilities for 10 days. Throughout the acclimation period, exposure, and recovery phases of the study, birds were fed a game bird diet formulated to Wildlife International, Ltd. specifications (Wildlife International 2003a; 2003b). Feed contained a minimum of 27% protein and 2.5% crude fat, a maximum of 5% crude fiber, and a vitamin mix.

Adult northern bobwhite quail were purchased from K & L Quail (Oroville, CA) for the reproduction study. The birds were phenotypically indistinguishable from wild quail. Prior to exposures to PFBS, quail were acclimated to laboratory conditions for a period of 5 weeks, during which they were observed for general condition and physical injuries. Food and water were provided ad libitum to both adults and offspring throughout the studies. The basal ration contained at least 27% protein, 2.5% fat, no more than 3.8% fiber, and approximately 1.1% calcium and 0.9% limestone. For adult breeding birds, an additional 5% (w/w) of limestone (approximately 38.5% Ca) was added to the diet to aid in egg shell formation. To ensure the health of juvenile and adult birds, all housing and husbandry practices were conducted as established by the National Research Council (NRC 1996).

Dietary Dosing

For acute dietary exposures, PFBS was added directly in the ration to which 2% corn oil had been added and both control and treated diets were mixed in a Hobart mixer (model AS200T). Nominal dietary test concentrations used in the acute studies were 1000, 1780, 3160, 5620, and 10,000 mg PFBS/kg, ww feed. In the chronic reproduction study, PFBS was mixed as a solid directly into the ration at nominal concentrations of 100, 300, and 900 mg PFBS/kg, ww feed to which 2% corn oil had been added to both control and treatments. To verify nominal concentrations and evaluate homogeneity and stability, feed preparations were extracted with methanol and analyzed by high-pressure liquid chromatography with triple quadrupole mass spectrometry (Wildlife International 2005). Internal standards were not used in the analysis; instead matrix spikes were used to evaluate recoveries. The method limit of quantification (LOQ) for the acute studies was set at 150 mg PFBS/kg, ww feed while that for the chronic study was 50 mg PFBS/kg, ww feed. Dose preparations for both sets of studies were found to be homogeneous, stable, and on average, all dose mixtures for each dose group were found to be within 10% of the nominal dose (data not shown).

Quantification of PFBS

Concentrations of PFBS in serum, liver, and eggs were with a solid-phase extraction (SPE) procedure followed by quantification by liquid chromatography/tandem mass spectrometry (Wildlife International 2005). Briefly, samples ranging from 2.0×10^{-2} to 1.0 mL or gram were diluted (fluids) or homogenized (tissue) at a 1:6, 1:10 or 1:20 dilution using reagent-grade water. An aliquot (up to 1 ml) of the diluent/homogenate was spiked with the appropriate surrogate or analyte mixture. Acetonitrile (ACN) was added to precipitate proteins and as the extraction solvent. The sample was then mixed for approximately 20 min and centrifuged at 2000 rpm for 10 min. The supernatant was diluted with water and passed through a preconditioned SPE (Sep-Pak 6cc trifunctional C_{18}; Waters, Milford, MA, USA) column in a Zymark Rapid Trace automated system (Hopkinton, MA, USA). The analytes were eluted from the column with 2.0 ml methanol and the eluant was transferred to auto-vials for chemical analysis. Separations were achieved using a Keystone Betasil (Thermo Electron, Waltham, MA USA) C_{18} (2×50 mm, 5 μm) analytical column and an ammonium/methanol mobile phase. PFBS was quantified at 299 *m/z* in a Hewlett Packard® series 1100 liquid chromatograph (Agilent, Palo Alto, CA, USA) equipped with an atmospheric pressure ionization (API) mass spectrometer detector (Agilent). Unless otherwise noted, all analytical results with feed, serum and liver are reported on a sample wet weight (ww) basis. The results from these analyses were considered quantitative to ± 20% based on the precision and accuracy of the standard curves.

Acute Exposures

Following hatching and acclimation, 10-day-old bobwhite chicks and mallard ducklings were sorted by weight and randomly assigned from each weight class to either the control (30 birds) or treatment (10 birds/treatment) groups. The groups were housed in brooding pens containing five birds each. Bobwhite quail chick pens (72 cm × 90 cm × 23 cm) were located in a thermostatically controlled room having a mean (± SD) ambient temperature and relative humidity of 27.1°C (± 1.7°C) and 54% (± 11%), respectively. Mallard duckling pens (62 cm × 90 cm × 25.5 cm) were located in a room with mean (± SD) ambient room temperature and relative humidity of 24.9°C (± 0.7°C) and 74% (± 5%), respectively. Each group of birds was identifiable by pen number and test concentration and leg bands identified individual birds. A 16-h light/8-h dark photoperiod was maintained for both acclimation and test phases.

Both water and feed were provided ad libitum during acclimation and test phases.

Juvenile birds were exposed for 5 days to either PFBS-treated or control diet. Following the exposure period, all groups were given untreated basal diet for three days. On day 8, one-half of the treatment and control birds were euthanized and tissue, liver, and blood samples were collected for analysis. The remaining birds were fed the basal diet until day 22, when the surviving birds were euthanized. Birds were observed daily for abnormal behavior, physical injury, or mortality.

Body weights were measured at test initiation and on days 5 and 8 for all birds. The remaining birds were weighed at days 15 and 22. Feed consumption was measured as the difference in the amount of feed given to each pen at the start and end of each study phase. The amount of the feed wasted by the birds was not measured; therefore the measured feed consumption values are presented as an estimate of total feed consumption. All birds sampled on days 8 and 22 were subjected to gross necropsy. Prior to necropsy, blood was drawn in heparin-containing syringes from each bird. The blood was separated and serum was collected. During necropsy, livers were removed and weighed. Samples of serum and liver were stored at –20°C for chemical analysis.

Chronic Exposure

After approximately a five-week acclimation phase, quail were exposed to PFBS in the diet. All birds were approximately 18 weeks old at study initiation with body weights ranging from 171 to 220 g. Each treatment group contained 16 replicate pens with each pen containing a pair of birds, one male and one female. Each pair was housed in $27 \times 51 \times 23$ cm pens constructed of wire mesh. The mean (± SD) ambient temperature during the study was 23.2 (± 1.0°C) with a mean relative humidity of 34 (± 17%). A light cycle of 8 h light/16 h dark was administered for the 7 weeks prior to photostimulation. At the beginning of week 8 the photoperiod was increased to 17 h of light per day to induce egg laying.

Upon the initiation of egg production, eggs were collected on a daily basis from all pens and stored in a cold room at (13 ± 0°C; mean relative humidity of 84 ± 6%) until incubation. All eggs laid in a weekly interval were considered to be one lot. At the end of each weekly interval, eggs were counted and candled (Speed King model no. 32 candling lamp) to detect egg shell cracks or abnormal eggs. Cracked or abnormal eggs were recorded and discarded and the remaining eggs were set for incubation. Eggs were incubated at an average temperature of 37.4°C with an average relative humidity of 54%. Eggs were candled on day 11 or 12 and again on day 21 to determine embryo viability. On day 21 of incubation, eggs were placed in a Petersime Hatcher (model no. S6H) and allowed to hatch. After hatching, chicks were housed in $72 \times 90 \times 23$ cm brooding pens. The light cycle for hatchlings was maintained at 16 h light/8 h dark and temperatures in the brooding pens were within the range 36.9–37.5°C. Hatchlings were fed an untreated diet and body weights were determined 14 days post-hatch.

Reproductive endpoints evaluated during this study included: egg production, embryo viability, hatchability, and hatchling health and survival. In addition, blood samples were collected from one 16-day-old chick selected indiscriminately from each pen. Following blood collection, adult birds and chicks were euthanized and subjected to gross necropsy. Liver, kidney, and gonad, were removed for histopathological evaluation. In addition, liver weights were determined and samples were collected for PFBS analysis. At study termination (week 21), blood samples were collected from surviving adult birds for PFBS analysis.

Feed consumption was measured weekly as the difference in weighed amount of feed given to each breeding pair at the start (day 0) and that which remained at the end of the week (day 7). Due to the experimental design, feed consumption of individual birds could not be determined nor could feed consumption be determined by sex. Since the amount of the feed wasted by the birds was not quantified, the measured feed consumption values are presented as an estimate of total feed consumption by pen.

Statistical Analyses

In the acute and chronic studies, treatment concentrations were expressed as both a concentration in the diet (mg PFBS/kg feed) and as an average daily intake (ADI) of PFBS (mg PFBS/kg bw/d) for each treatment group. The ADI was estimated on a pen basis using food consumption and adult body weights. Body weights were averaged over the duration of the exposure along with average measured concentrations for each treatment group (Wildlife International 2005). Differences in the responses of measurement endpoints among treatments were assessed by analysis of variance (ANOVA) followed by Bonferoni's *t*-test to compare values for each treatment group to that of the untreated controls (Gulley 1990). The criterion used for significance in all statistical tests was $p < 0.05$. Feed consumption and reproduction parameters were evaluated on a pen basis while body and liver weight were evaluated on an individual bird basis. In the chronic study, statistical analyses of body weights were conducted separately for males and females by ANOVA and Dunnett's multiple

comparison procedure was used to compare differences between the treatments and controls (Dunnett 1955). Data reported as percentages, such as the measures of reproduction, were examined using Dunnett's method following arcsine square root transformation.

Results

Dietary PFBS Characterization

Feed concentrations of PFBS were confirmed by instrumental analyses. The actual concentrations of PFBS in feed collected during the acute dietary exposures ranged from 93 to 96.4% of the nominal concentrations. Actual mean measured concentrations were 938, 1700, 2940, 5420, and 9440 mg PFBS/kg, ww feed for nominal exposures of 1000, 1780, 3160, 5620, and 10,000 mg PFBS/kg, ww feed, respectively. For the acute mallard study, the ADI values, based on body weight and feed consumption measured during the study, were 629, 845, 1393, 2190, and 3974 mg PFBS/kg bw/day. The ADI values for the acute quail study were 292, 492, 774, 1505, and 2304 mg PFBS/kg bw/day. In the quail reproduction study, the measured concentrations in feed samples collected during the study ranged from 98 to 105% of nominal values. Mean (± SD) measured concentrations of PFBS in feed were 105 ± 5.13, 299 ± 12.0, and 880 ± 16 mg PFBS/kg, ww feed for the 100, 300, and 900 mg PFBS/kg, ww feed nominal treatment levels, respectively. ADI values determined during the chronic exposure were 9.7, 30, and 88 mg PFBS/kg bw/day for the 100, 300 and 900 mg PFBS/kg, ww diet, respectively. No PFBS or any other co-eluting compounds were detectable at the limit of quantitation (LOQ) in the control feed.

Acute Toxicity to Juvenile Mallard

No treatment-related effects on survival or overt signs of toxicity were observed in any of the treatment groups or in the control group. As a result, a LC50 value could not be determined. In addition, all birds from all treatment groups were normal in appearance and behavior throughout the test. During the exposure phase (days 0–5), no statistically significant effect on mean mallard body weight gain were observed when compared to the control values (Fig. 2A). There was a statistically significant ($p < 0.05$) reduction in weight gain of juvenile mallards exposed to 10,000 mg PFBS/kg, ww feed when compared to the control at day 5. However, this effect did not persist and by day 8 weight gain approximated that measured in the controls. While a statistically significant reduction in weight gain for mallards from 3160 mg PFBS/kg, ww feed treatment (day 15–22) was also noted when compared to the controls, this effect was not concentration-dependent and when taken together with the fact that weight gain during the earlier phases of the study was similar to control values, this finding was deemed to be an artifact and not considered to be treatment-related.

Feed consumption in PFBS-treated mallards was not significantly different from that measured in the controls in any of the study phases. In addition, during the gross pathology evaluation, no remarkable findings were observed in PFBS-treated mallards when compared to controls. Finally, there were no apparent PFBS-related effects on mean absolute liver weight (Fig. 2C) or on mean relative liver weight (liver weight as a percentage of body weight; data not shown) for mallards collected on days 8 and 22 of the study.

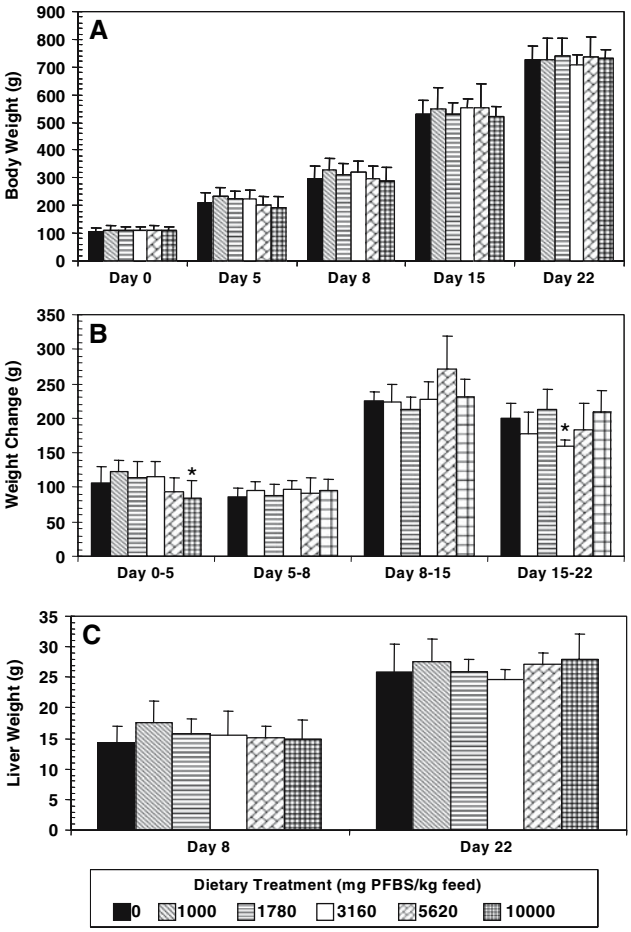


Fig. 2 Average body weight (A), weight change (B), and liver weight (C) for juvenile mallards exposed to PFBS in the diet. Weights reported as means ± standard deviations. * indicates statistically significant differences from the control group at $p < 0.05$ (Bonferoni's *t*-test)

Acute Toxicity to Juvenile Quail

No treatment-related mortalities or overt toxic effects were observed during any phase of the study. For this reason, an LC50 could not be determined. In addition, with the exception of incidental observations associated with physical injury, birds from all PFBS treatments were normal in appearance and behavior throughout the study. On days 5 and 8 of the study, mean body weight gains of quail exposed to 5,620 or 10,000 mg PFBS/kg, ww were significantly less when compared to control quail (Fig 3). However, by day 15 of the study the mean body weights of quail from these two treatments recovered and were not statistically different from control values. Body weight gain from day 0 to 5 was significantly reduced from control levels in quail exposed to 1000, 5620, and 10,000 mg PFBS/kg ww feed. However, body weight gain from these treatment groups recovered between days 5 to 8 such that they did not statistically differ ($p > 0.05$) from that of control values. While feed consumption of quail exposed to PFBS during the exposure phase of the study was slightly less than that measured in the controls, these differences were not statistically significant or dose related.

No overt signs of toxicity were observed in quail exposed to PFBS-treated feed when compared to untreated controls. While incidental observations of physical injury such as toe, foot, or ankle lesions were noted in all PFBS-treatment groups and the control, these effects were sporadic and attributable to pen-mate aggression. In addition, quail from both the PFBS treatments and the untreated controls were normal in appearance and behavior throughout the study. On day 8, absolute liver weights of quail exposed to 1000 or 10,000 mg PFBS/kg, ww feed were statistically ($p < 0.01$) less than those of the untreated controls (Fig. 3C). However, when liver weight was normalized to body weight, normalized liver weights of PFBS-treated quail were comparable to control values (data not shown). At day 22, there were no significant differences in absolute or relative liver weights between quail exposed to PFBS and the untreated controls.

Chronic Toxicity to Adult Quail

No PFBS-related mortality or overt toxicity was observed for quail exposed to 100, 300 or 900 mg PFBS/kg, ww feed. While three quail died, one from the control group (female) and one each from the 100 (male) and 300 (female) mg PFBS/kg ww feed groups, these deaths were not attributed to PFBS exposure. Incidental clinical observations made during the test occurred at approximately the same frequency for all groups and included

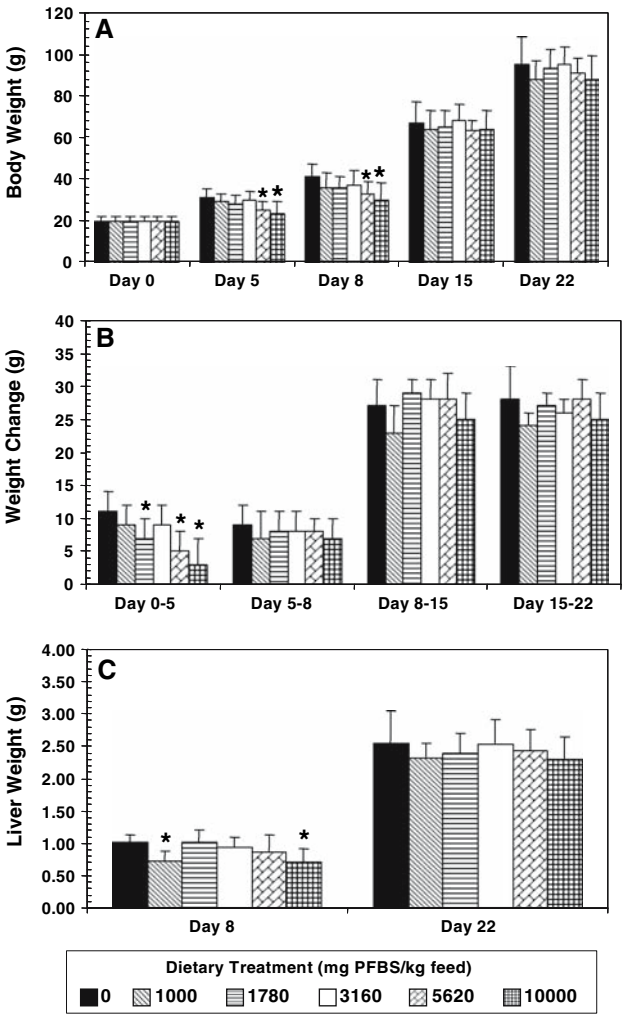


Fig. 3 Average body weight (A), weight change (B), and liver weight (C) for juvenile quail exposed to PFBS in the diet. Weights reported as means ± standard deviations. * indicates statistically significant differences from the control group at $p < 0.05$ (Bonferoni's *t*-test)

findings that were associated with injuries due to normal pen wear. Except for these incidental findings, all birds were normal in appearance and behavior throughout the study.

There were no statistically significant effects on body weight or weight gain of PFBS-treated adult quail when compared to controls (Table 1). There were also no treatment-related effects on feed consumption when compared to that of control quail (Table 1). While there was a statistically significant 15% increase in feed consumption observed in birds fed 300 mg PFBS/kg, ww feed at week 16, this difference was not considered treatment related. This conclusion was based on the observations that this effect had not been observed at any other time for this treatment group and that it was not dose-responsive. Finally, while absolute and body weight normalized liver weights of quail from the 900 mg PFBS/kg, ww feed

Table 1 Mean feed consumption and whole body and liver weights (± standard deviation) of northern bobwhite quail exposed to various concentrations of PFBS during the chronic exposure[a]

Nominal PFBS (mg/kg, ww feed)	Average daily intake[b] (mg PFBS/kg bw/day)	Feed consumption (g feed/bird/day)	Sex	Body weight (g, ww)		Liver weight (g)	
				Week 8	Week 21	Absolute	Relative
Control	NA	24 ± 2	M	203 ± 14	205 ± 20	4.13 ± 1.45	1.98 ± 0.57
			F	201 ± 15	238 ± 15	7.23 ± 0.64	3.04 ± 0.31
100	9.7	24 ± 2	M	208 ± 15	215 ± 10	4.07 ± 0.90	1.90 ± 0.42
			F	197 ± 13	237 ± 15	7.58 ± 1.58	3.20 ± 0.82
300	29.7	24 ± 2	M	208 ± 10	215 ± 17	4.19 ± 1.69	1.92 ± 0.63
			F	201 ± 12	238 ± 19	7.20 ± 1.11	3.03 ± 0.44
900	87.8	24 ± 3	M	209 ± 10	214 ± 16	3.93 ± 1.07	1.81 ± 0.36
			F	201 ± 15	230 ± 20	6.62 ± 1.40	2.83 ± 0.59

[a] Liver weight measured at study termination, week 21. Relative liver weight is absolute liver weight as a percentage of body weight

[b] Average daily intake based on body weight and feed consumption for weeks 1 to 21 of the study

Differences between control and PFBS treatments were not significant ($p > 0.05$)

treatment were slightly less than that measured in the controls, these differences were not statistically significant ($p > 0.05$) (Table 3).

There were no PFBS-related gross pathologies observed in the livers, kidneys, ovaries, or testes of adult male or female quail. There was a slight incidence of smaller diameter testis that was observed in the adult males from the control (2 of 16) and from the 100 (5 of 15), 300 (3 of 16), and 900 (5 of 15) mg PFBS/kg, ww feed treatments sampled at study termination. While a few males from all treatment groups and the controls exhibited some seminiferous tubular degeneration, nearly all the males from these groups exhibited normal spermatogenesis that was characterized by the presence of numerous mature spermatozoa in the seminiferous tubules. Since the presence of smaller testis was not dose-dependent and occurred at approximately the same frequency in the control and exposed males, these observations were not considered to be PFBS related.

Chronic Effects on Reproduction of Quail

There were no statistically significant, PFBS-related effects on quail reproductive performance when compared to control quail (Table 2). There was a slight delay in onset of egg production (approximately one week) for quail exposed to 900 mg PFBS/kg, ww feed. In addition, there was a slight but not statistically significant reduction in the total number of eggs laid by hens from the 900 mg PFBS/kg, ww feed treatment when compared to control quail. However, this difference was attributed to a single hen that exhibited foot lesions and that did not lay any eggs during the last five weeks of the study. When the data from that pen were excluded from the analysis, the number of eggs laid per hen from this treatment groups were comparable to that of the unexposed controls. Finally, there were no statistically significant effects observed on any reproductive endpoint measured during the study for PFBS-treated quail when compared to untreated controls (Table 3).

PFBS Concentrations in Liver, Blood Serum, and Eggs of Chronically Exposed Quail

PFBS was present in liver and blood serum of adult quail in a dose-dependent manner (Table 4). While there was a slight difference in liver and serum PFBS concentrations between male and adult female quail from each of the exposures, these differences were not statistically significant and were typically less than 1.5-fold. PFBS concentrations measured in blood serum were greater than that observed in the liver. The ratio between mean PFBS concentrations in blood serum and liver for both adult male and female quail ranged from 3.4 to 5.1 with a mean value of approximately 4. Serum PFBS concentration of both genders was approximately 6- to 13-fold less than that measured in diet while liver PFBS was approximately 30- to 56-fold less than that in the diet.

Concentrations of PFBS in eggs were directly proportional to dietary concentrations and were also dependent on when the eggs were laid during the study. Concentrations of PFBS in eggs laid during the seventh week (lot G) were approximately 1.6-fold greater than that measured in eggs during the second week (lot B) (Table 4). Based on PFBS concentrations measured in adult females, the egg-to-serum ratio was approximately 1.0 while the egg-to-liver ratio was approximately 3.4 for all treatment groups. In 14-day-old offspring, PFBS concentrations in blood serum and liver were proportional but less than that measured in adult female quail or eggs from the same treatment group (Table 4). For

Table 2 Reproductive performance of northern bobwhite quail during chronic exposure to PFBS in the diet

Reproductive parameter	Nominal PFBS (mg PFBS/kg, ww feed)			
	Control	100	300	900
No. replicates	15	15	15	16
Eggs laid[a]	58 ± 7	56 ± 8	60 ± 6	51 ± 11
Eggs set	52 ± 7	51 ± 8	54 ± 6	46 ± 12
Viable embryos	46 ± 11	48 ± 10	50 ± 7	44 ± 11
Live 3-week embryos	46 ± 11	48 ± 10	50 ± 7	43 ± 11
Hatchlings	45 ± 12	46 ± 10	47 ± 6	42 ± 11
14-day survivors	43 ± 11	43 ± 11	45 ± 7	40 ± 11
Eggs laid/hen/day	0.58 ± 0.1	0.57 ± 0.1	0.60 ± 0.1	0.51 ± 0.1
14-day-old survivors/hen	43 ± 11	43 ± 11	45 ± 7	40 ± 11
Normalized data (%)[b]				
Viable embryos/eggs set	89 ± 18	93 ± 10	92 ± 9	94 ± 6
Live 3-week embryos/viable embryos	99 ± 2	99 ± 1	99 ± 1	99 ± 1
Hatchlings/live 3-week embryos	96 ± 8	97 ± 4	94 ± 4	97 ± 3
Hatchlings/eggs set	86 ± 20	90 ± 11	86 ± 9	91 ± 7
14-day-old survivors/hatchlings	96 ± 3	93 ± 9	96 ± 4	94 ± 5
Hatchlings/max. set	71 ± 19	73 ± 16	74 ± 10	67 ± 18
14-day-old survivor/max. set	69 ± 18	68 ± 17	71 ± 11	63 ± 17

[a] Values represent means ± standard deviations and are based on 99 d of egg production

[b] Values represent pen means ± standard deviations for each experimental group

No statistical differences were seen between control and treatment groups at $p > 0.05$

Table 3 Body weight and liver weights of offspring from adult quail exposed to PFBS in the diet[a]

Nominal PFBS	Body weight (g, ww)[b]		Liver weight (g, ww)[b]	
(mg/kg feed)	Hatchling	Offspring	Male	Female
Control	6 ± 0	26 ± 2	1.013 ± 0.111	1.082 ± 0.118
100	6 ± 1	26 ± 3	1.113 ± 0.183	1.030 ± 0.129
300	6 ± 0	27 ± 2	1.207 ± 0.122	1.292 ± 0.287
900	6 ± 0	27 ± 3	1.107 ± 0.228	1.071 ± 0.169

[a] Body weight from hatchlings and 14-day-old survivors. Liver weights from 14-day-old survivors

[b] Data presented as mean and standard deviation

Differences between control and treatment groups were not statistically significant ($p > 0.05$)

instance, PFBS concentrations in the tissues of offspring were at least 480-fold less than those measured in eggs from the seventh week (lot G) for all treatment groups. This indicates that the contribution of PFBS from eggs to the chicks would not be expected to persist for a significant period of time due to either rapid loss from the tissues through elimination processes or through growth dilution.

Discussion

Unlike the effects observed in avian studies conducted with PFOS, PFBS was not observed to be acutely toxic to juvenile mallard and quail even at dietary concentrations of up to 10,000 mg PFBS/kg ww feed. Furthermore, when PFBS-related effect were noted on sublethal endpoints such as body and liver weight, these effects did not persist to study termination. For example, while there was a statistically significant reduction at day 8 in liver weight of quail from the 10,000 mg PFBS/kg, ww feed treatment group, liver weight at day 22 for this treatment group had returned to control levels. Furthermore, no significant differences in normalized liver weight were observed between PFBS-treated and control quail sampled on day 8 or 22 of the study. Since this liver finding was not associated with changes in any other pathological indicators in the liver,

Table 4 Average (± SD) concentrations of PFBS in liver and blood serum of adult and juvenile quail and eggs during a chronic dietary exposure.

Nominal PFBS (mg/kg feed)	Sex	Adult tissue concentrations[a] (μg PFBS/g, ww)		Offspring tissue concentrations[b] (μg PFBS/g, ww)		Egg concentrations[c] (μg PFBS/g, ww)
		Serum	Liver	Serum	Liver	
Control	M	<LOQ	<LOQ	<LOQ	<LOQ	Lot B:<LOQ
	F	<LOQ	<LOQ	<LOQ	<LOQ	Lot G: <LOQ
100	M	16.5 ± 10.3	3.25 ± 2.226	0.037 ± 0.015	0.021 ± 0.010	Lot B: 7.67 ± 2.05
	F	14.6 ± 8.4	3.25 ± 2.31			Lot G: 14.0 ± 5.50
300	M	27 ± 9.5	7.78 ± 3.32	0.057 ± 0.032	0.052 ± 0.062	Lot B: 23.6 ± 8.58
	F	37.8 ± 23.5	11.1 ± 6.03			Lot G: 31.4 ± 19.1
900	M	68.2 ± 21.3	15.7 ± 4.78	0.133 ± 0.074	0.111 ± 0.079	Lot B: 50.5 ± 27.9
	F	104 ± 84.6	29.6 ± 19.7			Lot G: 92.6 ± 31.8

[a] Adult serum and liver samples collected at study termination. Limit of quantification (LOQ) for serum and liver was 0.0022 and 0.0019 μg PFBS/g, ww, respectively

[b] Offspring liver and serum samples collected at day 16, post hatch. LOQ for serum and liver was 0.0022 and 0.0019 μg PFBS/g, respectively

[c] Egg from lot B collected from second week of egg sets; egg from lot G is from the seventh week. LOQ for egg was 0.0058 μg PFBS/g, ww. NA, analyzed

this suggests that these effects were not toxicologically significant or directly related to PFBS. Instead, they may have been the result of normal variation in liver weight related to potential malnutrition due to the high PFBS concentrations in the feed (USEPA 2002; Bailey *et al.* 2004). Based on the body weight endpoints from the acute studies, the NOAECs for quail and mallards were 3,160 and 5,620 mg PFBS/kg, ww feed, respectively.

In the quail reproduction study, no PFBS-related effects on body and liver weight or feed consumption were noted with any of the dietary treatments. Thus, while there was a slight reduction from control values in these endpoints in quail exposed to 900 mg PFBS/kg ww feed, these effects were not statistically significant and typically represented less than a 10% difference from control levels. Gross pathological and histopathological evaluation of the adult quail and their offspring also did not reveal any significant PFBS-related effects. While there was a slight incidence of reduced testis size in male quail from all treatment groups, the incidence was not dose responsive or statistically significant when compared to control values. In addition, the frequency of adult males with normal spermatogenesis was similar between all treatment groups and the controls. Thus, in conjunction with the fact that egg production and fertility were not effected in PFBS-treated quail compared to controls, this condition was considered to be the result of physiological regression, a normal process that has been observed in many birds post-reproduction (Rosenstrauch *et al.* 1994; Wilkelski *et al.* 2003).

When compared to the results from the quail dietary reproduction study previously conducted with PFOS, PFBS was found to be significantly less toxic. In the current quail reproduction study, no PFBS-related effects were noted on any lethal or nonlethal endpoints measured in the study and the dietary NOAEC was determined to be 900 mg PFBS/kg, ww feed (equivalent to an ADI of 87.8 mg PFBS/kg bw/day). In contrast, the NOAEC and LOAEC based on mortality for PFOS were 10 and 50 mg PFOS/kg, ww feed, respectively. Based on reproductive endpoints, the PFOS LOAEC was 10 mg PFOS/kg, ww feed (equivalent to 0.77 mg PFOS/kg bw/d) while a NOAEC was not determined (Newsted *et al.* 2007). When the chronic NOAEC for PFBS is compared to the LOEAC for PFOS in quail, PFBS is at least 90-fold less toxic than PFOS based on feed concentration. When compared on an average daily intake (ADI) basis, PFBS is at least 150-fold less toxic than PFOS. The difference in toxicity between PFBS and PFOS observed in the avian studies is not unexpected as the toxicity of PFAAs has been shown to be related to the number of perfluoroalkyl carbons (Goecke-Flora and Reo 1996; Upham *et al.* 1998; Hu *et al.* 2002; Martin *et al.* 2003a). For instance, while gap junction communication (GJIC) was inhibited in liver epithelial cells (WB-F344) exposed to PFOS (eight perfluorinated carbons), it was not inhibited by PFAAs with carbon chain lengths of less than five or more than 16. This result was also observed by Hu *et al.* (2002) where the optimal chain length to inhibit GJIC in WB-F344 and dolphin kidney cells (CDK) for carboxylates and sulfonates was determined to be 10 and eight carbons, respectively. GJIC was not inhibited by PFBS in any of these cell lines. Thus, the results from the PFOS and PFBS avian toxicity studies follow the same structure–activity relationship observed in the in vitro studies.

The observation that PFBS was present in blood and liver of adult quail at concentrations that were less than that measured in their PFBS-treated diets is consistent with the

results of both laboratory and field studies conducted with other species. Concentrations of PFBS in blood serum and liver were approximately 11- and 41-fold less than those in the diet, respectively. Thus, due to biodiminution between the diet and blood plasma and liver, bioaccumulation factors (BAF) for PFBS were less than 1.0. This result is consistent with the observation that in natural food chains there is little or no increase in PFBS concentration between trophic levels in aquatic and terrestrial organisms (Tomy *et al.* 2004; Kannan *et al.* 2005). In most cases, the ratio of PFBS concentrations in the higher trophic level to that in the lower trophic level were less than 1.0. Laboratory studies with several fish species also support the concept of biodiminution. In a bioconcentration study with rainbow trout, the estimated bioconcentration factor (BCF) was less than 1.0 (Martin *et al.* 2003a). In a dietary bioaccumulation study with rainbow trout, the bioaccumulation factor (BAF) for PFBS was also estimated to be less than 1.0 (Martin *et al.* 2003b). For bluegill exposed to PFBS in water, BCF values ranged from 0.113 to 0.16 in edible tissue and from 0.272 to 0.43 in inedible tissues depending on PFBS exposure concentration (Wildlife International 2001).

Bioaccumulation and biomagnification of PFBS via trophic transfer is not expected to be a significant factor in determining the long-term exposure potential of upper-trophic-level avian species. This conclusion is based on the fact that, in the current laboratory study, PFBS was not accumulated to any significant level; the concentration in the blood and liver of quail exposed to PFBS was at least 10-fold less than that measured in their diet. This conclusion contrasts with that observed in laboratory studies with PFOS in which PFOS has been shown to bioaccumulate into the serum and liver of adult male quail to levels that are approximately 14- and 9-fold greater than those measured in their diet, respectively (Newsted *et al.* 2006; 2007). Similarly, using field-based concentration data, many biomagnification factors for PFOS have been derived: that for common mergansers to fish is cited as 8.9 (Sinclair *et al.* 2006), glaucous gulls to arctic cod have a BMF of 9.0 (Tomy *et al.* 2004) while a BMF of 4–5 has been calculated for bald eagle to fish (Kannan *et al.* 2005). Thus, when the bioaccumulation potential of PFBS in aquatic organisms and avian species is taken into consideration relative to that observed for PFOS, the bioaccumulation potential of PFBS into upper-trophic-level avian species would not be significant.

Gender-specific differences in accumulation of PFBS from the diet into blood serum and liver of quail were not great and were mostly less than 1.6-fold. The only statistically significant gender-dependent differences in tissue concentrations were associated with the 900 mg PFBS/kg, ww feed group. In contrast, PFOS concentrations in male quail liver and serum were approximately 18- and 16-fold greater than that measured in female quail, respectively (Newsted *et al.* 2007). From these data it can be concluded that sex and reproductive condition are not important factors affecting the accumulation of PFBS in adult quail.

Potential Hazard of PFBS in the Environment

To date, few studies have detected PFBS in environmental samples. In a study conducted in Japan, no PFBS was detected in fish, birds or human samples (Taniyasu *et al.* 2003). In that study, the detection limit for liver was 7.5 ng PFBS/g ww and 151 ng PFBS/ml for blood. While these detection limits are relatively high in comparison to current achievable detection limits, PFBS at these levels would not pose a significant risk to avian species. These limits are approximately 3000- and 570-fold less than the concentrations of PFBS in liver and blood serum associated with the NOAEC values determined in this study, respectively. Furthermore, even if PFBS liver concentrations were to reach levels that were equivalent to greatest liver PFOS concentrations measured in piscivorous birds (Kannan *et al.* 2001), these concentrations would still be approximately 13-fold less than the PFBS no observed effect concentration measured in the laboratory studies. Thus, based on currently available data, concentrations of PFBS in the environment would not be expected to pose a significant hazard to birds.

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