ECOTOXICOLOGY, ENVIRONMENTAL FATE AND HEALTH TESTING OF PERFLUOROBUTANE SULFONATE

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EXECUTIVE SUMMARY

Introduction

This document describes the physical and chemical properties, degradation, ecotoxicology and mammalian toxicology information generated for perfluorobutane sulfonate (PFBS). PFBS is a chemical that is the potential degradation product of certain substances based on perfluorobutane sulfonyl fluoride chemistry. PFBS is a fully fluorinated four-carbon organic molecule produced synthetically by electrochemical fluorination, other processes, and from the degradation or metabolism of other four-perfluorocarbon products or derivatives.

PFBS is not metabolized but is excreted rapidly and has very low toxicity in acute and repeat-dose tests. Further, it does not affect reproductive function or prenatal development. Although it is persistent in the environment, PFBS does not accumulate in organisms. As a chemical with low toxicity that does not bioaccumulate, PFBS does not meet the criteria for designation of a PBT chemical under the USEPA PBT Chemical policy.

PFBS-based products fall into the broad category defined by PFAS (Perfluoro Alkyl Sulfonates, carbon chain length from C1 to C20 or greater). Because properties vary significantly depending on the carbon number or chain length, the environmental, health and safety characteristics of members of this class of substances must be reviewed on an individual basis. 3M has studied the potential hazards of perfluoroalkyl sulfonates with higher carbon numbers or chain lengths (e.g., C_6 and C_8), and this information has enabled 3M to focus its research with respect to PFBS.

Environmental Characteristics

PFBS is non-volatile and highly soluble in water. Thus, any PFBS in water would be expected to remain in the water column rather than volatilizing to air. PFBS does not partition to sediment. It does not bioconcentrate; the steady-state bioconcentration factor in bluegill sunfish was found to be less than 1. It is not degraded by hydrolysis or photolysis, although it is degraded by high-temperature incineration.

Acute and chronic ecotoxicology studies with a number of aquatic species show no or minimal toxicity at quite high concentrations (> 100 mg/L). Acute NOEC values for all species evaluated for ecotoxicity ranged from 127 to 5,620 ppm, while chronic NOEC values ranged from 200 to 502 ppm. Aquatic organisms tested include two invertebrate species and two fish species, as well as algae and wastewater treatment bacteria. The most sensitive aquatic species tested was the mysid shrimp, with the 96-hour acute no-observed-effect concentration (NOEC) determined to be 127 mg/L. Fifty percent clearance from fish was estimated at 1 to 3 days.

In acute avian feeding studies, no mortality was seen with mallard duck or bobwhite quail after exposures as high as 10,000 mg PFBS/kg feed. In a six-week pilot reproduction feeding study with five pairs of bobwhite quail, a NOEC concentration of 200 mg PFBS/kg feed for six weeks was determined based on egg production. A 21-week definitive reproduction study with 16 pairs of Bobwhite Quail determined the NOEC to be 900 mg/kg feed, which is equivalent to an average daily dose of 87.8 mg PFBS/kg body weight/day. No treatment-

related mortalities, overt signs of toxicity, histopathology, or treatment-related effects upon body or liver weight or feed consumption were seen at any of the concentrations tested. There were no treatment-related effects upon any of the reproductive parameters measured. The difference in egg production between the pilot and definitive studies is thought to be an artifact of length of exposure, sample size and the replacement of a hen during the pilot study.

Given the fact that PFBS did not bioconcentrate in organisms to levels greater than the concentrations to which they were exposed and the very low toxicity to aquatic and avian species, no adverse effects on the environment or biota are expected.

Human Health Hazard Assessment

Hazard Characterization

PFBS has a relatively short elimination half-life in the primate. A single-dose intravenous pharmacokinetic study in male and female cynomolgus monkeys showed a terminal-phase half-life of 3-4 days.

Acute toxicity tests indicate moderate eye irritation in rabbits, as expected with any surfactant. Studies show no skin irritation in rats and no allergic dermal contact sensitization reactions in guinea pigs.

PFBS produced no toxicity in rats following single oral gavage administration at 2,000 mg PFBS/kg body weight. Additionally, acute dermal exposure at 2,000 mg PFBS/kg body weight in rats showed no toxic effects.

PFBS was not mutagenic in the *Salmonella typhimurium/Escherichia coli* plate incorporation/preincubation mutation assay. PFBS did not induce chromosome aberrations in cultured chinese hamster ovary (CHO) cells with and without exogenous metabolic activation. These findings are consistent with the fact that PFBS is chemically non-reactive and cannot form metabolites that would be reactive with genetic material.

PFBS administered daily by gavage for 28 days at a dose of 900 mg/kg/day resulted in an increase in male liver and female kidney weight in rats. The increased liver and kidney weights are considered to be an adaptive rather than toxic response. Only minor effects on electrolytes (phosphorous and potassium) were observed at 300 mg PFBS/kg/day. The no-observable effect level (NOEL) for PFBS in this 28-day repeat dose study is 100 mg/kg/day.

A 90-day repeat dose oral gavage study at PFBS doses of 60, 200 and 600 mg PFBS/kg body weight was performed in rats. At the dose of 600 mg/kg/day, there were treatment-related microscopic changes in the stomach, nasal cavity and turbinates, and kidney tubules. The nasal and stomach effects are related to irritation from repeated exposure to PFBS, a strong surfactant at a relatively high dose. The microscopic kidney effects (primarily minimal to mild tubular hyperplasia and papillary edema) are likely consistent with a response to high local concentrations of PFBS, and there were no indications of functional impairment or damage. There was a slight decrease in red blood cells, hemoglobin and hematocrit at 600 mg PFBS/kg in the male rats. Hemoglobin and hematocrit were also reduced at 200 mg

PFBS/kg/day. The 90-day study also included a motor activity and functional observation battery to evaluate potential neurotoxic effects. PFBS had no neurotoxic effects. The NOEL for all effects was 200 mg/kg/day for the female rat, and 60 mg/kg/day for the male rat.

A two-generation reproductive study has been conducted, using PFBS doses of 30, 100, 300 and 1,000 mg/kg/day in rats. There were no effects on fertility or reproduction in either the P or the F1 generations. There were no microscopic changes in male or female reproductive organs, and no effects on sperm parameters, mating, estrous cycles, pregnancy, and natural delivery in the P or F1 generations.

There were no treatment-related effects on survival of pups in the two-generation study. Litter size and average pup body weight per litter did not differ significantly from controls in any dose group. In the F1 generation, body weight was reduced in males at 1,000 mg/kg/day. Preputial separation was slightly delayed (two days) at this dose, a finding consistent with the body-weight reduction. Essentially no effects were observed in the F1 females. F2 pups had normal body weights.

The NOEL for toxicity in the parental generations (P and F1) was 100 mg/kg/day, which is consistent with the 90-day repeat-dose study findings. In males, increased liver weight (absolute or relative) and corresponding increased incidence of adaptive hepatocellular hypertrophy were observed in the 300 and 1,000 mg/kg/day dose groups. Increased incidence of mild to minimal microscopic findings in the medulla and papilla of the kidneys, consistent with those seen in the 90-day oral study, were observed in the 300 and 1,000 mg/kg/day dose-group rats. The reproductive NOEL was >1,000 mg/kg/day in both generations.

In summary, PFBS exhibits little or no toxicity in laboratory animal testing, including a two-generation study. PFBS is rapidly cleared from the body, with an elimination half-life of hours in rats and a few days in primates. PFBS is not metabolized and cannot covalently modify biological compounds.

For all these reasons, the human health hazard of PFBS is extremely low.

Conclusion

This report is a technical summary of the ecological and mammalian toxicity and fate data accumulated for PFBS as of June 2005. Although PFBS is resistant to degradation and is persistent in the environment, results from environmental testing demonstrate that PFBS is not acutely or chronically toxic to aquatic or avian organisms at concentrations less than 100 ppm. The acute NOEC values for all species evaluated for ecotoxicity ranged from 127 to 5,620 ppm, while chronic NOEC values ranged from 200 to 900 ppm. PFBS does not bioconcentrate and does not bioaccumulate. Thus, adverse ecological effects are not expected. Results from numerous mammalian toxicity studies indicate that PFBS has low toxicity in both acute and repeat dose studies. Further, it does not affect reproductive function or prenatal development. PFBS is cleared from the body in fish and primates within days. Exposures are expected to be low. The data indicate the potential toxicity and ecological impacts of PFBS are minimal.

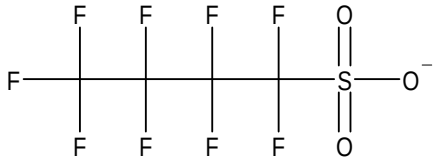
1.0 PHYSICAL/CHEMICAL PROPERTIES

Testing described in this document, except where noted, was conducted utilizing the potassium salt of PFBS (CAS No. 29420-49-3). Because the salt is transformed immediately to the anion when dissolved, the results describe the anion as well.

Identity:

Molecular formula: $C_4F_9SO_3^-$

Structural formula:



Synonyms: 1-Butanesulfonate, 1,1,2,2,3,3,4,4,4-nonfluoro

1.1 Vapor Pressure

Vapor pressure was evaluated in the laboratory utilizing the Spinning Rotor Guage method, following OECD guideline 104. Hexachlorobenzene and DDT were successfully used as method reference substances. The vapor pressure of the potassium salt of PFBS was below the method detection limit, and was reported as $<1.22 \times 10^{-5}$ Pa @ 20°C.[1]

1.2 Dissociation constant

Perfluoroalkyl sulfonic acids are considered to be strong acids (super acids) and will exist at 100% dissociation when dissolved in aqueous media. A literature citation and Hammett acidity value ($H_o = -13.2$) for PFBS have been reported for the acid dissociation value measured in non-aqueous media by UV spectrophotometry at 22° C.[2]

1.3 Solubility

The shake flask method (OPPTS 830.7840 and OECD 105) was used to determine the water solubility of the same lot of PFBS, potassium salt at two different laboratories. Both studies were conducted following Good Laboratory Practice guidelines. Preliminary and definitive tests were conducted at both laboratories, and the concentration of PFBS in the water was measured. At 20° C, solubility was reported as 46,200 mg/L, and at 22.5 – 24° C as 52,600 – 56,600 mg/L.[3,4]

The solubility of PFBS in methanol and acetone was also estimated using the shake flask method. These studies were conducted in one laboratory, and only the preliminary test was conducted. A sample of 10 mg of PFBS was visually determined to be dissolved in 100 µL of each solvent after shaking, vortexing, and sonicating.[4]

1.4 Surface Tension

Static surface tension measurement of PFBS in water, was measured at 37 dynes/cm using standard Wilhelmy Plate methodology (1 x 2 cm plate) on a Krüss K12 at ambient pressure and 21° C[5].

1.5 Critical Micelle Concentration

A critical micelle concentration for PFBS was determined to be 50,000 mg/L from a graph of the surface tension versus the log of the concentration. The measurements were made using standard methodology at ambient pressure and 21° C using a Krüss K12 and Dosimat 665[6].

PHYSICAL PROPERTIES

Table 1-1 Physical and Chemical Properties

Parameter	Report Date	Results
Vapor Pressure[1]	4/29/02	< 1.22 x 10^{-5} Pa @ 20 ºC
Dissociation Constant[2]	1976 (lit. value)	Hammett Value H_o = -13.2
Solubility in Pure Water[3,4]	8/30/00, 3/28/01	46,200 mg/L at 20°C 52,600 - 56,600 mg/L at 22.5 – 24° C
Solubility in Methanol[4]	3/28/01	> 10%
Solubility in Acetone[4]	3/28/01	> 10%
Surface Tension[5]	1/14/2002	37 dynes/cm
Critical Micelle Concentration[6]	1/14/2002	50,000 mg/L

2.0 ENVIRONMENTAL FATE

2.1 Degradation

Laboratory studies of hydrolysis, photolysis and biodegradation were not carried out to evaluate the degradability of PFBS. PFBS is expected to be stable under environmental conditions based on its chemical structure and by analogy to the stability of longer chain perfluoroalkyl sulfonates. Given the strength of the chemical bonds in the molecule and the complete fluorination, PFBS is not expected to biodegrade or to undergo hydrolysis or photolysis[7,8].

Incineration studies were conducted utilizing a laboratory-scale simulation of a hazardous waste incinerator to evaluate the destruction of two perfluorobutanesulfonyl polymers and PFBS salt at temperatures of up to 900° C. Quantifiable amounts of PFBS were not formed during the combustion of the polymers. Results of chemical analyses indicated that, with the exception of stable C_1 and C_2 fluorocarbons, fluorinated organic intermediates are unlikely to be emitted during the incineration of PFBS or perfluorobutane sulfonamides. Thermal degradation under high temperature conditions, such as those occurring during incineration is the only known degradation mechanism for PFBS[9].

Table 2-1 Degradation of PFBS		
Parameter	**Report Date**	**Results**
Hydrolysis[7]	7/22/02	Half life estimate > 41 years
Photolysis[8]	7/22/02 (amended 3/24/04)	Half life estimate ≥ 3.7 years
Biodegradation	Not Tested	Non-biodegradable
Thermal Degradation[9]	1/7/03	No PFBS was formed during combustion studies of two perfluorobutanesulfonyl polymers. Results suggest the C-S bond was completely destroyed and did not reform.

2.2 Partitioning

The air/water partition coefficient (K_{AW}) was calculated using the laboratory-generated vapor pressure and water solubility data (see Table 1-1). The result from the water solubility study conducted at 20° C was used in this calculation. The log K_{AW} was found to be < -10.4.[10]

A soil adsorption/desorption study was conducted following OECD Guideline 106. Three soils (loam, clay loam, and clay), one sediment and one washed, powdered, lyophilized NIST sludge were used in the study. Results from the Tier 1 and 2 testing demonstrated that PFBS did not adsorb to the walls of the test vessels. In Tier 3 testing, the three soils and one sediment were tested at a solid to solution ratio of 1:1, while the sludge was tested at a ratio of 1:5. Study results demonstrated no adsorption of PFBS to soils or sediment and minimal adsorption to sludge. Freundlich isotherm calculations were performed using the sludge results only. The sludge adsorption K_f was found to be 0.3 and the desorption K_f was 0.001. The initial PFBS concentration in solution was found to be independent of the determined sorption value. The study results indicate that PFBS tends not to sorb to soils, sediments, and sludge.[11]

A flow-through biocencentration study following OECD Guideline 305 was conducted using juvenile bluegill sunfish (*Lepomis macrochirus*) at exposure concentrations of 0.53 and 5.2 mg/L. The uptake and depuration periods were 28-days and 16-days, respectively. Water, edible, and non-edible fish tissues were analyzed for PFBS concentration. Concentrations in whole fish were calculated based on concentrations in edible and non-edible portions. Calculations of bioconcentration factor (BCF) at apparent steady state and BCFK (kinetic bioconcentration factor) were completed using concentrations of PFBS in edible tissue, non-edible tissue, and whole fish, for both exposure concentrations. BCF values at apparent steady state in edible tissues ranged from 0.16 – 0.21, in non-edible tissues from 0.43 – 0.51, and in whole fish from 0.30 – 0.38. BCFK values in edible tissues ranged from 0.18 – 0.73, in nonedible tissues from 0.50 – 0.86, and from 0.36 – 1.1 in whole fish. Time to 50% clearance was estimated to be from 1.3 – 2.9 days, using BIOFAC computer software.[12]

Table 2-2 Partitioning Test Results		
Parameter	**Report Date**	**Results**
Log Air-Water Partition Coefficient (log K_{AW}), calculated from water solubility and vapor pressure[10]	6/18/02	< −10.4
Soil and Sediment Adsorption/Desorption[(a)11]	3/08/01	No adsorption to any soil or sediment seen. (K_f = 0.0) Highly mobile
Activated Sludge Adsorption/Desorption[(a)11]	3/08/01	Freundlich K_f(ads) = 0.3 Freundlich K_f(des) = 0.001 Highly mobile
Bioconcentration (Bluegill Sunfish, steady-state BCF) Exposed to 0.53 mg/L[12]	5/09/01	Edible Tissue BCF: 0.21 Non-edible Tissue BCF: 0.51 Whole Fish BCF: 0.38
Bioconcentration (Bluegill Sunfish, steady-state BCF) Exposed to 5.2 mg/L[12]	5/09/01	Edible Tissue BCF: 0.16 Nonedible Tissue BCF: 0.43 Whole Fish BCF: 0.30

[(a)]Soil types utilized were clay, clay loam, loam, river sediment, powdered and dried activated sludge from NIST.

The water solubility and soil, sediment, and sludge adsorption/desorption data indicate that any PFBS discharged to a water source would tend to remain in the water column as opposed to binding to sediment.

Bioconcentration test data indicate that PFBS will not partition preferentially from water into fish tissues, and therefore, that PFBS will not bioaccumulate or biomagnify in fish.

The very low vapor pressure and calculated air/water partition coefficient indicate that volatility of the compound is insignificant. Therefore, atmospheric dispersion of PFBS is considered unlikely.

3.0 Ecotoxicology Studies

3.1 Microbial Systems

PFBS was not toxic to wastewater treatment bacteria at 1,000 mg/L, the highest concentration tested. The study was conducted following OECD guideline 209, and utilized activated sludge from a wastewater treatment plant that receives waste from predominantly domestic sources. After 3 hours of exposure, a concentration-response curve was not evident over 7 nominal test concentrations of PFBS spanning from 1.0 to 1,000 mg/L. The 3-hour EC_{50} was determined to be > 1,000 mg/L, with 8.2% inhibition in respiration seen at 1,000 mg/L. [13]

3.2 Algae

PFBS inhibited algal growth only at very high doses (greater than 1,077 mg/L). Testing was conducted using the freshwater green alga, *Selenastrum capricornutum*. Cells were exposed for 96 hours, with microscopic counts taken at 24, 48, 72, and 96 hours. The NOEC and EC_{50} values were calculated using three methods to determine inhibition: 1) cell density; 2) area under the growth curve; and, 3) average specific growth rate. Exposure concentrations were measured at 0, 72 and 96 hours.

The data indicate PFBS was algistatic at the highest level tested; i.e., growth resumed when aliquots of the algae in the maximally inhibited concentration was placed in fresh growth media. Observations of algae cells during the studies found that there were no signs of aggregation, flocculation or adherence of the cells to the flasks after exposure. Calculations utilizing cell density and area under the curve resulted in lower effective concentrations than those using average specific growth rate. However, as the rate of growth, not cell mortality, appeared to be affected in these studies, algae NOEC (1,077 mg/L) and EC_{50} (5,733 mg/L) values reported here were calculated using the average specific growth rate.[14]

3.3 Acute Toxicity to Aquatic Invertebrates

The static acute toxicity of PFBS to a freshwater (*Daphnia magna*) and a marine (*Mysidopsis bahia*) aquatic invertebrate were determined. In the daphnid study, two replicates, each containing 10 daphnids, were exposed for 48-hours. Exposure concentrations were determined at 0, 24, and 48 hours. The effect concentrations were calculated using mean measured concentrations. The 48-hour NOEC and EC_{50} were determined to be 886 mg/L and 2,183 mg/L, respectively.[15]

The marine mysid study was also conducted in duplicate, with 10 mysids per vessel exposed for 96 hours. Exposure concentrations were determined at 0, 48, and 96 hours. The mysid 96-hour NOEC and EC_{50} were determined to be 127 mg/L and 372 mg/L, respectively.[16]

3.4 Chronic Toxicity to Aquatic Invertebrates

A static-renewal survival, growth and reproduction toxicity study was conducted utilizing *Daphnia magna.* There were no adverse effects on survival, reproduction, or growth at concentrations $\leq$ 502 mg/L after 21 days (NOEC = 502 mg/L). Survival was reduced at 1,876 mg/L, while growth and reproduction were reduced at 995 mg/L. Mean measured concentrations were determined from fresh and previous solutions during each week of the test.

In the course of this study, the young produced by the control, 60, 121, 247, 502 and 995 mg/L exposure groups were removed from the test chambers on Day 14. Due to reduced survival at the 1,876 mg/L concentration, there were insufficient offspring from this treatment group to study. They were exposed to the same concentrations to which the respective first-generation adults were exposed. Survival was monitored for 48 hours. After 48 hours of exposure, survival in all treatment groups (control, 60, 121, 247, 502, and 995 mg/L) was 100%. The results of the daphnid second-generation acute exposure indicated a NOEC of 995 mg/L.[17]

3.5 Acute Toxicity to Fish

Two species of fish were evaluated for 96-hour static acute toxicity: fathead minnow (*Pimephales promelas*) and the bluegill sunfish (*Lepomis macrochirus*). The fathead minnow was more sensitive, with an LC_{50} of 1,938 mg/L and a NOEC of 888 mg/L. An LC_{50} of 6,452 mg/L and a NOEC of 2,715 mg/L were reported in the bluegill study. At 96 hours, all surviving fish of both species appeared normal. Exposure concentrations were measured at 0, 48, and 96-hours.[18,19]

3.6 Acute Avian Feeding Studies

Study Design

Acute feeding studies were conducted using 8-day-old mallard ducks (*Anas platyrhynchos*) and 10-day-old northern bobwhite quails (*Colinus virginianus*). Each species was offered the dosed feed for 5 days, followed by untreated feed until Day 22. Doses were reported on a nominal concentration basis for five dose levels (1,000; 1,780; 3,160; 5,620; and 10,000 ppm) plus the negative control group. There were 12 animals per PFBS treatment group and 30 for the negative controls. Homogeneity of test substance concentrations in diet was verified. On Day 8, one-half of the treatment and control bird groups were sacrificed, subjected to gross necropsy, and liver weights were determined. Liver and sera samples were taken from quail exposed to the two highest concentrations (5,620 and 10,000 mg/kg) and were analyzed for PFBS to help determine the concentrations to use in pending reproduction studies. The remaining half of the birds continued without further treatment until Day 22, when the birds were sacrificed, subjected to gross necropsy, and liver weights were obtained.

Results: Mallard Duck (*Anas platyrhynchos*)

There was no mortality from PFBS in mallard ducks at any of the doses tested. The dietary LC_{50} value for mallard ducks was > 10,000 mg/kg feed. Based on a statistically significant ($p < 0.01$) reduction in body-weight gain at the 10,000 mg/kg feed concentration on Day 5 of exposure, the NOEC was 5,620 mg PFBS/kg feed. The mallards exposed to 10,000 mg PFBS/kg feed gained 84 g in weight, while the control birds gained 107 g. There were no overt signs of toxicity or treatment-related effects on feed consumption or liver weights at any of the concentrations tested. No treatment-related necropsy findings were observed. Mallard liver and serum were not analyzed for PFBS.[20]

Results: Bobwhite Quail (*Colinus virginianus*)

There was no treatment-related mortality from PFBS in bobwhite quail at any of the doses tested. The dietary LC_{50} value for quail was > 10,000 mg/kg feed. Based on statistically significant ($p < 0.01$) reductions in body-weight gain after 5 days at the 5,620 and 10,000 mg PFBS/kg feed concentration, the NOEC was 3,160 mg PFBS/kg feed. Quails at the 10,000 mg PFBS/kg feed dose gained 3 grams, those at the 5,620 dose gained 5 grams, while the control quails gained 11 grams after 5 days of exposure. There were no overt signs of toxicity or treatment-related effects on feed consumption or liver weights at any of the concentrations tested. No treatment-related necropsy findings were observed.[21]

PFBS concentrations found in liver and sera taken from the individuals sacrificed on Day 8 are shown in Table 3-1. PFBS did not accumulate to any significant degree in the liver or serum of the bobwhite quail. Doses of 10,000 mg/kg in feed resulted in levels of 1.3 ppm in the serum and liver. [22] Despite a nearly two-fold difference in dose, there does not appear to be any proportional difference in serum and liver PFBS concentrations at the two concentrations studied.

Table 3-1. **Summary of Mean Analytical Results for PFBS in Quail Liver Tissue (mg/kg wet) and Serum (mg/L) at Day 8**

Nominal PFBS Conc., mg/L	Day 8 Percent Mortality	Day 8 PFBS Liver Conc., mg/L	Day 8 PFBS Serum Conc., mg/L
Negative Control	0	<LOQ (0.0256 ppb)	<LOQ (0.0254 ppb)
5,620	0	1.36	2.67
10,000	0	1.34	1.26

3.7 Avian Pilot (Range-Finding) Reproduction Feeding Study

Five pairs of adult Northern Bobwhite Quail were each exposed to PFBS at nominal dietary concentrations of 75, 200, 550, or 1,500 mg PFBS/kg in feed for 6 weeks. The birds were observed for mortality, behavior, signs of toxicity, and egg production. At the end of treatment, all birds were euthanized and subjected to gross necropsy. Liver weights were also obtained. Studies demonstrating homogeneity of test substance concentrations in diet were conducted and the results served as verification of test substance concentrations. None of the control diet samples showed any indication of the presence of the test substance.

No treatment-related mortalities or overt signs of toxicity were observed at any of the concentrations tested. All necropsy findings were considered to be unrelated to treatment. There were no treatment-related effects on body weight or feed consumption at the 75, 200, or 550 mg/kg level. There were slight but consistent reductions in male body weight and feed consumption in the 1,500 mg/kg treatment group. There were reductions in mean egg production at the 550 and 1,500 mg/kg test concentrations and reductions in female liver weight at the 1,500 mg/kg test concentrations. The NOEC in this study was determined to be 200 mg/kg based on egg production. It should be noted that the egg production was significantly lower in Pen 322 than that of the other pens in 1500 ppm dose group. In this pen, the female was euthanized on day 3 due to non-treatment related neck injury. This female was replaced with another that had been acclimated with the other birds to the test conditions. However, egg production in this pen consistently lagged behind that of the other pens exposed to 1500 ppm in the diet. Egg production for the other four pens was higher than that than that seen in the 550 ppm dose group. In a study with such a small sample size (n = 5 females per dose), a change in response from one animal can significantly affect statistical evaluation and may not be representative of a true effect. It is uncertain if a true dose-response relationship is demonstrated in this study. The results of this study were used to set feed concentrations for a definitive reproduction study with the Bobwhite Quail.[23]

3.8 Avian Definitive Reproduction Feeding Study

Study Design

Sixteen pairs of adult Northern Bobwhite Quail were each exposed to PFBS at nominal dietary concentrations of 0 (control), 100, 300 and 900 mg PFBS/kg feed for 21 weeks. The birds were observed for mortality, behavior, signs of toxicity, eggshell thickness and egg production. Hatching success and hatchling survivability were also monitored. At the end of treatment, all surviving adult birds were euthanized and subjected to gross necropsy. Samples of liver, kidney and gonad from all adult birds and selected offspring from each pen were collected and submitted for histopathological examination. Liver weights were also obtained. Samples of sera and liver were obtained from adults and select offspring at the end of the study and were analyzed for PFBS concentrations. Egg homogenates (at least seven eggs per dose) were also analyzed. Studies demonstrating homogeneity of test substance concentrations in diet were conducted and the results served as verification of test substance concentrations. None of the control diet samples showed any indication of the presence of the test substance.

Results: Bobwhite Quail (*Colinus virginianus*)

No treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body or liver weight or feed consumption were seen at any of the concentrations tested. Except for incidental findings, all birds appeared to be normal in appearance and behavior throughout the study. There were no treatment-related effects upon any of the reproductive parameters measured, including egg production, hatching success and hatchling survivability. All necropsy and histopathological findings were incidental and considered to be unrelated to treatment. The overall estimated daily doses in this study (calculated utilizing feed consumption and body weight) were 0 (negative control), 9.7, 29.7, and 87.8 mg PFBS/kg body weight/day. The NOEC (no-observed-effect concentration) for Northern Bobwhite quail exposed to PFBS in the diet was 900 mg PFBS/kg diet, the highest concentration tested. This corresponds to an average daily dose of 87.8 mg PFBS/kg body weight/day.[24]

PFBS concentrations found in liver and serum samples are shown in Table 3-2. Low levels were detected in negative control liver (range 0.00945 – 0.129 mg/kg) and serum (0.0209 – 2.8 mg/kg). None of the egg samples from the negative control group contained measurable levels of PFBS. Concentrations in the liver, serum, eggs, and offspring tended to increase with increasing dose. Doses at the NOEC of 900 mg/kg in feed resulted in mean PFBS levels of 16 – 30 ppm in adult liver and 68 to 104 ppm in adult serum. Offspring liver and serum values were less than 0.4 ppm at all doses. Mean concentrations in egg homogenates from the 900 ppm dose ranged from 51 to 92 ppm.[25]

Table 3-2. **Summary of Mean (and Range) PFBS Concentrations in Quail Liver Tissue, Serum and Egg Homogenates at Study Termination**

Nominal PFBS Conc., mg/kg feed	PFBS Liver Conc., mg/kg	PFBS Serum Conc., mg/kg	PFBS Egg Conc., mg/kg*
100, male	**3.25** (0.808 – 8.89)	**16.5** (5.81 – 37.7)	
100, female	**3.52** (0.396 – 8.19)	**14.6** (2.49 – 33.1)	**7.65** (lot B), **14.0** (lot G) (4.92 – 10.8 lot B), (8.44 – 26.2 lot G)
100, offspring	**0.0211**** (<0.0137 – 0.0301)	**0.0369**** (<0.0278 – 0.0552)	
300, male	**7.78** (1.42 – 14.2)	**27.9** (14.2 – 42.9)	
300, female	**11.1** 3.57 – 23.5)	**37.8** (12.2 – 102)	**23.6** (lot B), **31.4** (lot G) (13.3 – 36.5 lot B), (16.1 – 73.1 lot G)
300, offspring	**0.0515**** (0.0138 – 0.200)	**0.0567** (0.0245 – 0.119)	
900, male	**15.7** (6.73 – 23.7)	**68.2** (34.3 – 98.8)	
900, female	**29.6** (9.73 – 77.4)	**104** (30.5 – 370)	**50.5** (lot B), **92.6 (**lot G) (33.8 – 118 lot B), (52.6 – 137 lot G)
900, offspring	**0.111** (0.0340 – 0.313)	**0.133** (0.0413 – 0.320)	

*Eggs were collected twice during the study

**The mean was calculated for only those samples in which PFBS was detected.

Table 3-3 Ecotoxicity of PFBS		
Parameter	**Result[a]**	**Units**
Wastewater Bacteria (OECD 209)		
3-hour EC50	> 1,000[b]	mg/L
Inhibition at highest concentration tested (1000 mg/L) = 8.2%		
***Selenastrum capricornutum* (freshwater green algae)**		
96-hour NOEC (growth rate)	1,077	mg/L
96-hour ErC_{10} (95% confidence interval)	1,674 (1,482 – 1,839)	mg/L
96-hour ErC_{50} (95% confidence interval)	5,733 (5,659-5,817)	mg/L
***Daphnia magna* (freshwater water flea)**		
Acute 48-hour NOEC	886	mg/L
48-hour EC_{50} (95% confidence interval)	2,183 (1,707 – 3,767)	mg/L
21-day Semi-static Life-cycle Test NOEC	502	mg/L
21-day Semi-static Life-cycle Test LOEC	995	mg/L
***Mysidopsis bahia* (mysid shrimp)**		
Acute 96-hour NOEC	127	mg/L
Acute 96-hour LC_{50} (95% confidence interval)	372 (314 – 440)	mg/L
***Pimephales promelas* (fathead minnow)**		
Acute 96-hour NOEC	888	mg/L
Acute 96-hour LC_{50} (95% confidence interval)	1,938 (888 – 3,341)	mg/L
***Lepomis macrochirus* (bluegill sunfish)**		
Acute 96-hour NOEC	2,715	mg/L
Acute 96-hour LC50 (95% confidence interval)	6,452 (5,252 – 9,433)	mg/L
***Anas platyrhynchos* (mallard duck)**		
Dietary (5-days) acute NOEC (body weight gain)	5,620[b]	mg/kg[c]
Dietary (5-days) acute no mortality concentration	10,000[b]	mg/kg[c]
Dietary (5-days) LC_{50}	> 10,000[b]	mg/kg[c]
***Colinus virginianus* (bobwhite quail)**		
Dietary (5-days) acute NOEC (body weight gain)	3,160[b]	mg/kg[c]
Dietary (5-days) acute no mortality concentration	10,000[b]	mg/kg[c]
Dietary (5-days) LC_{50}	> 10,000[b]	mg/kg[c]
Dietary pilot (6 week) reproduction NOEC (mean egg production)	200[b]	mg/kg[c]
Dietary definitive (21-week) reproduction NOEC (survival, reproduction)	900[b]	mg/kg[c]

[a]All results calculated using mean measured concentrations except where noted
[b] Results based on nominal concentrations; sample well characterized
[c]Reported as mg PFBS per kg feed

3.9 Summary of Ecotoxicology

PFBS exerted minimal toxicity to the wide range of organisms studied. The most sensitive species tested was the mysid shrimp, *Mysidopsis bahia*, with a 96-hour acute NOEC of 127 mg/L and an acute LC_{50} of 372 mg/L. This acute LC_{50} value is well above 100 mg/L, the

concentration threshold above which the USEPA's OPPT classifies chemicals to be of low concern for TSCA 8(e) reporting. This concentration (100 mg/L) is also recommended by the OECD Guidelines for the Testing of Chemicals as the highest to be used in acute aquatic toxicity limit tests.

3.10 Environmental Hazard Evaluation

Laboratory data indicate that the environmental hazard of PFBS is low. The most sensitive endpoints in aquatic and terrestrial acute and chronic toxicity testing are at concentrations greater than 100 parts per million.

PFBS does not appear to bioconcentrate, and therefore, exposure through the food chain is unlikely.

Based on the very low to negligible hazard, the rapid clearance as demonstrated in the fish bioconcentration and monkey studies, and the low exposure potential, no adverse environmental or ecological effects are expected from PFBS.

4.0 MAMMALIAN TOXICITY

PFBS has been evaluated (as the potassium salt) in a wide variety of toxicity studies including acute, repeat-dose oral, mutagenicity, developmental toxicity, reproductive toxicity and pharmacokinetics. PFBS has low acute and repeat-dose toxicity, is not bioaccumulative, and does not affect reproductive function or prenatal development.

4.1 Acute Toxicity of PFBS

Dermal Toxicity Study of PFBS:

PFBS was studied to determine the acute effects of a single dermal application of 2,000 mg PFBS/kg body weight to the skin of Sprague-Dawley rats (male and female, 8 weeks old, 5 rats in each dose group) followed by a 14-day recovery. The test material was applied as a powder uniformly over an area that was approximately 10% of the body surface area. The test material was held in contact with the skin with a porous gauze dressing and non-irritating tape for a 24-hour exposure period. Clinical observations were for 14 days. Clinical observations included red material around the eyes, nose, and mouth. Treatment with 2,000 mg PFBS /kg produced no other treatment-related adverse clinical observations, mortality, changes in body weight, or gross pathology findings. The dermal LD_{50} of PFBS is > 2,000 mg/kg, which classifies PFBS as "practically non-toxic" by the dermal route of administration.[26]

Acute Oral Toxicity (Rat):

PFBS was studied to determine the potential of the material to induce lethality in the rat. Male and female Sprague-Dawley rats were exposed by gavage to PFBS in aqueous carboxymethyl cellulose at PFBS doses of 500, 1,000, and 2,000 mg/kg body weight and then observed for a 14-day recovery period. There were 5 rats in each dose group. There were no indications of hypoactivity, ataxia, and corneal opacity. Single oral gavage

administration of 2,000 mg/kg of PFBS did not induce any adverse clinical signs, mortality, and changes in body weights or gross findings in rats. The oral LD_{50} of PFBS in the rats is greater than 2,000 mg/kg.[27]

4.2 Primary Eye and Skin Irritation

PFBS caused moderate eye irritation effects in rabbits. PFBS did not produce skin irritation or allergic skin reactions in studies with rats.

Primary Eye Irritation:

PFBS was studied to determine its potential to induce irritation in the eyes of three 16-week-old female New Zealand white rabbits. Within one hour after application of approximately 80 mg of PFBS powder, excessive tearing was noted in all three exposed rabbits. The tearing persisted throughout the remainder of the study. Maximum average ocular scores for 24- and 72-hours post-dose were 30 and 35, respectively. Ocular irritation scores at the 7-day reading < 10. Some effects were noted in one animal 21 days after exposure. The resulting scores indicate that PFBS is classified as a moderate eye irritant.[28]

Primary Skin Irritation:

PFBS was studied to determine its potential to cause irritation of the skin of three 14-week-old female New Zealand white rabbits. PFBS powder was applied to a small area (approximately 6 cm^2) of skin and covered with a gauze patch for 4 hours. Scoring of the dose site was performed at the time of patch removal and at approximately 24, 48 and 72 hours after patch removal. The Draize scoring system was used. During the study, no adverse findings were reported, with the exception of one rabbit exhibiting decreased appetite on study days 2 to 4.[29]

PFBS did not induce erythema, edema, or other skin effects during this study (Score 0.0/8.0). PFBS is classified as "non-irritating" to skin.

Delayed Contact Hypersensitivity Study in Guinea Pigs (Maximization Test):

No allergic response was produced by PFBS in guinea pigs using the Maximization Protocol to assess delayed contact hypersensitivity potential. The material is considered not a skin sensitizer.[30]

4.3 Genotoxicity

Bacterial Reverse Point Mutation Assay (Ames):

PFBS was tested in the *Salmonella typhimurium/Eschericia coli* plate incorporation-preincubation mutation assay in the presence and absence of induced rat-liver S-9, a bioactivating system. The definitive mutation assay, using the plate incorporation method, was performed with the four standard *Salmonella typhimurium* tester strains, specifically TA98, TA100, TA1535, and TA1537, and the *Escherichia coli* strain WP2uvrA. The results of the mutation assay were negative. Consequently, a second (confirmatory) mutation assay

was performed to re-affirm the results using the preincubation method of treatment. The results of the mutation assays indicated that PFBS did not induce any significant increase in the number of revertant colonies for any of the tester strains in the presence or absence of induced rat liver S-9. The positive and negative controls fulfilled the requirements of the test. Under the conditions of this study, PFBS was not mutagenic in the *Salmonella typhimurium/Escherichia coli* plate incorporation/preincubation mutation assay.[31]

Chromosomal Aberration:

PFBS was tested for its potential to induce chromosome aberrations in cultured chinese hamster ovary (CHO) cells with and without exogenous metabolic activation in the chromosome aberration assay (OECD 473 OPPTS 870.5375, with DMSO as dosing vehicle). PFBS was not considered to be a clastogenic agent in CHO cells.[32]

4.4 Repeat Dose Toxicity Studies

28-Day Repeat Dose Oral Toxicity:

Eight-week-old male and female Charles River Sprague-Dawley rats (10 rats/sex/dose) were dosed daily by gavage for 28-days with PFBS at dose levels of 100, 300 and 900 mg/kg/day body weight. An extra 5 rats/sex in the 0 and 900 mg/kg groups constituted a 14-day recovery group. This study resulted in no mortalities, abnormal clinical observations, and changes in body weight gain or food consumption.

A neurobehavioral battery (peripheral neuropathy, motor activity and audio-visual) was negative, except for tail-flick response in males at 900 mg/kg/day that suggested hyper-excitability.

There were some hematological findings that were judged to be incidental. At doses of 300 and 900 mg/kg/day there was a significant decrease in serum phosphorus and potassium. An increase in serum chloride was also noted. An increase in relative and absolute liver weight was observed in males at the 900 mg/kg/day dose. Kidney-weight effects in females were also seen at 900 mg/kg/day. No associated gross or microscopic findings were observed. The organ weight parameters were similar to controls after a 14-day recovery in the 900 mg/kg/day dose group. The increases in kidney weights were considered to be an adaptive response given the lack of any histological change. The no-observable effect level (NOEL) for PFBS in this 28-day repeat dose study was 100 mg/kg/day.[33]

90-Day Repeat Dose Oral Gavage Toxicity Study:

Male and female Charles River Sprague-Dawley rats (10 rats/sex/dose) were dosed daily by gavage for 90-days with PFBS at dose levels of 60, 200, and 600 mg/kg/day body weight. Observations for clinical signs of toxicity were made daily. Food consumption and body weights were recorded weekly and at the end of the study. A functional observation battery and motor activity assessment were conducted with a satellite group of rats (5/sex). Upon termination of exposure, histological examination was performed on all prepared tissues from the control and 600 mg/kg/day group. Additional microscopic examinations were performed

on the nasal cavities and turbinates, stomachs, and kidneys of the male and female rats in the 60 and 200 mg/kg dosage groups.

There were no treatment-related mortality or body weight changes. Chromorhinorrhea observed periorally and urine stained abdominal fur were observed in males at the 600 mg/kg/day dose. Motor activity and functional observation battery results were similar to controls, indicating that PFBS had no neurotoxic effects.

All rats appeared normal at sacrifice. At necropsy the red blood cells (RBC), hemoglobin concentration, and hematocrit values were reduced in males receiving 200 and 600 mg/kg/day; however, there were no microscopic findings in bone marrow. Total protein and albumin was decreased in females at the 600 mg/kg/day dose. There were no significant changes in clinical chemistry, including cholesterol, glucose, triglycerides, and serum alkaline phosphatase, suggesting no changes in liver function.

Treatment-related microscopic changes were only observed at the 600 mg/kg/day dose (both sexes, stomach and kidneys). The stomach lesions consisted of hyperplasia and some necrosis of the mucosa with some squamous metaplasia. The stomach effects were likely due to a cumulative direct irritation effect resulting from gavage of PFBS, which is a strong surfactant. The microscopic findings in the kidneys consisted primarily of minimal-to-mild hyperplasia of the epithelial cells of the medullary and papillary tubules and the ducts in the inner medullary region in both male and female rats. The incidence of tubular hyperplasia in high-dose males and females were 8/10 and 6/10, respectively, with 13 of the 14 responses being minimal or mild (one moderate). Minimal papillary edema was observed in high-dose males (3/10) and females (3/10). A single incident (1/10) of moderate papillary necrosis was observed in a male. Although these observations can be related to treatment, there were no corresponding changes in absolute or relative kidney weights. Likewise, clinical chemistry parameters related to kidney function, including blood-urea-nitrogen (BUN), creatinine, sodium and potassium remained unchanged. These kidney findings are likely due to a response to high concentration of PFBS in tubules and ducts, and did not result in any physiological impairment. The clear NOEL for the kidney microscopic findings is 200 mg/kg. Therefore, the microscopic findings in the kidney at 600 mg/kg represent a No Observable Adverse Effect Level (NOAEL).

Microscopic changes of an “equivocal and uncertain nature” were observed in the nasal cavity and turbinates. This incidence was very low and sporadic in both the male and female rats in the 200 mg/kg and 600 mg/kg dose group. The observed effects are not indicative of a systemic toxic effect and were likely due to local irritation of PFBS on the nasal mucosal membranes. This effect is not critical in evaluating risk in humans.

The No Observable Effect Level (NOEL) for the female rat is 200 mg/kg/day, the highest dose tested. The NOEL for the male rat is 60 mg/kg per day, as a result of reductions in RBC, hematocrit, and hemoglobin at 200 and 600 mg/kg/day.[34]

4.5 Developmental Toxicity Study

A prenatal developmental toxicity study used timed-mated Charles River Sprague-Dawley rats that were gavage dosed on gestation days (GD) 6-20 with either 0, 100, 300 or 1,000 mg

PFBS/kg body weight, 25 rats/group. Aqueous carboxymethyl cellulose was used as the vehicle. Dosages were adjusted daily to account for body-weight changes. Female rats were observed for viability at least twice each day. Rats were also examined for clinical observation of effects of the test substance, abortions, premature deliveries, and mortality. Body weights and feed consumption were recorded throughout the study. All surviving rats were sacrificed on GD 21. Gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. The gravid uterus was excised and weighed. The number of *corpora lutea* in each ovary was recorded. The uterus of each rat was examined for pregnancy, and the number and distribution of implantation sites, live and dead fetuses, and resorptions. Selected fetuses were examined for soft-tissue alteration and skeletal alterations.

Maternal body-weight gains were significantly reduced for the entire gestation period at 1,000 mg/kg/day, and maternal body weights were reduced on GD 20 and 21. Absolute and relative feed consumption values were also reduced in this dose group throughout the entire dosing period.

Fetal body weights were significantly reduced in the 1,000 mg/kg/day group. No other fetal or litter parameters were affected at any dose level when compared to the controls. Neither were there any gross external, soft-tissue or skeletal fetal alterations noted in any group. There was no evidence of maturational delays.

The maternal NOEL for PFBS is 300 mg/kg/day based on reduced body-weight gains and feed consumption at the high dose (1,000 mg/kg/day). The developmental NOEL is 300 mg/kg/day based on reduced fetal body weights at the high dose.[35]

4.6 Oral Two-Generation Reproduction Study

A two-generation reproduction study was performed with Sprague-Dawley rats at oral (gavage) PFBS dose levels of 30, 100, 300, and 1,000 mg/kg/day. The parental (P) and F1 generation had 30 rats per sex in each group. The experimental design, based on OECD 416 and OPPTS 870.8300 guidelines, is described in Attachment I to this document. In this study, the P generation commenced receiving daily doses of PFBS at approximately 6 weeks of age and received at least 70 doses before cohabitation of males and females. Mated females were separated, allowed to litter and rear the F1 generation until litters were weaned at lactation day 22. Parental rats were euthanized and a necropsy performed either after cohabitation (males) or after weaning their litters (females). Thirty male and thirty female rats were selected at weaning from the F1 litters in each dose group to serve as the rats that would be used for mating to produce the F2 generation. The remaining F1 pups were euthanized and a necropsy performed at 22 days of age. After cohabitation, the mated F1 females were allowed to litter and rear their F2 young until lactation day 22. At that time the litters and dams were euthanized and a necropsy performed.

The P and F1 generation rats were observed for viability at least twice each day of the study. Body weights of the P generation male rats were recorded weekly during the dosage period and on the day of sacrifice. Reproductive endpoints evaluated for the parental generation were: 1) duration of gestation; 2) fertility index; 3) gestation index; 4) number and sex of offspring per litter; 5) number of implantation sites; 6) general condition of the dam and litter during the postpartum period; 7) litter size; 8) viability, viability index, lactation index, and

percent survival; and 9) sex ratio. Litters were examined after delivery to identify the number and sex of pups, stillbirths, live births and gross alterations.

At necropsy adult P and F1 generations were examined for gross lesions. The following organs were individually weighed and organ-to-body weight and organ-to-brain weights ratios were calculated: brain; kidneys; spleen; ovaries; testes; thymus; liver; adrenal glands; pituitary; uterus with oviducts and cervix; left and right epididymis; prostate; and seminal vesicles. Histological examinations were performed on tissues from ten randomly selected rats per sex from the control and high-dose groups. The right *vas deferens* was used to analyze percent sperm motility. The caudal and distal midsection of the right epididymis was used for total sperm count evaluations.

All F1 pups not selected for production of the F2 generation were sacrificed on day 22. The urinary bladder and lungs of all F1 generation pups were perfused as with neutral buffered 10% formalin. The following organs were weighed from the first randomly selected pup per sex per litter: brain; spleen; liver; thymus; and kidneys. F2 pups were sacrificed on day 22, and a gross necropsy was performed.

Results for P Generation Male Rats:

There were no male P generation treatment-related deaths. Chromorhinorrhea was seen at 1,000 mg/kg/day and also in a few males in the 300 mg/kg/day group. An increased incidence of urine-stained abdominal fur occurred in the 1,000 mg/kg/day group only. Group mean body-weight gains were significantly reduced in the 1,000 mg/kg/day dosage group on days 43 to 50, and 50 to 57, and a mean body-weight loss occurred in this group on days 105 to 112. Group mean terminal body weights were unaffected by dosages of the test substance as high as 1,000 mg/kg/day. No treatment-related changes in absolute and relative food consumption were noted.

All mating and fertility parameters were unaffected by dosages of PFBS as high as 1,000 mg/kg/day. No treatment-related microscopic changes were observed in the reproductive organs of male P generation rats, and all sperm parameters evaluated were unaffected by dosages of PFBS as high as 1,000 mg/kg/day.

No gross lesions were observed at necropsy. Liver weights (absolute and relative to body weight and brain weight) were significantly increased in the 300 and 1,000 mg/kg/day dose groups.

The microscopic incidence of mild, adaptive enlargement of liver cells (hypertrophy) and minimal to mild proliferation of kidney medullary/papillary tubular and ductular epithelial cells (hyperplasia) was increased in the 300 and 1,000 mg/kg/day dose groups. In addition, primarily minimal focal papillary edema and one incident of moderate focal papillary necrosis was noted in the 1,000 mg/kg/day dose group. These kidney findings are likely due to a response to high concentration of PFBS in tubules and ducts, and did not result in any physiological impairment.

The NOEL for the P generation male rats is 100 mg/kg/day based on an adaptive hypertrophy of liver cells and hyperplasia of kidney medullary/papillary tubular and ductular epithelial cells observed at 300 mg/kg/day.

Results for P Generation Female Rats:

There were no treatment-related P generation female rat deaths. Clinical observations, made during the cohabitation period, included chromorhinorrhea and excess salivation (effecting 5 of 30 dams) in the 1,000 mg/kg/day group. Mean body-weight gain was reduced in the 1,000 mg/kg/day dose group during the last week of dosing prior to mating (pre-cohabitation days 64-70) and during the first week of gestation. Mean body weights in the 1,000 mg/kg/day dose group were lower than controls on days 8 and 11 of lactation. Mean terminal body weights were comparable to controls in all treated groups. Some excess salivation was noted in 5 of 30 animals in the high-dose group. There were no treatment-related gross pathological findings.

Estrous cycle evaluation did not reveal any differences among the five dose groups. All mating and fertility parameters were unaffected by doses of PFBS as high as 1,000 mg/kg/day. Pregnancy occurred in 25 of 29 rats in each dose group. Natural delivery observations were unaffected by doses of PFBS as high as 1,000 mg/kg/day. No microscopic changes were observed in the reproductive organs of any female P generation rat given doses as high as 1,000 mg/kg/day.

The absolute weight of the brain was significantly reduced in the 1,000 mg/kg/day dosage group.

Treatment-related microscopic findings were observed in the kidneys of female rats in the 300 and 1,000 mg/kg dose groups. These findings consisted of minimal-to-mild hyperplasia of the tubular and ductular epithelium of the inner medulla/papilla, and primarily minimal focal papillary edema. In addition, three incidents of minimal-to-moderate focal papillary necrosis were observed in the 300 mg/kg/day dose group; although, none were observed in the 1,000 mg/kg/day dose group.

The NOEL for the P generation female rats is 100 mg/kg/day based on the microscopic observation of medullary/papillary epithelial hyperplasia, edema, and necrosis in the kidneys observed at 300 mg/kg/day.

F1 Generation Male Rats:

There were no treatment-related F1 generation deaths. Some excess salivation was observed in the 1,000 mg/kg dose group. Body-weight gains for the F1 generation male rats were reduced with statistical significance during the second week of dosing in the 1,000 mg/kg/day dose group and, although not statistically significant, were generally less than controls during most of the period prior to mating. Mean body weights were always less than controls in the high-dose group, frequently with statistical significance from day 36 to termination. As a result, terminal body weights of the F1 generation male rats were significantly reduced only in the high-dose group. Although absolute liver weights were not increased in the high-dose group, the ratio of the liver weight-to-body weight was

significantly increased in the high-dose group. The absolute weight of seminal vesicles was reduced in the high-dose group; however, this reduction was not apparent when compared on the basis of organ weight to body weight ratios. Absolute food consumption was unaffected in all dose groups; although, due to lesser mean body weight, relative feed consumption was typically higher in the high-dose group, sometimes with statistical significance.

As in the P generation males, microscopic examination of the liver and kidneys of F1 generation male rats revealed minimal-to-mild treatment-related effects in the 300 and 1,000 mg/kg/day dose groups.

Preputial separation of F1 generation male was slightly delayed (1.6 days on average as compared to controls) in the 1,000 mg/kg/day group. This observation was considered to be associated with the reduced body weights in the 1,000 mg/kg/day dosage group at the time of sexual maturation. Dosages as high as 1,000 mg/kg/day did not effect any mating and fertility parameters evaluated in the F1 generation male rats. No treatment-related microscopic changes were observed in the reproductive organs on any male F1 generation rat from any dose group. There were no treatment-related effects on sperm parameters in any dose group.

The NOEL for the F1 generation male rats is 100 mg/kg/day based on minimal-to-mild microscopic findings in liver and kidney cells observed at 300 mg/kg/day.

F1 Generation Female Rats:

No treatment-related F1 generation deaths occurred. Other than body weight increases (as opposed to decreases) in all dose groups, microscopic findings in the kidneys similar to the P-generation, and excessive salivation (affecting 4 of 30 dams) at the 1,000 mg/kg/day level, all observations were considered unrelated to PFBS administration.

Body weights for the F1 generation female rats were unaffected by dosages of the test substance as high as 1,000 mg/kg/day, and absolute and relative feed consumption were normal at all doses. All necropsy observations were normal in all dose groups. Absolute organ weights and the ratios of the organ weights to terminal body weights and to the brain weights were comparable to control values and did not differ significantly.

Treatment-related microscopic changes consistent with those observed in the P-generation female rats were observed in the kidneys of F1-generation female rats in the 300 and 1,000 mg/kg/day dosage groups. Unlike the P generation females, there was no incidence of necrosis.

The age of vaginal patency for the F1 generation female rats was comparable at all doses as were estrous cycle evaluations. All mating and fertility parameters were unaffected at all dose groups.

No treatment-related microscopic changes were observed in the reproductive organs of any female F1 generation rat even at 1,000 mg/kg/day. Pregnancy occurred in 24 to 28 rats in each dosage group. Natural delivery observations were unaffected by dosages of the test substance as high as 1,000 mg/kg/day.

The number of pups surviving per litter, the percentage male pups, litter size, and average pup body weight per litter were comparable and did not differ significantly for any of the dosage groups when compared to the control group. The NOEL for the F1 generation female rats is 100 mg/kg/day based on an increased incidence of minimal-to-mild microscopic findings in the kidney observed at 300 mg/kg/day.

Results for the F2 Generation Pups:

No adverse clinical or necropsy observations for F2 generation pups were attributable to dosages of PFBS as high as 1,000 mg/kg. F2 generation weaning rats had normal body weights. None of the significant differences in organ weight or organ weight ratios for the F2 generation pups were considered treatment-related because they were not dosage-dependent.

Conclusions for the 2-Generation Reproduction Study:

The P generation paternal NOEL is 100 mg/kg/day based on adaptive liver weight increase and primarily minimal-to-mild hyperplasia of medullary/papillary tubular and ductular epithelium of the kidney. The maternal NOEL is 100 mg/kg/day based on primarily minimal to mild hyperplasia of the medullary/papillary tubular and ductal epithelium in the kidney. All of these changes are believed to be a response to the high concentrations of surfactant in the kidney due to rapid elimination of high administered doses. The reproductive NOEL in the male and female P generation is > 1000 mg/kg/day, the highest dose of the study.

The F1 generation paternal NOEL is 100 mg/kg/day based on primarily minimal adaptive hypertrophy of liver cells. The F1 generation maternal NOEL is 100 mg/kg/day based on increased minimal to mild hyperplasia of the tubular and ductular epithelium of the medulla and papilla of the kidney. The developmental NOEL in the F1 generation is 300 mg/kg/day based on body weight effects and slightly delayed sexual maturation in males which is believed to be a function of body weight effects. The reproductive NOEL in the male and female F1 generation rats is > 1,000 mg/kg/day.

The F2 pup NOEL is > 1000 mg/kg/day.[36]

4.7 Pharmacokinetic Studies

Single-Dose Intravenous Pharmacokinetic Study in Cynomolgus Monkeys:

A single dose of 10 mg/kg was given to three cynomolgus monkeys/sex and samples (urine and sera) were collected over 14 days, with an additional serum collection after 31 days. Maximal serum concentrations occurred in the two- or four-hour samples, and ranged from 29,060 to 61,740 ng/mL (ng/mL = parts per billion, weight/volume). After 14 days, one serum measurement was below the quantitation limit of 1 ng/mL, and the other five were near the quantitation limit. All serum samples from Day 31 were below a quantitation limit of 0.5 mg/mL. Mean (± SD) values of pharmacokinetic parameters for males and females, respectively, were: terminal half-life in serum, 4.0 ± 1.9 and 3.5 ± 3.1 days; total body clearance, 511 ± 245 and 368 ± 207 mL/day/kg; volume of distribution at steady state, 254 ±

31.5 and 255 ± 29.5 mL/kg; and, area under the curve to infinity, 24258 ± 14918 ng·day/mL. Urine was the primary route of excretion. Elimination kinetics for males and females were similar.[37]

***In Vitro* Protein Binding Studies:**

Protein binding characteristics of PFBS have been studied *in vitro*.[38] Saturation of plasma protein binding was studied by adding 500 µg/mL PFBS to rat, monkey (cynomolgus), and human plasma and determining the percent bound to protein. The percent bound to rat, monkey, and human plasma was 84.1, 91.8, and 88.4, respectively. The percent binding to human plasma protein fractions was also studied by adding 10 µg/mL PFBS to protein fractions made up at physiological concentration. PFBS was found to only bind to albumin at 93.5%. No binding occurred with α-globulin, γ-globulin, α-2-macroglobulin, fibrinogen, transferrin, or β-lipoprotein. Thus, the lack of protein binding of PFBS results in a higher level of free PFBS in the plasma and therefore a higher urinary elimination and lack of bioconcentration.

PPARα Activation:

Activation of the PPARα was studied in male rats given five daily doses of 1,500 mg PFBS/kg body weight by measuring liver palmitoyl CoA oxidase activity. Livers were removed on the day of the last dose and the activity measured. Although a final report on this study is pending, the results demonstrated that, under the study conditions, PFBS did increase palmitoyl CoA oxidase activity compared to controls.[39]

4.8 Toxicological Hazard Evaluation

In summary, PFBS exhibits little or no toxicity in laboratory animal testing, including a mutagenicity and clastogenicity assay, repeat dose assays including a developmental toxicity study, a 90-day oral gavage study, and a full two-generation reproduction study. This low toxicity is due to the chemicals resistance to bio-activation (cannot covalently modify biological compounds) and rapid elimination. For all these reasons PFBS does not present a significant toxicological hazard.

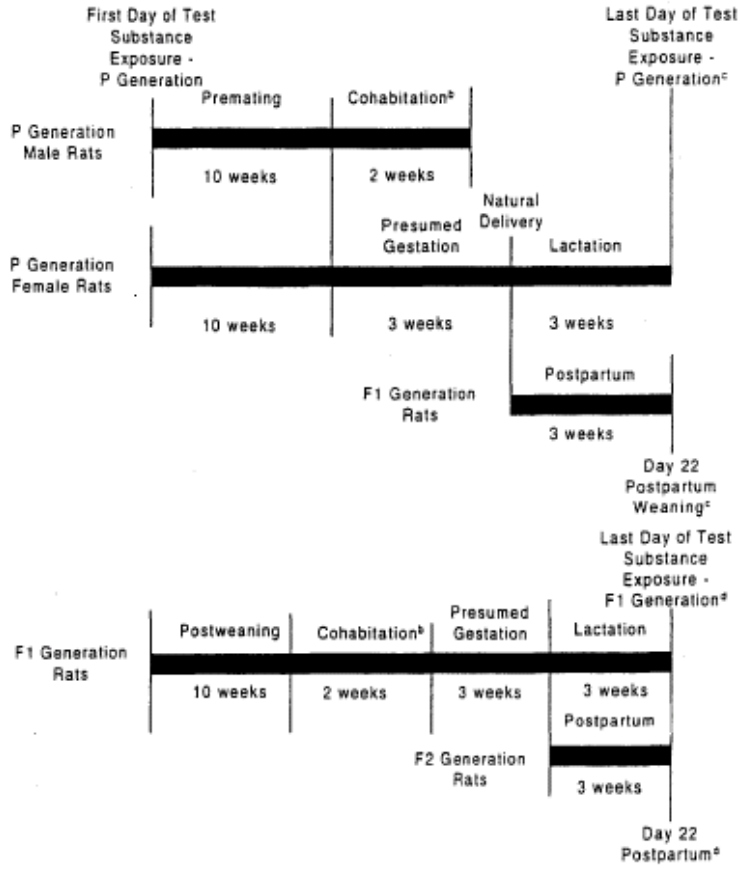
Table 4-1 **Mammalian Toxicity of PFBS**		
Parameter	Result	Units
Dermal Toxicity (rat)		
LD_{50}	> 2000	mg/kg
Acute Oral Toxicity (rat)		
LD_{50}	> 2000	mg/kg
Eye Irritation (rabbit)	Moderately irritating	
Dermal Irritation (rabbit)	Non-irritating	
Guinea Pig Sensitization (Maximization Test)	Not a skin sensitizer	
Bacterial Mutagenicity Assay (Ames)	Not a bacterial mutagen	
Chromosomal Aberration (CHO cells)	Non-clastogenic	
Repeat Dose Toxicity (rat)		
28 day Oral Toxicity (rat), NOEL	100	mg/kg/day
90 day Oral Toxicity (rat), Female NOEL Male NOEL	200 60 (see text)	mg/kg/day
Developmental Toxicity (rat)		
Maternal NOEL Paternal NOEL	300 300	mg/kg/day
Two-generation reproduction summary (rat) P generation Male NOEL P generation Female NOEL P generation Reproductive NOEL P generation Post-natal NOEL F1 generation Male NOEL F1 generation Female NOEL F1 generation Developmental NOEL F1 generation Reproductive NOEL F2 generation Pup NOEL	 100 100 > 1000 > 1000 100 100 300 > 1000 >1000	 mg/kg/day

5.0 CONCLUSION

This report is a technical summary of the ecological and mammalian toxicity and fate data accumulated for PFBS as of June 2005. Although PFBS is resistant to degradation and is persistent in the environment, results from environmental testing demonstrate that PFBS is not acutely or chronically toxic to aquatic or avian organisms at concentrations less than 100 ppm. The acute NOEC values for all species evaluated for ecotoxicity ranged from 127 to 5,620 ppm, while chronic NOEC values ranged from 200 to 502 ppm. PFBS does not bioconcentrate and does not bioaccumulate. Thus, adverse ecological effects are not expected. Results from numerous mammalian toxicity studies indicate that PFBS has low toxicity in both acute and repeat dose studies. Further, it does not affect reproductive function or prenatal development. PFBS is cleared from the body in fish and primates within days. Exposures are expected to be low. The data indicate the potential toxicity and ecological impacts of PFBS are minimal.

Attachment I

TWO-GENERATION REPRODUCTION STUDY[a]



Dosage Period

a. For additional details see "Tests, Analyses and Measurements" section of the protocol.
b. Male rats sacrificed after determination of sufficient number of pregnancies; sperm evaluation.
c. P generation female rats and F1 generation rats not selected for continuation on study sacrificed.
d. F1 generation dams and F2 generation litters sacrificed.

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