FINAL REPORT

Development of Toxicity Reference Values (TRVs) for Birds Exposed to PFOS, PFOA and Associated Mixtures of Fluorinated Compounds

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14. ABSTRACT
Per- and polyfluoroalkyl substances (PFAS) are ubiquitous contaminants in the environment. They were used extensively in the formulation of aqueous film forming foams (AFFF). The Department of Defense used these AFFF formulations since approximately 1970 for fire-training and emergency response. Two manufacturing techniques for PFAS produced varying individual components. The 3M company produced PFAS, and subsequently AFFF, using electrochemical fluorination while Ansul produced AFFF using DuPont's fluorotelomer based PFAS. Due to their use and storage, release of these chemicals to the environment is significant. In the environment, exposure to PFAS from wildlife is probable in areas contaminated with AFFF. However, to date, there are few studies that have examined the effects of PFAS in avian species that provide data meeting the criteria for development of toxicity reference values (TRVs) used to evaluate environmental risks posed by AFFF use.

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List of Acronyms

3M	Minnesota Mining and Manufacturing Company
6:2 FtTAoS	6:2 fluorotelomer thioamido sulfonate
6:2 FTS	6:2 fluorotelomer sulfonate
8:2 FTS	8:2 fluorotelomer sulfonate
ADI	average daily intake
AFFF	aqueous film forming foam
ANOVA	analysis of variance
BMCx	benchmark dietary concentration at the x% response level
BMCLx	lower 95% confidence limit of BMCx
BMDx	benchmark dose at the x% response level
BMDLx	lower 95% confidence limit of BMDx
bw	body weight
DOD	Department of Defense
EDx	effective dose for x % of the population
FTA	fire training activities
h	hours
LC/MS/MS	liquid chromatography tandem mass spectrometry
LOAEL	lowest observed adverse effect level
LC50	lethal dietary concentration for 50% of the experimental population
LD50	lethal dose for 50% of experimental population
LT50	lethal time for 50% of experimental population
MSU	Michigan State University
NOAEL	no observed adverse effect level
PFAS	perfluoroalkyl substances
PFHxS	perfluorohexane sulfonate
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
SAS	Statistical Analysis System
TRV	toxicity reference value
USACHPPM	US Army Center for Health Promotion and Preventative Medicine

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Abstract

Introduction

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous contaminants in the environment. They were used extensively in the formulation of aqueous film forming foams (AFFF). The Department of Defense used these AFFF formulations since approximately 1970 for fire-training and emergency response. Two manufacturing techniques for PFAS produced varying individual components. The 3M company produced PFAS, and subsequently AFFF, using electrochemical fluorination while Ansul produced AFFF using DuPont's fluorotelomer based PFAS. Due to their use and storage, release of these chemicals to the environment is significant. In the environment, exposure to PFAS from wildlife is probable in areas contaminated with AFFF. However, to date, there are few studies that have examined the effects of PFAS in avian species that provide data meeting the criteria for development of toxicity reference values (TRVs) used to evaluate environmental risks posed by AFFF use.

Technical Approach

This study assessed the acute toxicity of two PFAS, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), individually and in combination as well as two AFFF formulations in Japanese quail (*Coturnix japonica*). Additionally, this project determined the chronic toxicity of PFOS and one AFFF formulation, and developed a TRV for Japanese quail. Acute studies involved feeding 10-day old quail feed dosed with 9 dietary treatments of PFOS, PFOA, PFOS + PFOA, 3M AFFF and Ansul AFFF. For the chronic exposure juvenile quail were fed 6 dietary treatments of either PFOS or 3M AFFF contaminated feed. Those quail were paired and reproductive effects observed.

Results

From the acute exposure average daily doses resulting in 50% mortality at day 5 were 38 (34–43), 68 (63–74), 55 (51–59), and 130 (103–164) mg PFOS, PFOA, PFOS + PFOA, and PFOS in 3M AFFF kg body weight^{-1} d^{-1}. Ansul AFFF did not result in any mortalities. Dietary concentrations resulting in 50% mortality at day 5 were 351(275–450), 496 (427–575), 398 (339–468), and 467 (390–559) mg PFOS, PFOA, PFOS + PFOA, and PFOS in 3M AFFF kg feed^{-1}. From the chronic exposure PFOS or AFFF PFOS did not have a significant effect on egg production and had a variable effect on hatchability and chick body weight. Chick survivability, considered the critical effect, was significantly decreased beginning at 8.7 mg PFOS and 11 mg AFFF PFOS kg^{-1} feed.

Benefits

The no observed adverse effect level (NOAEL) for PFOS was 4.1 mg kg feed^{-1} (0.55 mg kg body weight^{-1} d^{-1}) and 5.0 mg AFFF PFOS kg feed^{-1} (0.66 mg kg body weight^{-1} d^{-1}) resulting in average toxicity reference values (TRVs) of 0.25 mg kg feed^{-1} and 0.034 mg kg bw^{-1} d^{-1}.

Publications

Bursian, S. J., Roberts, J., Harr, K., Link, J.E., McCarty, M., Simcik, M.F. Dietary Exposure of Japanese Quail (Coturnix japonica) to Perfluorooctane Sulfonate (PFOS) and a Legacy Aqueous Film Forming Foam Containing PFOS: Effects on Reproduction and Chick Survivability and Growth. *Environmental Toxicology and Chemistry* **2021** https://doi.org/10.1002/etc.5138.

Bursian, S. J., Link, J.E., McCarty, M., Simcik, M.F. The Subacute Toxicity of PFOS and/or PFOA and Legacy Aqueous Film Forming Foams to Japanese Quail (*Coturnix japonica*) chicks. *Environmental Toxicology and Chemistry*. **2020** doi:10.1002/etc.4684

Executive Summary

Introduction

Perfluoroalkyl substances (PFAS) are a broad chemical class consisting of a fully fluorinated carbon chain and several different end groups including sulfonate, carboxylate, sulfonamidoalkyls and alcohols. These compounds were used in mixtures of AFFF as fire extinguishing agents both as an emergency measure as well as for fire training activities (FTA) by the US military at many installations around the country. The US military has the largest stockpile of AFFF, accounting for approximately 29% in 2004 (Place and Field 2012). The AFFF formulations sold by 3M, which account for 75% of the total AFFF stockpiled on military bases, contain fluorochemicals synthesized by electrochemical fluorination while the remaining stockpiled AFFF contain telomerization-based fluorochemicals (Place and Field, 2012). PFOS was an active ingredient utilized in the 3M AFFF formulations until the phase-out of PFOS and related chemicals in 2000 through 2002. Current AFFF formulations are based on telomer fluorosurfactants that do not contain PFOS (Place and Field 2012, Gewurtz, Bhavsar et al. 2014). As a result of their use in AFFF, PFAS have been released into the environment during firefighting activities. Subsequent contamination of biota, surface water and groundwater proximate to these installations has since been widely reported (Moody and Field 1999, Moody and Field 2000, Gewurtz, Bhavsar et al. 2014, Arias E, Mallavarapu et al. 2015). Despite the documentation of PFAS as environmental contaminants, relatively few studies dealing with their ecotoxicity have been conducted and to date, most of these studies have focused on single compounds (PFOS and/or PFOA), rather than commercial formulations containing other PFAS (Backe, Day et al. 2013).

Effluents of AFFF resulting from fire emergency responses, firefighting training activities and equipment maintenance typically were not contained or pre-treated prior to release into waste-water treatment systems or the environment (Moody et al. 2003). These effluents are considered a primary source of poly- and perfluoroalkyl substances (PFAS) in biota, surface water and ground water at specific Department of Defense facilities (Moody and Field 1999, Moody and Field 2000, Moody et al. 2003, Gerwurtz et al. 2014, Arias et al. 2015, Anderson et al. 2016). The presence of the long chain perfluoroalkyl acids PFOS and PFOA are of particular concern in terms of both human health as well as health of the environment because they are persistent, bioaccumulate, and have induced toxic effects in laboratory animals (Giesy and Kannan 2002, Kannan 2011).

There are few studies that have examined the effects of PFAS in avian species that provide data meeting the criteria for development of toxicity reference values (TRVs) (US Army Center for Health Promotion and Preventative Medicine 2000) used to evaluate environmental risks posed by use of chemicals, including military-related chemicals such as PFAS associated with AFFF. Data from studies utilizing an ecologically relevant exposure scenario, exposure to the chemical via the feed or water, are preferred for development of TRVs to be used in ecological risk assessments. Japanese quail (*Coturnix japonica*) administered PFOA via the drinking water had suppression of T cell-mediated immunity, which however did not translate into compromised disease resistance (Smits and Nain 2013), a relevant endpoint for development of TRVs. The reproductive effects of dietary PFOS were examined in mallards (*Anus platyrhynchos*) and northern bobwhites (*Colinus virginianus*) by Newsted et al. (2007). The lowest dietary concentration used was the lowest observed adverse effect level (LOAEL) for northern bobwhites and there were no treatment-related effects, other than adult mortality at the greatest dietary concentrations, for mallards. Using data from this reproduction study (Newsted

et al. 2007), an avian TRV for PFOS (Newsted et al. 2005) was published prior to publication of the reproduction study. The authors of the TRV publication stated that a more accurate estimate of potential risk could be achieved if more toxicity data were available. Most recently, the reproductive effects of a 1.2:1 mixture of PFOS and perfluorohexane sulfonate (PFHxS) administered to northern bobwhites via the drinking water were evaluated by Dennis et al. (2020).

Objectives

The overall objective of this project was to develop avian ecotoxicity information for compounds associated with aqueous film forming foam (AFFF) in birds. Our specific objectives were to determine the acute toxicity of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) separately and in combination, in Japanese quail and to determine the acute toxicity of other perfluoroalkyl substances relative to PFOS in Japanese quail using two historic formulations of AFFF, notably 3M and Ansul formulations, which represent electrochemical fluorination and fluorotelomer technologies. Our final objective was to develop toxicity reference values (TRVs) for PFOS and AFFF in Japanese quail based on chronic feeding studies.

Technical Approach

The objectives were accomplished by performing three tasks:

Task 1: ACUTE EXPOSURE TO PFOS, PFOA, PFOS + PFOA

Hypothesis: *Acute dietary exposure of Japanese quail to PFOS, PFOA or a combination of PFOS plus PFOA for five days will have an adverse effect on survivability allowing derivation of LD50, LC50 and LT50 values.*

Japanese quail eggs were incubated at the Michigan State University (MSU) Poultry Science Research and Teaching Center (East Lansing, MI). Resulting hatchlings were raised in a brooder battery until 10 days of age at which time birds were sorted by weight and randomly assigned from each weight class to one of nine treatment groups (20 birds per group). Once assigned to treatment groups, birds were individually identified. Groups were housed in separate compartments of a second brooder battery in an environmentally controlled room with a photoperiod of 17 hours (h) light:7 h dark. Feed and water were available ad libitum. Ten-day-old birds were fed PFOS (0, 8.7, 17.6, 35.1, 70.3, 141, 281, 562, or 1125 µg/g feed), PFOA (0, 43.5, 88, 175, 350, 700, 1400, 2810, or 5625 µg/g feed), or PFOS plus PFOA (at the same concentrations used for the individual compounds) in their diet for five days and then placed on clean feed. Treatment groups were housed such that birds fed lower concentrations of PFOS, PFOA or PFOS plus PFOA were maintained above birds fed higher concentrations to prevent cross-contamination of feed. The three trials were run sequentially. On day 8, half of the birds (if there were enough survivors) in each group were euthanized by cervical dislocation, sampled for blood by cardiac puncture and the liver removed and weighed. The remaining birds were continued on clean feed for an additional 14 days at which time surviving birds were euthanized by cervical dislocation, sampled for blood by cardiac puncture and the liver removed and weighed as before. Serum samples for each dietary concentration of PFOS, PFOA and PFOS plus PFOA at day 8 and day 22 were analyzed for PFOS and/or PFOA by LC/MS/MS at the University of Minnesota. Individual body weights and feed consumption for each compartment were determined on days 5, 8, 15 and 22.

Endpoints included LC50 (dietary concentration that results in 50% mortality of a population for a given exposure time), LD50 and LT50 (the exposure time that results in 50% mortality of a population for a given dose). Other endpoints included body weight gain, feed consumption, liver weight and PFOS and/or PFOA concentrations in serum.

Task 2: ACUTE EXPOSURE TO 3M or ANSUL AFFF

Hypothesis: *Acute dietary exposure of Japanese quail to 3M or Ansul aqueous film forming foam for five days will have an adverse effect on survivability allowing derivation of LD50, LC50 and LT50 values.*

We had previously obtained historical formulations of both 3M and Ansul AFFF from local fire departments and selected those that were also used for military applications. Ten-day-old Japanese quail were fed nine dietary treatments of either 3M or Ansul AFFF. Dosage of 3M AFFF was based on the PFOS and PFOA concentration in the AFFF. Targeted concentrations of PFOS/PFOA based on analysis of the AFFF were 0/0, 70/0.88, 144/1.8, 192/2.4, 240/3.0, 280/3.5, 420/5.3, 560/7.0, and 1120/14 mg PFOS/PFOA kg feed^{-1}. Dosage of Ansul AFFF was based on the 6:2 fluorotelomer thioamido sulfonate (6:2 FtTAoS) concentration in the AFFF. Nine dietary treatments were formulated to contain Ansul AFFF at 0, 1.5, 2.9, 5.8, 12, 23, 46, 92, and 184 mL/kg feed given a concentration of 6100mg 6:2 FtTAoS/L AFFF. Other PFAS analyzed in the Ansul were present at levels orders of magnitude lower, with only 6:2 fluorotelomer sulfonate (6:2FTS), PFOA, and 8:2 fluorotelomer sulfonate (8:2FTS), showing any significant concentration at 7.3, 5.5 and 4.3 µg/mL, respectively (Appendix). Endpoints were the same as for Task 1.

Task 3: CHRONIC EXPOSURE OF JAPANESE QUAIL TO PFOS OR AFFF

Hypothesis: *Dietary exposure of breeding pairs of Japanese quail to PFOS or AFF for a total of 18 weeks will have an adverse effect on reproduction and survivability of offspring.*

Japanese quail eggs were obtained from the Michigan State University (MSU) Poultry Teaching and Research Center (East Lansing, MI) breeding flock. Eggs were incubated and hatched at the facility. At hatching, quail were moved to a brooder battery. Birds were fed experimental diets for 4 weeks. Treatment groups were arranged in the brooder so that the lowest concentrations of PFOS were at the top of the battery and the highest concentrations were at the bottom to avoid cross contamination of the feed. At 4 weeks of age, birds were sorted by sex within treatment and then randomly paired (male/female), individually identified with a plastic wing tag, weighed and moved to breeder/layer pens. Birds were maintained on 8 h light and 16 h dark until 8 weeks of age when photoperiod was increased over 2 weeks to 17 h light and 7 h dark to induce egg laying at 10 weeks of age. Light intensity in the room was approximately 20 lux.

Birds were weighed every 2 weeks and feed consumption was measured weekly for each breeding pair. When egg laying began at 10 weeks of age, eggs were collected daily between 0800 and 0900. Individual eggs were labeled using non-toxic felt tip surgical markers with hen identification number, date and dietary concentration and then placed in an egg cooler. Eggs in

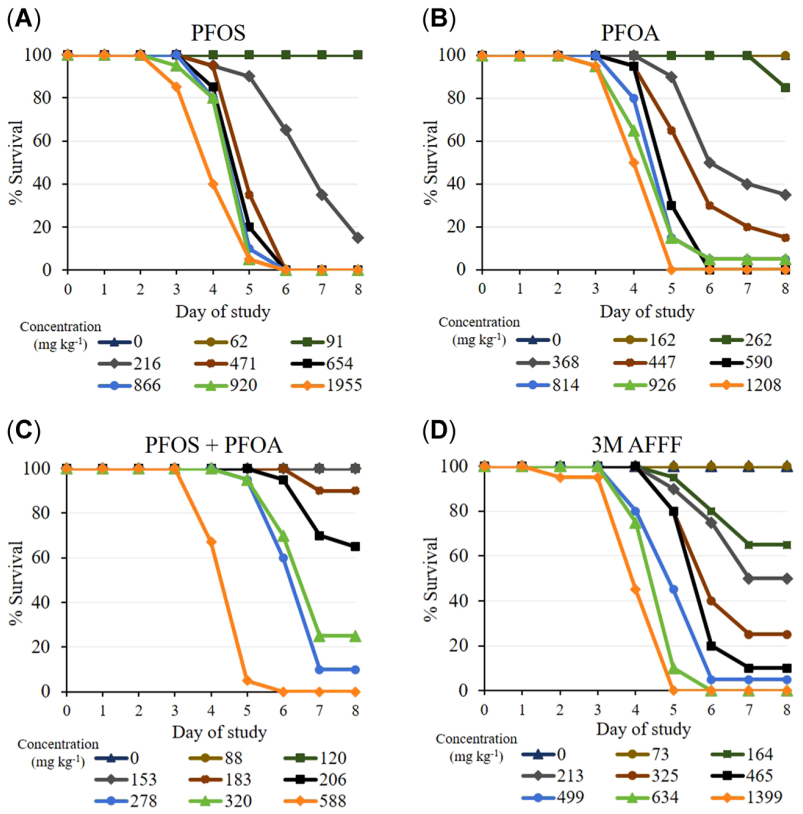
the egg cooler were set in a rotary incubator at 1-week intervals. Eggs with damaged shells were considered non-viable and excluded from hatchability calculations. Weekly, the yolk and albumin from these eggs were separated and frozen for subsequent PFAS analysis. On day 14 of incubation, eggs were placed in hatching baskets by hen number and transferred to a hatcher. Beginning on day 17, hatchlings with dry feathers were removed from the hatcher, weighed and transferred to the brooder battery by dietary concentration twice a day. Eggs remaining on the afternoon of day 18 were considered unhatched. All unhatched eggs were opened on the afternoon of day 18 and stage of embryo development was determined and recorded. Embryo age at death was categorized as less than 4 days, 4 to 7 days, 8 to 10 days, 11 to 14 days, greater than 14 days, dead pip or live pip. Embryos that were developed enough to visualize anatomical structures were examined for abnormalities. Unhatched eggs with no gross indication of embryo development were assumed to be infertile and were not included in hatchability calculations. Live hatchlings were raised in the same brooder unit and under the same conditions used to raise the parental birds. Offspring were raised for 14 days on non-contaminated Game Bird and Turkey Startena® crumbles that had been ground to reduce particle size. Birds were checked twice daily and weighed at hatch and on days 7 and 14. On day 14, a subsample of 10 chicks per dose group were randomly selected for necropsy. Chicks were euthanized by cervical dislocation and blood was immediately obtained by cardiac puncture. Livers were removed, weighed and frozen (-20^{o} C) in glass vials for subsequent PFAS analysis. Blood was allowed to clot prior to centrifugation at 2000 x g for 10 min at room temperature. Serum was separated and frozen at -20^{o} C. These procedures were repeated for each of the 10 hatches.

At 20 weeks of age, surviving adult birds were weighed, euthanized by cervical dislocation and blood immediately collected by cardiac puncture. Birds were necropsied and half of the liver and the kidneys were removed, weighed and placed in 10% neutral buffered formalin for subsequent histological examination. The remaining half of the liver and serum were frozen at -20^{o}C for subsequent PFAS analysis. At least 2 sections of liver and 2 sections of kidney were placed in a single cassette for each quail. If whole kidneys were available, cranial and caudal lobes were sampled. Samples were sent to URIKA Pathology for processing and evaluation. Hepatic and renal tissues were examined by a board certified pathologist and lesions were graded on a severity scale of 0 to 4 according to the following criteria: 0 = no lesion recognized; 1 = minimal lesions, 1 to 3 foci or small foci of a few cells; 2 = mild lesions, increased number of foci or more of the lesion; 3 = moderate lesions, more lesions (2-3 per 10x field of vision); 4 = severe lesions, the majority of cells and/or extensive regions involved with the lesions.

Results and Discussion

Acute Exposure Study

There was a distinct dose-response relationship between PFOS, PFOA, PFOS + PFOA and 3M AFFF exposure and both feed intake and body weight for adult quail. No significant decrease in feed consumption or body weight occurred in the Ansul AFFF exposed quail. There was also significant mortality of adult quail exposed to PFOS, PFOA, PFOS + PFOA and 3M AFFF starting as early as day 3 and at levels as low as 152 µg PFOS g feed^{-1}. No mortality occurred at any exposure level for Ansul AFFF in this study. When comparing the levels of exposure PFOS was more toxic than PFOA, PFOS and PFOA toxicity appeared additive and AFFF exposure was less toxic than equivalent levels of PFOS. But when considering internal dose, AFFF and PFOS showed similar toxicity.



Effect of perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), PFOS + PFOA, and 3M aqueous film-forming foam (AFFF) on offspring survivability at day 8 following a 5-d dietary exposure.

(A) Day 8 survivability of Japanese quail (Coturnix japonica) chicks at each concentration of dietary PFOS after a 5-d dietary exposure. (B) Day 8 survivability of Japanese quail chicks at each concentration of dietary PFOA after a 5-d dietary exposure. (C) Day 8 survivability of Japanese quail chicks at each concentration of dietary PFOS + PFOA after a 5-d dietary exposure. (D) Day 8 survivability of Japanese quail chicks at each concentration of PFOS provided by 3M AFFF after a 5-d dietary exposure.

Doses of PFOS, PFOA, PFOS + PFOA and AFFF PFOS that resulted in 50% lethality ranged from 38 to 145 mg kg body weight^{-1} d^{-1} and dietary concentrations that resulted in 50% lethality ranged from 389 to 550 mg kg feed^{-1}. The time required for 50% mortality (LT50) on day 8 across dietary concentrations was similar for each treatment (PFOS: 5.7 to 3.4 d; PFOA: 5.9 to 3.8 d; PFOS + PFOA: 7.4 to 4.0 d; AFFF PFOS: 7.6 to 4.3 d).

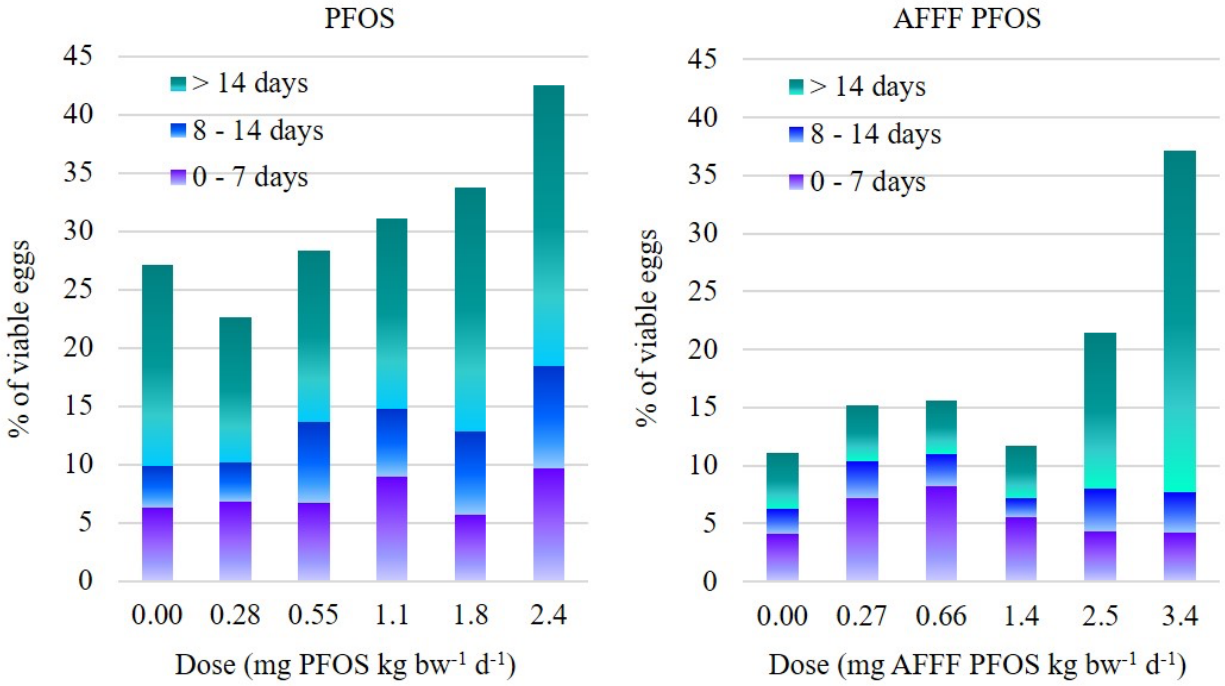
Chronic Exposure Study

No adult mortality occurred as a result of exposure to PFOS or 3M AFFF in the chronic exposures.

When exposed to chronic levels of PFOS and 3M AFFF quail feed intake was variable. Females had significantly higher feed consumption than males, and it was unaffected by exposure levels. Male quail exhibited significantly less consumption starting at the 18 mg PFOS kg feed^{-1} and 27 mg kg feed^{-1} levels when compared to controls. Like the acute exposure, this feed consumption had a commensurate effect on body weight. Females showed no effect on body weight either during growth or at necropsy. Males showed significant decreased weight

gain with chronic PFOS exposure starting at 19 mg AFFF PFOS kg feed^{-1} and significantly lower body weight at necropsy starting at 14 mg PFOS kg feed^{-1} and 11 mg AFFF PFOS kg feed^{-1}.

The chronic exposure had no effect on egg production, but did correspond to a significant reduction in hatchability at 18 mg PFOS kg feed^{-1}. The greatest reproductive effect observed was on embryo mortality. In general, there was a dose-related increase in embryo mortality for both PFOS and AFFF PFOS. In the PFOS trial, the greatest mortality occurred after day 14 of incubation across all groups with the proportions being similar (31.7 - 38.8%). In the 3M AFFF trial, embryo mortality occurred primarily in the first 7 d of incubation across treatment groups with exception of the greatest feed concentration that had the greatest proportion of embryos dying after 14 d of incubation. There was a significant increase in the number of embryos dying after day 14, dead pips and live pips at 27 mg AFFF PFOS kg feed^{-1} (ADD = 3.4 mg kg body weight^{-1} d^{-1}) compared to controls.



Percent of viable eggs that experienced embryo mortality

Chronic exposure to PFOS and AFFF also had significant effects on chick survivability, chick body weights, chick liver weights and adult liver and kidney pathology. There was reduced survivability through the first 7 days at feed concentrations of 8.7 and 18 mg PFOS kg feed^{-1} and beginning at 11 mg AFFF PFOS kg feed^{-1}. Chick body weights showed a significant decrease compared to controls at feed concentrations as low as 2.1 mg PFOS and 2.1 mg AFFF PFOS kg feed^{-1}. The only effect on adult liver weights were observed at 27 mg AFFF PFOS kg feed^{-1} where liver weight was significantly larger than controls. For 14-day old chicks absolute and relative liver weights increased for PFOS exposed groups, but no significant changes were observed in 3M AFFF exposed groups. Liver pathology results indicated extramedullary hematopoesis in males from the PFOS trial and females from the AFFF trial, but canalicular cholestasis, myofibroblast proliferation and heterophilic inflammation were not observed.

Kidney pathology results indicated tubular regeneration severity in females exposed to PFOS, glomerulopathy in both males and females exposed to PFOS, and significant tubular degeneration in females exposed to AFFF PFOS.

Benchmark modeling using the US Environmental Protection Agency benchmark dose (BMD) software (version 3.1.1; http://www.epa.gov/ncea/bmds) was attempted to derive the lower 95% confidence limit of the BMD (BMDL) for a 20% decrease in chick survivability for PFOA and AFFF PFOS. However, all models were judged by the BMD software as "questionable". For this reason, it was decided that the no adverse effect levels (NOAELs) for the critical effect of chick survivability would be used. No Adverse Effect Levels (NOAELs) associated with chick survivability were determined to be 4.1 mg PFOS kg feed^{-1} (0.55 mg PFOS kg body weight^{-1} day^{-1}) and 5.0 mg AFFF PFOS kg feed^{-1} (0.66 mg AFFF PFOS kg body weight^{-1} day^{-1}). Toxicity Reference Values (TRVs) were averaged for PFOS and AFFF and determined to be 0.25 mg PFOS kg feed^{-1} and 0.034 mg PFOS kg body weight^{-1} day^{-1}. These values are similar to TRV values reported by Newsted et al (2005) for bobwhite quail.

Implications for Future Research and Benefits

Acute Exposure Study

Based on dietary concentrations related to mortality, feed consumption and body and organ weight endpoints, the results of the present study indicate that PFOS is acutely more toxic to Japanese quail than PFOA. Results also suggest that the acute toxicities of PFOS and PFOA are additive. AFFF PFOS was less toxic than PFOS and PFOA based on dietary concentrations and 6:2 FtTAoS provided by Ansul AFFF was not toxic at concentrations fed. However, examination of hepatic concentrations of PFOS, AFFF PFOS and PFOA in birds that died on trial suggests there is a tissue threshold for mortality and the threshold for AFFF PFOS is less than the thresholds for PFOS and PFOA and equivalent to PFOS + PFOA.

Chronic Exposure Study

Examination of the effects of dietary PFOS and a legacy AFFF containing PFOS AFFF PFOS on reproduction and chick survivability and growth in Japanese quail determined that the NOAELs associated with chick survivability, which is considered the critical effect, were 4.1 mg PFOS kg feed^{-1} (0.55 mg PFOS kg body weight^{-1} d^{-1}) and 5.0 mg AFFF PFOS kg feed^{-1} (0.66 mg AFFF PFOS kg body weight^{-1} d^{-1}). Toxicity reference values were calculated by averaging the PFOS and AFFF PFOS values and dividing by a total uncertainty factor of 18. Resulting TRVs are 0.25 mg kg feed^{-1} and 0.034 mg kg bw^{-1} d^{-1}, which are similar to TRVs reported by Newsted et al. (2005) for northern bobwhites.

NOAELs, LOAELs and TRVs for Japanese quail and northern bobwhites based on avian reproduction studies assessing the effects of PFOS administered in the feed or drinking water

Measure of PFOS exposure	Japanese quail – dietary[a]			Northern bobwhite – dietary[b]			Northern bobwhite – drinking water[c]	
	NOAEL[d]	LOAEL[d]	TRV[d,e]	NOAEL	LOAEL	TRV[f]	NOAEL	LOAEL
Feed or water concentration (mg kg $^{-1}$ or mg L^{-1})	4.6	9.9	0.26	-	10	0.27	18.7×10^{-3}	5.96×10^{-4}

ADD (mg kg bw^{-1} d^{-1}	0.61	1.3	0.034	-	0.77	0.021	8.50×10^{-5}	2.45×10^{-3}
Adult female serum (mg L^{-1})	18	35	1.0	-	8.7	0.24	-	-
Adult female liver (mg kg^{-1})	10	18	0.56	-	4.9	0.14	-	-
Chick serum (mg L^{-1})	5.3	13	0.30	-	-	-	-	-
Chick liver (mg kg^{-1})	3.1	6.0	0.17	-	-	-	-	-
Egg (mg kg^{-1})[g]	33	63	1.8	-	62	1.7	-	-

[a]Present study.
[b]Newsted et al. (2005, 2007).
[c]Dennis et al. (2020).
[d]Values are based on the average of PFOS and AFFF PFOS values.
[d]TRVs calculated by dividing NOAEL values by a total uncertainty factor of 18.
[f]TRVs calculated by dividing LOAEL values by a total uncertainty factor of 36.
[g]Based on concentrations in eggs layed during week 10.
NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level; TRV = toxicity reference value; PFOS = perfluorooctane sulfonate; AFFF = aqueous film forming foam; PFHxS = perfluorohexanesulfonate; ADD = average daily dose; bw = body weight

Development of Toxicity Reference Values (TRVs) for Birds Exposed to PFOS, PFOA and Associated Mixtures of Fluorinated Compounds

1. Objective

The overall objective of this project was to develop avian ecotoxicity information for compounds associated with aqueous film forming foam (AFFF) in birds. Our specific objectives were to determine the acute toxicity of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) separately and in combination, in Japanese quail and to determine the acute toxicity of other perfluoroalkyl substances relative to PFOS in Japanese quail using two historic formulations of AFFF, notably 3M and Ansul formulations, which represent electrochemical fluorination and fluorotelomer technologies. Our final objective was to develop toxicity reference values (TRVs) for PFOS and AFFF in Japanese quail based on chronic feeding studies.

2. Background

Aqueous film forming foam (AFFF) formulations, developed by the U.S. Navy and 3M Company in the mid-1960s to extinguish hydrocarbon-fuel fires resulting from fire-training and fire emergency situations (Moody and Field 1999), are mixtures of fluorinated surfactants. The characteristics of these fluorinated surfactants are dictated by the process used to generate them. Electrochemical fluorination was used by 3M through the early 2000s for their formulations. This process results in fully fluorinated perfluoroalkyl sulfonic acids (PFSAs), such as perfluorooctane sulfonate (PFOS) and other CF_2 homologues, such as perfluorooctanoic acid (PFOA), as well as various perfluoroalkyl sulfonamides and their derivatives (Buck, Franklin et al. 2011, Anderson, Long et al. 2016). Seventy-five percent of the total supply of AFFF on military installations consist of products resulting from electrochemical fluorination (Place and Field 2012). The remaining 25% of AFFF belonging to the military consist of telomerization-based fluorochemicals composed of carbon chains that are not fully fluorinated, having homologues of varying C_2F_4 units (Place and Field 2012, Anderson et al. 2016). The fluorotelomers exclusively degrade to PFOA and other perfluoroalkyl carboxylic acids (PFCAs) while perfluoroalkyl sulfonamides and their derivatives can degrade to PFOS and other PFSAs (Anderson et al. 2016).

Effluents of AFFF resulting from fire emergency responses, firefighting training activities and equipment maintenance typically were not contained or pre-treated prior to release into waste-water treatment systems or the environment (Moody, Hebert et al. 2003). These effluents are considered a primary source of poly- and perfluoroalkyl substances (PFAS) in biota, surface water and ground water at specific Department of Defense facilities (Moody and Field 1999, Moody and Field 2000, Moody et al. 2003, Gerwurtz et al. 2014, Arias et al. 2015, Anderson et al. 2016). The presence of the long chain perfluoroalkyl acids PFOS and PFOA are of particular concern in terms of both human health as well as health of the environment because they are persistent, bioaccumulate, and have induced toxic effects in laboratory animals (Giesy and Kannan 2002, Kannan 2011).

Toxicity reference values are used in the evaluation of environmental risks posed by use of chemicals, including military-related chemicals such as PFAS associated with AFFF. These values are measures of toxicity that evaluate the likelihood of effects in individual organisms that may be relevant to a population of organisms (US Army Health Promotion and Preventive Medicine: USACHPPM 2000). According to USACHPPM Technical guide 254: Standard Practice for Wildlife Toxicity Reference Values, criteria used to select toxicity data relevant to development of TRVs include: (1) the critical effects chosen should be clearly linked to factors that influence population sustainability, such as mortality, reproduction, development, growth, behavior relevant to reproduction, feeding and predator avoidance and decrease resistance to disease; (2) exposure duration should be defined and the effect level should be expressed as a no observed adverse effect level (NOAEL), lowest observed adverse effect level (LOAEL) or effective dose x (EDx) where x is less than 50% of the experimental population; (3) the exposure pathway should closely match the pathway that contributes most to exposure in the field; (4) the study design relative to appropriate exposure pathways in the environment must be valid; (5) the quality of the study must be such that the variability in response is relevant, the bioavailability of the substance in the field and the substance used in the study must be comparable, doses administered are appropriately quantified with minimal variability, sufficient information is provided to repeat the study and data corroborate with other similar data (USACHPPM 2000).

Relatively few studies have examined the effects of PFAS in avian species, and even fewer studies provide data that meet the criteria listed above for development of TRVs. Custer *et al* (2012) reported that reduced hatching of tree swallow (*Tachycineta bicolor*) eggs in east central Minnesota was associated with egg PFOS concentrations as low as 150 ng/g. While reduced hatchability reported in this field study is a relevant effect, field studies typically do not provide appropriate cause and effect data that can be used to develop TRVs (USACHPPM 2000). Many of the studies evaluating the effects of PFAS in birds have involved injection of PFOS and/or related compounds into eggs with an assessment of hatchability and survivability or changes in biochemical endpoints (Molina Elizabeth, Balander et al. 2006, O'Brien, Carew et al. 2009, Peden-Adams, Stuckey et al. 2009, O'Brien, Kennedy et al. 2010, Norden, Westman et al. 2012, Stroemqvist, Olsson et al. 2012, Norden, Berger et al. 2016). In those egg injection studies assessing the effects of PFOS on hatchability and survivability, which are both relevant endpoints for development of TRVs, there was considerable variation in the effective doses between studies. Molina *et al*. (2006) injected chicken (*Gallus gallus domesticus*) eggs with 0.1, 1.0, 10 or 20 µg PFOS/g egg via the air cell prior to incubation, reporting a lethal dose for 50% of experimental population (LD50) of 4.9 µg PFOS/g egg and a LOAEL of 0.1 µg PFOS/g egg, based on hatchability. Pathological changes in the liver characterized by bile duct hyperplasia, periportal inflammation and hepatic cell necrosis occurred at doses as low as 1.0 µg PFOS/g egg. A dose-dependent decrease in the number of embryos pipping and an LD50 of 93 µg/g egg were reported for chicken eggs injected with 0.1, 5.0 or 100 µg PFOS/g egg via the air cell prior to incubation (O'Brien *et al*. 2009a). This LD50 value is approximately 20 times greater than the LD50 value reported by Molina *et al*. (2006). Contrary to the Molina *et al*. (2006) study, hatchability was not affected in chicken eggs injected with 1.0, 2.5 or 5.0 µg PFOS/g egg via the air cell prior to incubation, but there were increases in spleen (all doses) and liver (2.5 and 5.0 µg PFOS/g egg) masses and immunological effects (all doses) in 14-day-old hatchlings (Peden-Adams *et al*. 2009). Strömqvist *et al*. (2012) injected chicken eggs via the air cell with 20 µg PFOS or 20 µg PFOA/g egg at 15 days of incubation, which induced mortality. It is not possible to compare the results of this study with the studies referenced above because of the differences in study design. Injection of chicken eggs with 5, 20 or 40 µg PFOA/g egg (site of injection and stage of incubation were not specified) resulted in a significant decrease in hatchability and an increase in developmental defects (splayed legs) at all doses (Yanai, Dotan et al. 2008). However, the authors did not report an LD50 value and only a LOAEL is available because of study design.

While egg injection studies can be used to assess the relevant endpoints of mortality, development and growth, and data can be used to identify NOAELs, LOAELs and EDs, this exposure pathway does not mimic what occurs in the wild, the transfer of chemical from the adult female to the egg. Development of accurate TRVs to be used in ecological risk assessments requires data from studies utilizing an ecologically relevant exposure scenario, exposure to the chemical via the feed or water.

There are three studies that employed oral exposure of birds to PFOS or PFOA and examined endpoints relevant to development of TRVs. Smits and Nain (2013)exposed Japanese quail to 1 or 10 µg PFOA/ml via drinking water for eight weeks to assess effects on T cell, B cell and innate immunity. Daily intake was estimated to be 0.2 and 2.1 µg PFOA/g body weight (bw). The authors reported that there were no clinical signs of toxicity and no effects on feed and water intake, but there was T cell immunosuppression at the high dose. However, the PFOA-induced suppression of T cell-mediated immunity did not translate into compromised

disease resistance, which is a relevant endpoint for development of TRVs. Newsted *et al.* (2006) evaluated the acute oral toxicity of PFOS in juvenile mallards (*Anus platyrhynchos*) and northern bobwhites (*Colinus virginianus*). Ten-day-old birds were provided feed containing PFOS (8.7 to 1125 µg PFOS/g feed) for five days and then clean feed for 17 days. The LD50 was determined to be 150 µg/g bw/day (cumulative dose of 750 µg/g bw over the 5-day period) for mallards and 61 µg/g bw/day (cumulative dose of 305 µg/g bw) for bobwhites. It was stated that the hepatic concentration of PFOS associated with mortality was at least 50 times greater than PFOS concentrations in the livers of wild birds. In a subsequent chronic study by Newsted *et al*. (2007), mallard and northern bobwhites were administered 0, 10, 50 or 150 µg PFOS/g feed for a total of 21 weeks, including at least seven weeks prior to initiation of egg laying. Eggs were incubated and hatchlings were maintained on clean feed for 14 days. The two highest dietary concentrations resulted in significant adult mortality within seven weeks and were discontinued. The lowest feed concentration (10 µg PFOS/g feed) was identified as the LOAEL for northern bobwhites based on decreased survivability of 14-day-old chicks. There were no treatment-related effects, other than adult mortality, reported for the mallards.

Of the studies cited, the Newsted *et al*. (2007) study is the most appropriate for development of TRVs. Newsted *et al*. (2005) derived an avian toxicity reference value for PFOS based on the LOAEL of 10 µg PFOS/g feed and an overall uncertainty factor of 36. The individual uncertainty factors used for the calculation of a generic trophic level IV avian predator TRV for PFOS consisted of an intertaxon extrapolation factor of 6, an uncertainty factor of 2 to account for the fact that a LOAEL was used instead of a NOAEL and an exposure duration uncertainty factor of 3. The authors stated that as additional toxicity data became available, it was anticipated that the magnitude of uncertainty factors would decrease and a more accurate estimate of potential risk would be achieved.

We conducted a series of toxicity trials designed to generate avian ecotoxicity information for compounds associated with AFFF in birds. The subacute toxicities of PFOS, PFOA, PFOS plus PFOA and two legacy AFFF formulations manufactured by 3M and Ansul representing electrochemical fluorination and fluorotelomer technologies, were initially determined in Japanese quail, which is recognized as a surrogate for wild avian species (Bursian et al. 2020). Subsequently, two reproduction trials were conducted following the design used by Newsted et al. (2007) to assess the effects of PFOS and PFOS provided by the legacy 3M AFFF (AFFF PFOS) on egg production, hatchability and chick survivability for development of TRVs. The design used is similar to the US Environmental Protection Agency's (USEPA) Avian Reproduction Test (OCSPP 850.2300) (US Environmental Protection Agency 2012) and the Organisation for Economic Co-operation and Development's (OECD) Avian Reproduction Test (OECD 206) (Organisation for Economic Co-operation and Development 1984). The results of the acute and reproduction trials are reported here.

3. Materials and Methods

3.1 Acute Study

3.1.1 PFOS Diet preparation.

Nine dietary treatments were formulated to contain 0, 70, 141, 281, 562, 843, 1125, 1687, or 2250 mg of heptadecafluorooctanesulfonic acid (PFOS) kg^{-1} feed (PFOS potassium salt, ≥ 98%, Sigma-Aldrich). Feed used throughout the trial was Home Fresh Multi-Flock Turkey N Game Starter (Kent Nutrition Group). A stock suspension of PFOS was made by adding 2250 mg to 150 mL anhydrous ethanol (Koptec) and placing in an ultrasonic water bath (Branson 2200) to break up PFOS particles. After sonicating for 6 h, the suspension was mixed on a magnetic stir-plate for approximately 15 h. To prepare PFOS suspensions for the different treatment diets, appropriate volumes of the stock PFOS suspension and anhydrous ethanol were combined resulting in the desired concentration of PFOS kg^{-1} feed in a volume of 150 mL kg^{-1} feed. The control diet contained only anhydrous ethanol. To distribute the PFOS suspension evenly throughout the feed, the appropriate volume of the suspension for each treatment was removed while stirring on a magnetic stir-plate and immediately dribbled into a stainless steel mixing bowl (4.26 L) of a stand mixer (KitchenAid) containing the appropriate amount of feed while the feed was mixing. The PFOS-containing feed was mixed with a paddle-type flat beater for 10 min at the lowest speed. After mixing, the diet was poured on to a methanol-cleaned stainless steel or porcelain tray at a depth of approximately 1 cm and allowed to dry overnight in a fume hood to evaporate the ethanol. Feed samples were collected from various locations on the trays into 14 mL polypropylene tubes (Corning) and sent to the University of Minnesota for chemical analysis. Each diet was transferred to an open 9.5 L polypropylene bucket that was placed in a fume hood for an additional 24 h. Buckets were then sealed until the beginning of the trial.

3.1.2 PFOA Diet preparation.

Diets containing perfluorooctanoic acid (PFOA, 95%, Sigma-Aldrich) were prepared in a manner similar to the PFOS trial, except the solubility of PFOA in anhydrous ethanol eliminated the need to sonicate and mix prior to addition of the PFOA solution to the feed. The nominal dietary concentrations for the PFOA trial were 0, 200, 350, 500, 625, 750, 1000, 1250, and 1500 mg kg^{-1} feed.

3.1.3 PFOS +PFOA Diet preparation.

Diets containing both PFOS and PFOA had nominal concentrations of 0 + 0, 50 + 50, 75 + 75, 100 + 100, 135 + 135, 150 + 150, 175 + 175, 200 + 200 and 400 + 400 mg PFOS + PFOA kg^{-1} feed. A stock solution of PFOS was prepared by adding 400 mg PFOS to 150 mL anhydrous ethanol and sonicating and mixing as described for the PFOS trial above. The concentration of PFOS was low enough that it dissolved after mixing overnight. PFOA (400 mg) was then added to the PFOS stock solution resulting in a PFOS + PFOA solution. Treatment diets were prepared as described for the PFOS trial.

3.1.4 3M Lightwater AFFF Diet preparation.

Nine dietary treatments were formulated to contain 3M Lightwater 6% AFFF (manufactured April, 1990, lot #98-0211-1393-5) at 0, 8.8, 18, 24, 30, 35, 53, 70, or 140 mL kg $feed^{-1}$. Targeted concentrations of PFOS/PFOA based on analysis of the AFFF were 0/0, 70/0.88, 144/1.8, 192/2.4, 240/3.0, 280/3.5, 420/5.25, 560/7.0, 1120/14 mg PFOS/PFOA kg feed-

[1]. The AFFF concentrate was mixed with double deionized water to provide the concentrations needed. Treatment diets were prepared as described for the PFOS trial.

3.1.5 Ansul Ansulite AFFF Diet preparation.

The Ansulite AFFF (packaged September, 2002; log #4219) is a telomerization-based product and thus consists of shorter perfluorinated carbon chains. Therefore, diet formulations for this product were based on the 6:2 fluorotelomer thioamido sulfonate (FtTAoS) concentration assuming that the Ansul AFFF contained 6,100 mg 6:2 FtTAoS/L (Backe, Day et al. 2013). Nine dietary treatments were formulated to contain Ansul AFFF at 0, 1.5, 2.9, 5.8, 12, 23, 46, 92, and 184 mL/kg feed. Targeted concentrations of 6:2 FtTAoS were 0, 9, 18, 35, 73, 140, 279, 559, and 1118 mg kg feed^{-1}. Diets were mixed and stored as described for the PFOS trial.

3.1.6 Birds, housing, and sample collection

Ten-d-old Japanese quail, raised at the Michigan State University Poultry Teaching and Research Center (Lansing, Michigan USA), were randomly assigned to the 9 dietary treatments in the PFOS, PFOA, PFOS + PFOA, 3M AFFF and Ansul AFFF trials described in *Diet Preparation*. Birds were individually tagged, weighed, and placed in a chick brooder (Petersime). Chicks were housed in pens (100.3 x 68.6 x 25.4 cm) constructed of 1 cm^2 stainless steel mesh, 20 chicks per pen and 1 pen per treatment. Allotment of chicks to treatments was rotated between treatments to avoid any selection bias. Pre-weighed plastic feeders (33 x 8.9 x 5 cm) were filled with treatment feed to approximately 1.27 cm below the rim of the feeder and the weight of the container with feed was recorded. To control wastage, feed was covered with a 1.27 x 1.27 cm grid of galvanized wire that sank with the level of feed in the feeder as the chicks ate. Feeders were placed in the appropriate brooder pens so that the lowest concentrations were at the top of the battery and the highest concentrations were at the bottom to avoid cross contamination of feed. Water nipples (4 per pen) were adjusted initially to a height of 6.5 cm from the floor of the pen and then raised appropriately as the quail grew. Birds were checked at 0800 and 1600 each day. Moribund birds were euthanized by cervical dislocation. Euthanized birds and those found dead were necropsied at each observation time with the liver being collected, weighed and frozen in a glass scintillation vial with a foil lined lid for chemical analysis.

Chicks were fed their respective treatment diets for 5 d and then switched to control feed contained in 63.5 x 10 x 6 cm stainless steel feeders that hung on the outside of the brooder pen for an additional 18 d. On d 5 when diets were changed, feed and birds were weighed in order to calculate feed intake and bird body weight gain. Feed and birds were weighed again on d 8, 15 and 23.

Ten birds from each treatment were euthanized on d 8 unless the group had suffered mortalities during the first 8 d of the study. In treatments with mortalities, half of the remaining live birds were necropsied on d 8 and the remaining birds were necropsied on d 23. Birds were euthanized by cervical dislocation and blood was collected from the heart using a 1-mL tuberculin syringe with a 22-gauge 2.54-cm needle. Livers were removed, weighed and frozen (-20^{o}C) for subsequent chemical analysis. Blood was allowed to clot and then centrifuged at 1500 x g for 10 minutes (Heraeus Biofuge Pico microcentrifuge) at room temperature. Serum was separated and frozen at -20^{o}C for subsequent chemical analysis. Liver and serum samples were shipped overnight on dry ice to the University of Minnesota for analysis.

3.1.7 PFAS analyses

Feed and bird livers were extracted, and serum was directly injected onto the HPLC/MS/MS for PFAS determination. Triplicate 2 g feed subsamples were placed in 50 mL polypropylene centrifuge tubes (Corning) and spiked with PFHxS as a surrogate of analytical recovery. Approximately 20 mL of Optima grade methanol (Fisher Scientific) was added to each tube, and the tubes were shaken at maximum deflection on a Burrell Model 75 Wrist-Action Shaker for 30 minutes. Samples were then centrifuged at approximately 904 RCF on a Dynac Centrifuge (Clay-Adams) for 20 minutes. This process was repeated three times. The eluates were combined and brought to dryness under a gentle stream of pre-purified N_2 gas using a OA-SYS heating system (setting 5) and N-EVAP 111 nitrogen evaporator (Organomation Associates, Inc.). The samples were reconstituted in 2 mL Optima grade methanol using a Fisher vortexer at 1000 rpms. The reconstituted samples were centrifuged again as described before to remove any remaining solids and transferred by Pasteur pipet to 2.0 mL SafeSeal polypropylene microcentrifuge tubes (BioScience, Inc.) for storage at -20° C before analysis.

Livers were weighed, and approximately 100 mg subsamples were taken for analysis. Livers were added to 15 mL conical polypropylene centrifuge tubes containing 10mL of methanol and an additional 100 ng PFHxS surrogate standard in the PFOS acute trial. Samples were homogenized at 30,000 rpms using a PRO 250 and PRO SC-250 homogenizer and motor (Pro Scientific, Oxford CT, USA) equipped with Multi-Gen adapter and a Multi-Gen 7XL generator probe. Samples were sonicated for 30 minutes in a Mettler Ultrasonic Cleaner and centrifuged at 904 RCF for 20 minutes. Eluate was collected and diluted to 100 mL using 70 mL HPLC water and brought to volume with methanol.

All samples/extracts were spiked with known amounts of internal standards. For PFOS determination, ^{13}C mass labeled PFOS ($^{13}C_4$PFOS) was added. For PFOA analysis ^{13}C mass labeled PFOA ($^{13}C_4$PFOA). Samples were injected onto an Agilent 1200 HPLC (Hewlett-Packard, Palo Alto, CA) equipped with a 4 x 2.0 mm C_{18} guard column (Phenomenex, Torrance, CA) and 50 x 2.1 mm, 3 mm Betasil C_{18} analytical column (Thermo Fisher Scientific, Waltham, MA). The column temperature was kept constant at 20°C. The flowrate was 200 µL min^{-1}, and the injection volume was 10 µL. Mobile phase A consisted of 90:10 HPLC water:Optima methanol (v/v) and mobile phase B of Optima methanol. The aqueous and organic phases both phases both contained 2mM ammonium acetate. Sample matrix consisted of a 70:30 water:methanol (v/v) mixture matching initial HPLC conditions to prevent peak splitting for both liver and serum samples. An API 4000 (AB Sciex, Concord, ON, Canada) triple quadrupole mass spectrometer with an electrospray ionization source (negative) was used for PFAS determination. The instrument was operated with a declustering potential of -70 for PFOS and -27 for PFOA, and a collision energy of -80 for PFOS and -14 for PFOA. Each analyte was determined using multiple reaction monitoring with transitions of 499 to 99 and 413 to 169 for PFOS and PFOA, respectively. The masses were determined using the relative response factor method on triplicate calibration standards run every 6 samples. This is a one point calibration, which was determined to be sufficient after determining the linearity of response of the instrument. Surrogate recoveries of feed samples randed form 70-105% and average 83 ± 7%. No correction for surrogate recoveries was made. Because liver samples required orders of magnitude dilution prior to instrumental analysis, no surrogate was feasible.

3.1.8 Endpoints and statistical analysis

Endpoints included feed intake, body weight, lethal dose for 50% of the population (LD50), feed concentration resulting in 50% mortality (LC50), time required to result in 50% mortality (LT50), survivability through the first 8 d of the trial, absolute and relative (as a percent of body weight) liver weight, and serum and hepatic concentrations of PFOS and/or PFOA. For feed intake, the sample unit was pen and there was 1 pen per treatment. Feed intake was calculated as average daily feed intake based on feed disappearance and number of birds in the pen each day. The statistical unit for body weight, organ weight and tissue contaminant concentration was individual bird. Feed intake, body weight and body weight gain data were checked for normal distribution utilizing PROC UNIVARIATE in SAS (SAS Institute). If data were not normally distributed, they were log transformed before statistical analysis using PROC MIXED in SAS. Some body weight gain data were negative values and could not be log transformed. In this case, data were transformed using log-modulus transformation described by Wicklin (2011) based on the method of John and Draper (1980). The average daily dose (ADD) of treatment chemical expressed as mg kg body weight^{-1} d^{-1} was calculated as described by Newsted et al. (2006) using the following formula (Equation 1):

(1) ADD (mg kg body weight^{-1} d^{-1}) = [mean feed consumption (g) ÷ mean body weight (g)] x dietary concentration (mg kg feed^{-1})

The dose resulting in 50% lethality (LD50) after 5 d, the feed concentration resulting in 50% lethality (LC50) after 5 d, and time required to result in 50% lethality (LT50) after 8 d at each feed concentration were calculated using probit analysis (Finney 1952). Lethal dose and LC values were based on mortality occurring within the first 5 d of the trial. Survivability was calculated as the percent of live birds at the end of the first 8 d of each trial. Percent survivability and relative liver weight (liver weight expressed as percent of body weight) were arcsine transformed prior to PROC MIXED analysis. Liver weights had a non-normal distribution and thus were log transformed prior to PROC MIXED analysis in SAS. All means were compared to the control utilizing Dunnett's multiple comparison test. Means presented are back transformed with a 95% confidence interval.

The protocol for this study was approved by the Michigan State University Institutional Animal Care and Use Committee.

3.2 Chronic Exposure Study

3.2.1 PFOS Diet preparation.

Six dietary treatments were formulated to contain 0, 2.5, 5.0, 10, 15, or 20 mg heptadecafluorooctanesulfonic acid (PFOS) kg feed^{-1} (PFOS potassium salt, ≥ 98%, Sigma-Aldrich), accounting for chemical purity. Feed for the initial 10 weeks of the study was Purina Game Bird and Turkey Startena® crumbles. The composition of the basal diet is given in Table 1. For inclusion in the feed, PFOS was added to the appropriate volume of anhydrous ethanol (Koptec) and mixed on a magnetic stir plate until completely dissolved. Sufficient feed to last 10 weeks was weighed and placed in a 113 kg capacity, methanol cleaned, stainless steel paddle mixer (Wenger). While feed was mixing, the PFOS/ethanol solution was sprayed onto the feed using a hand-held pump-mist plant sprayer (Chapin). After the PFOS/ethanol solution was applied, the feed was mixed for an additional 10 min. Experimental diets were mixed from lowest to highest concentration. Diets were spread out to a depth of approximately 3.2 cm on

tables lined with plastic sheeting and allowed to dry for 92 h, being turned midway through the drying period. When dry, feed was sampled for chemical analysis and then stored in labeled, color-coded plastic storage barrels until fed. After approximately 8 weeks, treatment diets were prepared as described above but feed was changed to Purina Game Bird Breeder Layena® crumbles to accommodate egg laying. The composition of this feed is given in Supplemental Table 1. The layer ration was fed from 10 to 20 weeks of age. On average, the test substance was incorporated into the feed 11.5 d prior to the beginning of the trial. Feed samples were not evaluated for stability of the test substance because PFAS are resistant to oxidative and reductive stresses. There were no predefined acceptability criteria for percent recovery or homogeneity.

Table 1. Guaranteed Analysis of Purina Gamebird and Turkey Startena® and Gamebird Chow - Gamebird Breeder Layena®[a]

	Gamebird and Turkey Startena®	Gamebird Breeder Layena®
Nutrient	Analyzed amount	
Crude protein, minimum (%)	30.0	20.0
Crude fat, minimum (%)	2.5	2.5
Crude fiber, maximum (%)	6.5	7.0
Lysine, minimum (%)	1.5	0.9
Methionine, minimum (%)	0.5	0.3
Calcium (Ca), minimum (%)	1.0	2.8
Calcium (Ca), maximum (%)	1.5	3.2
Phosphorus (P), minimum (%)	0.8	0.8
Salt (NaCl), minimum (%)	0.25	0.3
Salt (NaCl), maximum (%)	0.75	0.7
Selenium (Se), minimum (ppm)	0.65	---[b]

[a]Purina Animal Nutrition.
[b]Not given.

3.1.2 3M Lightwater AFFF Diet preparation.

Diet preparation was essentially the same as described in *Dietary preparation PFOS*. However, the AFFF formulation (3M Lightwater 6% AFFF manufactured April 1990, lot #98-0211-1393-5), unlike PFOS, was a liquid and thus was diluted with water to make the 6 solutions that were added to the feed. Concentrations of 3M AFFF were calculated based on previous analysis of the product and were intended to provide PFOS at concentrations similar to dietary PFOS concentrations presented in *Dietary preparation PFOS*. The nominal concentrations of AFFF PFOS were 0, 2.5, 5.0, 10, 15, and 20 mg PFOS kg feed^{-1}.

3.1.3 Birds and housing

Japanese quail eggs were obtained from the Michigan State University (MSU) Poultry Teaching and Research Center (East Lansing, MI) breeding flock. Eggs were incubated and hatched at the facility. At hatching quail were moved to a brooder battery (Petersime) with 12 pens arranged in 6 levels (2 pens per level). Hatchlings were housed 27 birds per pen (100 x 69 x 25 cm constructed of 1 cm^2 stainless steel mesh) with 2 pens per treatment. The 256 cm^2 of floor space per bird in the present trial was slightly less than the recommendation of 300 cm^2 for

this species. Approximately 33% of the floor area of each pen was heated by an incandescent bulb regulated by a wafer thermostat. Temperature within this semi-enclosed area ranged between 32.2 and 38.7° C. The remaining floor area did not have a heat source. Chicks could move freely between the 2 areas and the twice-daily observations indicated uniform spacing of the birds throughout the pen. The temperature in the study room was maintained at 21.1° C. Humidity in the room was not monitored.

Birds were fed the experimental diets for 4 weeks. Treatment groups were arranged in the brooder so that the lowest concentrations of PFOS were at the top of the battery and the highest concentrations were at the bottom to avoid cross contamination of the feed. When initially placed in the brooder, chicks were introduced to water from the drinker nipple and provided feed scattered on the floor of the brooder pen. Additionally, a plastic feed trough (33 x 8.9 x 5 cm) covered with a 1.27 x 1.27 cm grid of galvanized wire (to control feed wastage) was provided in the pen for the first week. Subsequently, birds were fed from stainless steel trough-type feeders (63.5 x 10 x 6 cm) containing a grid that were attached to the outside of the pens. Initially water nipple (4 per pen) height was set to 6.5 cm from the floor of the pen and then raised accordingly as the chicks grew.

Birds were checked daily at 0800 and 1600 as were water nipples and temperature at each level of the brooder. The photoperiod was maintained at 8 h light and 16 h dark. Light intensity measured in the brooder was 25 lux. Cannibalism, foot pecking or aggression among chicks was not observed.

At 4 weeks of age, birds were sorted by sex within treatment and then randomly paired (male/female), individually identified with a plastic wing tag (Ketchum; 25 x 18 mm), weighed and moved to breeder/layer pens. Birds were housed in a single room in 2 64-cage batteries (Alternative Design). Cages in each battery were arranged in 4 levels of 8 cages per level totaling 32 cages per side. Each cage (28 x 30 x 25 cm) was equipped with 1 poultry water nipple and housed 1 pair of birds (16 pairs per dose). A stainless-steel trough-type feeder was attached to the outside of the cage. The floor of the pen was sloped so that eggs laid rolled to the front and outside the cage. Treatments were arranged in the battery similar to the brooder battery with lower dietary concentrations above higher concentrations. Birds were maintained on 8 h light and 16 h dark until 8 weeks of age when photoperiod was increased over 2 weeks to 17 h light and 7 h dark to induce egg laying at 10 weeks of age. Light intensity in the room was approximately 20 lux.

3.1.4 Reproduction

Birds were weighed every 2 weeks and feed consumption was measured weekly for each breeding pair. When egg laying began at 10 weeks of age, eggs were collected daily between 0800 and 0900. Individual eggs were labeled using non-toxic felt tip surgical markers with hen identification number, date and dietary concentration and then placed in an egg cooler (15-17^{o}C). Eggs in the egg cooler were set in a rotary incubator (Petersime) at 1-weeks intervals. The incubator was maintained at 37.6^{o} C and 51.9% relative humidity. Eggs with damaged shells were considered non-viable and excluded from hatchability calculations. Weekly, the yolk and albumin from these eggs were separated and frozen for subsequent PFAS analysis. On day 14 of incubation, eggs were placed in hatching baskets by hen number and transferred to a Surepip

hatcher (Agro Environmental Systems) maintained at 37.6° C and 62.6% relative humidity. Beginning on day 17, hatchlings with dry feathers were removed from the hatcher, weighed and transferred to the brooder battery by dietary concentration twice a day. Eggs remaining on the afternoon of day 18 were considered unhatched.

3.1.5 Embryo mortality

All unhatched eggs were opened in the afternoon of day 18 and stage of embryo development was determined and recorded. Embryo age at death was categorized as less than 4 d, 4 to 7 d, 8 to 10 d, 11 to 14 d, greater than 14 d, dead pip or live pip. Embryos that were developed enough to visualize anatomical structures were examined for abnormalities. Unhatched eggs with no gross indication of embryo development were assumed to be infertile and were not included in hatchability calculations.

3.1.6 Offspring rearing

Live hatchlings were raised in the same brooder unit and under the same conditions used to raise the parental birds. Offspring were raised for 14 d on non-contaminated Game Bird and Turkey Startena® crumbles that had been ground to reduce particle size. Birds were checked twice daily and weighed at hatch and on days 7 and 14.

3.1.7 Offspring necropsies

On day 14, a subsample of 10 chicks per dose group were randomly selected for necropsy. Chicks were euthanized by cervical dislocation and blood was immediately obtained by cardiac puncture (1-mL syringe, 22-gauge, 2.54-cm needle). Livers were removed, weighed and frozen (-20° C) in glass vials for subsequent PFAS analysis. Blood was allowed to clot prior to centrifugation at 2000 x g for 10 min (Heraeus Instruments) at room temperature. Serum was separated and frozen at -20° C. These procedures were repeated for each of the 10 hatches.

3.1.8 Adult necropsies

At 20 weeks of age, surviving adult birds were weighed, euthanized by cervical dislocation and blood immediately collected by cardiac puncture (1-mL syringe, 22-gauge, 2.54-cm needle). Birds were necropsied and half of the liver and the kidneys were removed, weighed and placed in 10% neutral buffered formalin for subsequent histological examination (Urika LLC). The remaining half of the liver and serum were frozen at -20°C for subsequent PFAS analysis.

3.1.9 Pathology

At least 2 sections of liver and 2 sections of kidney were placed in a single cassette for each quail. If whole kidneys were available, cranial and caudal lobes were sampled. Samples were sent to URIKA Pathology for prosessing and evaluation. Hepatic and renal tissues were examined by a board certified pathologist and lesions were graded on a severity scale of 0 to 4 according to the following criteria: 0 = no lesion recognized; 1 = minimal lesions, 1 to 3 foci or small foci of a few cells; 2 = mild lesions, increased number of foci or more of the lesion; 3 = moderate lesions, more lesions (2-3 per 10x field of vision); 4 = severe lesions, the majority of cells and/or extensive regions involved with the lesions.

3.1.10 PFAS analyses

Feed, livers, yolks and albumin were extracted, and serum was directly injected onto a high performance liquid chromatography-tandem mass spectromter (HPLC/MS/MS) for PFAS determination. Feed extraction was performed as described in previous assessment of subacute effects (Bursian, Link et al. 2020). Using mass-labeled surrogate standards was cost prohibitive given the high PFAS content of livers. High extractability of PFOS, however, was demonstrated in the subacute study using PFHxS as a surrogate standard (Bursian et al 2020). It was not feasible to use PFHxS in this manner in the present study given its presence in the 3M AFFF mixture. Whole livers were weighed and placed in conical polypropylene centrifuge tubes containing 10 mL methanol and homogenized at 30,000 revolutions per minute (rpm) using a PRO 250 and PRO SC-250 homogenizer and motor (Pro Scientific) equipped with Multi-Gen adapter and a Multi-Gen 7XL generator probe. Homogenates were sonicated for 30 min in a Model 5800 CPXH Series heated ultrasonic cleaning bath (Fisher) at 115˚ C and centrifuged at 4,500 relative centrifugal force (rcf) for 15 min in an Eppendorf 5804 centrifuge. The entire eluate was collected and brought to 25 mL using HPLC water. All samples/extracts were spiked with known amounts of internal standards. For PFOS determination, ^{13}C mass labeled PFOS ($^{13}C_4$PFOS) was added. For PFHxS analysis ^{13}C mass labeled PFHxS ($^{13}C_3$PFHxS) was used.

Yolk samples were weighed in scintillation vials, 5 mL of HPLC water was added and the samples were homogenized as described for livers. The sample was then transferred to a 50 mL conical polypropylene centrifuge tube. The scintillation vial was rinsed with an additional 5 mL of HPLC water, and the rinsate was collected. The scintillation vials were allowed to dry and then weighed. Yolk mass was considered the difference between the two measures. Approximately 35 mL of Optima methanol was added to the centrifuge tubes, and the tubes were sonicated at 115˚ C for 30 min. The samples were centrifuged at 4,500 rcf for 15 minutes, and the supernatant was brought to 50 mL using HPLC water.

Albumin samples were weighed in 15 mL polypropylene centrifuge tubes. Internal standard was spiked into the sample, and approximately 10 mL methanol was added. The samples were homogenized and centrifuged as previously described and evaporated to approximately 1 mL under a gentle stream of pre-purified N_2 gas using an OA-SYS heating system (setting 5) and N-EVAP 111 nitrogen evaporator (Organomation Associates). It was not necessary to correct for extraction efficiency since the internal standard was added before the extraction process. Extraction efficiency in the 3M AFFF trial, however, was calculated for quality control purposes by adding $^{13}C_8$PFOS before extraction and $^{13}C_4$PFOS before compound analysis: 62 ± 27% (average ± relative standard deviation).

Samples were injected onto an Agilent 1200 HPLC (Hewlett-Packard) equipped with a 4 x 2.0 mm C_{18} guard column (Phenomenex) and 50 x 2.1 mm, 3 mm Betasil C_{18} analytical column (Thermo Fisher Scientific). The column temperature was kept constant at 20°C. The flowrate was 200 µL min^{-1}, and the injection volume was 10 µL. Mobile phase A consisted of 90:10 HPLC water:Optima methanol (v/v) and mobile phase B of Optima methanol. The aqueous and organic phases both contained 2mM ammonium acetate. Sample matrix consisted of a 70:30 water:methanol (v/v) mixture matching initial HPLC conditions to prevent peak splitting for both liver and serum samples. An API 4000 (AB Sciex) triple quadrupole mass spectrometer with an electrospray ionization source (negative) was used for PFAS determination. The instrument was

operated with a declustering potential of -70 for PFOS and -PFHxS, and a collision energy of -80 for PFOS and PFHxS. Each analyte was determined using multiple reaction monitoring with transitions of 499 to 99 and 399 to 99 for PFOS and PFHxS, respectively. The masses were determined using the relative response factor method on triplicate calibration standards run every 6 samples. This is a one-point calibration that was determined to be sufficient after determining the linearity of response of the instrument.

The method detection limit (MDL) was determined according to 40 CFR 136 Appendix B (Federal Code of Regulations 2019) in which at least 7 subsamples of a matrix are spiked with a low-level standard and the MDL is taken as 3 times the standard deviation of the mean concentration determined. Since there was background PFOS and PFHxS in all of the samples, 3 times the standard deviation of the mean of the control samples was used as a conservative estimate of the MDL. The method detection limits are presented in Table 2.

Table 2. Method Detection limits for feed, adult and chick serum and livers and egg yolk and albumin

	PFOS	AFFF PFOS	AFFF PFHxS
Feed (µg g^{-1})	-	0.008	-
Adult serum (µg mL^{-1})	0.3	0.5	0.03
Adult liver (µg g^{-1})	0.6	0.4	0.03
Offspring serum (µg mL^{-1})	0.05	0.10	0.008
Offspring liver (µg g^{-1})	0.30	0.20	0.01
Egg yolk (µg g^{-1})	0.05	0.40	0.04
Egg albumin (µg g^{-1})	0.02	0.001	0.0002

3.1.11 Spike Recoveries

For quality assurance, control liver, control yolk and control serum composite were spiked with known amounts of PFOS, and AFFF to determine analytical recovery of our methods. Control liver samples were spiked in triplicate with PFOS or 3M AFFF at 6 levels to obtain nominal concentrations of 0, 18, 36, 65, 95, and 84 µgg–1 PFOS or 0 + 0, 2.0 + 25, 4.0 + 50, 8.1 + 100, 12 + 150, and 16 + 200 µgg–1 AFFF PFHxS + AFFF PFOS. These concentrations are representative of those determined for males in both chronic trials but encompass the same order of magnitude as those determined for females. The individual recovery ranges for PFOS, AFFF PFHxS, and AFFF PFOS were 75 to 103%, 81 to 106%, and 86 to 118%, respectively. Mean recoveries were 88% (10% relative standard deviation [RSD]), 94% (8% RSD), and 100% (10% RSD). control yolk samples were spiked in triplicate with PFOS at 6 levels to obtain nominal concentrations of 0, 19, 32, 54, 76, and 98 µgg–1 PFOS. These concentrations are representative of those determined in both chronic trials. The individual recovery range for PFOS was 51 to 109%. Mean recovery was 66% (21% RSD). Likewise, 8 control yolk samples were spiked with AFFF to obtain nominal concentrations 0 + 0, 2.9 + 36, 4.6 + 57, 6.9 + 86, 9.2 + 114, 11.5 + 143, 13.8 + 171, and 16.1 + 200 µgg–1 AFFF PFHxS + AFFF PFOS. The recovery range was 75 to 112% and 72 to 115%, and the mean recovery was 91% (14% RSD) and 91% (14% RSD) for AFFF PFHxS and AFFF PFOS, respectively. A composite control serum sample consisting of serum from 32 quail was divided into 0.33-mL subsamples and spiked with PFOS in triplicate to achieve nominal concentrations of 0, 27, 54, 108, 162, and 216 µgmL–1. The recovery range for PFOS was 61 to 113%, and the mean recovery was 97% (15% RSD). A composite sample of

serum was similarly taken from 60 quail and dosed with AFFF to achieve nominal concentrations of 0 + 0, 2.1 + 25, 4.1 + 50, 8.2 + 100, 16.4 + 200, and 24.6 + 300 µgmL–1 AFFF PFHxS and AFFF PFOS, respectively. The recovery range was 68 to 107% and 78 to 117%, and the mean recovery was 83% (16% RSD) and 99% (14% RSD) for AFFF PFHxS and AFFF PFOS, respectively (Supplemental Data, Table S2). One AFFF PFHxS sample (129%) and 2 AFFF PFOS samples fell outside of the acceptability criterion range of 50 to 120%. A table of the results are provided in Appendix

3.1.12 Endpoints and statistical analysis

End points included feed intake, adult body weight, adult body weight gain, egg production, hatchability, embryo mortality, chick survivability, chick body weights, chick and adult liver weights, liver and kidney pathology and serum, liver and egg PFOS concentrations. Analysis of variance (ANOVA) and Dunnett's multiple comparison procedure were used to evaluate differences between treatment and control groups for most endpoints. Non-normal data were log transformed for analysis and then back-transformed for table presentation. Statements of significance are based on $p < 0.05$. Feed consumption and reproduction parameters were evaluated on a cage basis while embryo mortality, chick survivability, body and organ weights, pathology and PFOS concentrations were evaluated on an individual bird basis. Percentage data (reproduction data and relative organ weight expressed as percent of body weight) were evaluated using Dunnett's adjustment after arcsine square root transformation. Since quail were group housed for the first 4 weeks of the study, daily feed intake was estimated based on feed disappearance of the pen and the number of birds in the pen. From week 4 to 20 when birds were housed as pairs, feed intake for the pair was calculated by feed disappearance in the pen and then adjusted to estimate feed intake per bird. Body weights of male and female birds were similar until the end of week 9 so during this period the feed disappearance of the pen was divided by two to estimate feed intake per bird. After week 9, females gained more weight than males and it was assumed they were also consuming more feed. To estimate feed intake per bird from week 10 to 20, the body weight ratio of male to female within each pen was calculated and applied to the amount of feed that disappeared. Average daily dose (ADD) of PFOS or AFFF PFOS (mg PFOS or AFFF PFOS kg body weight^{-1} d^{-1}) for each treatment group was calculated by dividing average daily feed intake by body weight (Bursian et al. 2020). Adult body weight gains were averaged over the duration of the exposure at each dietary concentration. The non-parametric Kruskal-Wallis test was used to test for differences in liver and kidney lesion severity scores among treatment groups.

4. Results and Discussion

4.1 Acute Exposure Study

4.1.1 Dietary concentrations and average daily dose

The ADDs through the first 5 d of the trial for PFOS, PFOA, and the combination as well as the 3M AFFF trial were based on analyzed feed concentrations (see Equation 1), whereas the ADDs for the Ansul AFFF trial were based on nominal concentrations of 6:2 FtTAoS as reported by Backe et al. (2013). Analyzed dietary concentrations in the PFOS trial were 0, 62, 91, 216, 471, 654, 866, 920, and 1955mgkg feed^{-1} and ranged from 55 to 89% of nominal concentrations. Corresponding ADDs were 0, 11, 17, 36, 57, 49, 58, 45, and 102mg PFOS kg body weight^{-1} d^{-1}. Analyzed concentrations in the PFOA trial were 0, 162, 262, 368, 447, 590, 814, 926, and 1208mg kg feed^{-1}, ranging from 72 to 81% of nominal concentrations. Corresponding ADDs were 0, 30, 52, 66, 59, 77, 95, and 74mg PFOA kg body weight^{-1} d^{-1}. It was not possible to calculate an ADD for the greatest feed concentration because all birds died prior to day 5. In the PFOS + PFOA trial, analyzed concentrations were 0 + 0, 43 + 45, 58 + 62, 74 + 79, 92 + 90, 104 + 102, 134 + 145, 155 + 164, and 296 + 292mg PFOS + PFOA kg feed^{-1} and ranged from 68 to 88% of nominal concentrations. Corresponding ADDs were 0 + 0, 8.5 + 8.7, 11 + 12, 15 + 16, 22 + 22, 20 + 19, 20 + 22, 25 + 27, and 32 + 31mg PFOS + PFOA kg body weight^{-1} d^{-1}. Analyzed concentrations of PFOS in the 3M AFFF trial (referred to as AFFF PFOS) were 0, 73, 164, 213, 325, 465, 499, 634, and 1399mg kg feed–1, ranging from 104 to 166% of nominal concentrations. Corresponding ADDs were 0, 15, 38, 50, 75, 125, 156, and 186mg PFOS kg body weight^{-1} d^{-1}. An ADD for the greatest feed concentration was not calculated because all birds died prior to day 5. Components of the 3M AFFF and their relative proportions were PFOS (91%), PFHxS (7%), perfluorobutane sulfonate (1%), and PFOA (1%). Feed containing the Ansul AFFF was analyzed for the presence of 6:2 FtTAoS, but because an authentic standard is not available, the concentration could not be determined (Hites and Jobst 2018). The ADDs of 6:2 FtTAoS corresponding to the nominal dietary concentrations of 0, 1.5, 2.9, 5.8, 12, 23, 46, 92, and 184mg 6:2 FtTAoS kg feed^{-1} were 0, 1.9, 3.3, 6.8, 14, 27, 52, 100, and 192mg kg body weight^{-1} d^{-1}.

4.1.2 LD50s, LC50s, LT50s

Exposure of Japanese quail to PFOS and PFOA singly or in combination in the feed for 5 d resulted in mortality as did exposure to AFFF PFOS. Exposure to Ansul AFFF did not result in any mortalities. The greatest dietary concentration resulting in no mortalities through d 8 in the PFOS trial was 91 mg kg feed^{-1}, 162 mg kg feed^{-1} for PFOA, 74 + 79 mg kg feed^{-1} for the PFOS + PFOA trial and 73 mg PFOS kg feed^{-1} for the 3M AFFF trial. At greater concentrations in each trial, typical clinical signs included lethargy, drooped wings, incoordination, and recumbency. The number of live birds at the different dietary concentrations of PFOS, PFOA, PFOS + PFOA, and AFFF PFOS at 5, 8, 15, and 23 d are presented in Table 3, with mortality curves in Figure 1. After day 8 (3 d after being switched to clean feed), there were only 2 treatment related mortalities recorded, both in the PFOS + PFOA trial. One bird in the 155 + 164mg PFOS + PFOAkg feed–1 group died on day 11 and one bird in the 74 mg + 79 mg PFOS + PFOA kg feed^{-1} group died on day 15. The lowest feed concentrations resulting in no survivors were 471 mg PFOS kg feed^{-1}, 590 mg PFOA kg feed^{-1}, 296 + 292 mg PFOS + PFOA kg feed^{-1} and 634 mg AFFF PFOS kg feed^{-1}. Doses of PFOS, PFOA, PFOS + PFOA and AFFF PFOS that resulted in 50% lethality (Table 4) ranged from 38 to 145 mg kg body weight^{-1} d^{-1} and dietary concentrations that resulted in 50% lethality ranged from 389 to 550 mg kg feed^{-1}. The time

required for 50% (based on both day 5 and day 8 values) in days (LT50) at each dietary concentration of PFOS, PFOA, PFOS + PFOA and AFFF PFOS is presented in Table 5. The range of LT values on day 8 across dietary concentrations was similar for each treatment (PFOS: 5.7 to 3.4 d; PFOA: 5.9 to 3.8 d; PFOS + PFOA: 7.4 to 4.0 d; AFFF PFOS: 7.6 to 4.3 d).

Table 3. Number of live Japanese quail (*Coturnix japonica*) exposed to dietary PFOS, PFOA, PFOS + PFOA, 3M AFFF or Ansul AFFF for 5 d at benchmark periods during the 23-d studies

	Day of study				
Analyzed dietary concentration (mg kg feed^{-1})	0	5	8	15*	23*
PFOS					
0	20	20	20	10	10
62	20	20	20	9	9
91	20	20	20	10	10
216	20	16	3	3	3
471	20	7	0	0	0
654	20	2	0	0	0
866	20	1	0	0	0
920	20	1	0	0	0
1955	20	0	0	0	0
PFOA					
0	20	20	20	10	10
162	20	20	20	10	10
262	20	19	17	8	8
368	20	17	7	3	3
447	20	10	3	0	0
590	20	5	0	0	0
814	20	3	1	0	0
926	20	1	1	0	0
1208	20	0	0	0	0
PFOS + PFOA[a]					
0 + 0	20	20	20	10	10
43 + 45	20	20	20	10	10
58 + 62	20	20	20	10	10
74 + 79	20	20	20	10	9
92 + 90	20	20	18	9	9
104 + 102	20	19	13	6	6
134 + 145	20	19	2	2	2
155 + 164	20	19	5	2	2
296 + 292	20	2	0	0	0
3M AFFF[b]					
0	20	20	20	10	10
73	20	20	20	9	9
164	20	20	13	7	7

213	20	18	10	4	4
325	20	16	5	2	2
465	20	15	2	1	1
499	20	9	1	1	1
634	20	2	0	0	0
1399	20	0	0	0	0
Ansul AFFF[c]					
0	20	20	20	10	10
9	20	20	20	10	10
18	20	20	20	10	10
35	20	20	20	10	10
73	20	20	20	10	10
140	20	20	20	10	10
279	20	20	20	10	10
559	20	20	20	10	10
1118	20	20	20	10	10

[*]Half of the birds were sacrificed at day 15, so the maximum number of birds would be 10.
[a]Dietary concentrations are expressed as the sum of PFOS and PFOA.
[b]Dietary concentrations are based on PFOS concentrations (analysis indicated that 3M AFFF was 91% PFOS).
[c]Dietary concentrations are nominal concentrations of 6:2 fluorotelomer thioamido sulfonate (FtTAoS).
PFOS = perfluorooctane sulfonate; PFOA = perfluorooctanoic acid; AFFF = aqueous film forming foam.

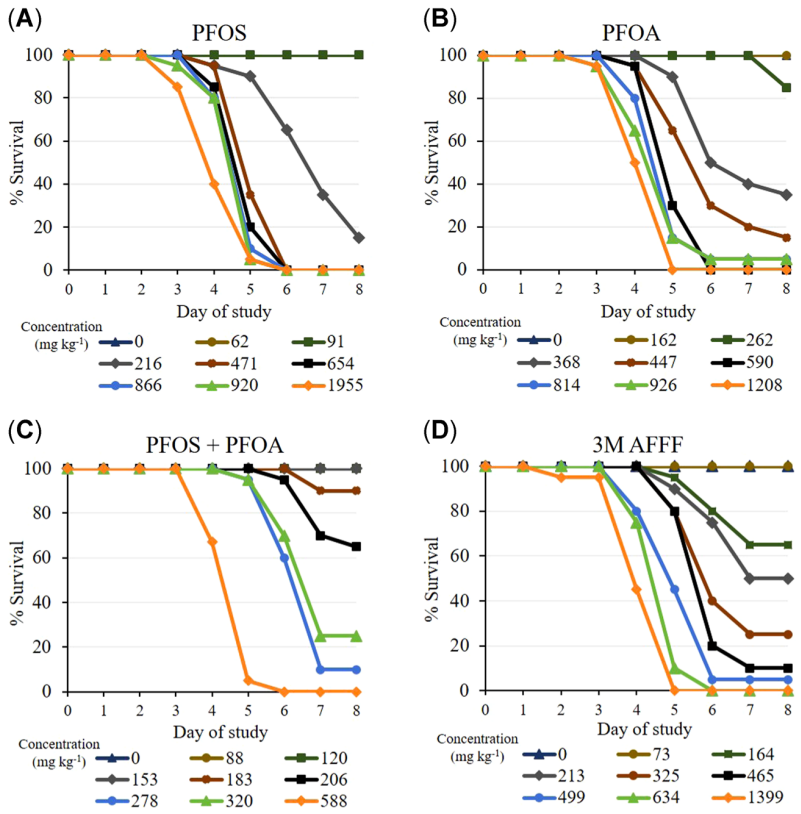


Figure 1. Effect of perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), PFOS + PFOA, and 3M aqueous film-forming foam (AFFF) on offspring survivability at day 8 following a 5-d dietary exposure.
(A) Day 8 survivability of Japanese quail (Coturnix japonica) chicks at each concentration of dietary PFOS after a 5-d dietary exposure. (B) Day 8 survivability of Japanese quail chicks at each concentration of dietary PFOA after a 5-d dietary exposure. (C) Day 8 survivability of Japanese quail chicks at each concentration of dietary PFOS + PFOA after a 5-d dietary exposure. (D) Day 8 survivability of Japanese quail chicks at each concentration of PFOS provided by 3M AFFF after a 5-d dietary exposure.

Table 4. Estimates of ADD50 and LC50 for Japanese quail chicks exposed to dietary PFOS, PFOA, PFOS + PFOA, or AFFF for 5 d

	Effect Metric	Day	Estimated LC50	95% CI	Slope
PFOS	ADD50 (mg kg body wt^{-1} d^{-1})	5	38	34-43	7.7
		8	--[a]	--	--
	LC50 (mg kg feed^{-1})	5	351	275-450	4.1
		8	--	--	--
PFOA	ADD50 (mg kg body wt^{-1} d^{-1})	5	68	63-74	11
		8	49	49-50	525
	LC50 (mg kg feed^{-1})	5	496	427-575	5.9
		8	323	294-355	18

PFOS + PFOA[b]	ADD50 (mg kg body wt^{-1} d^{-1})	5	55	51-59	14
		8	68	45-105	-1.9
	LC50 (mg kg feed^{-1})	5	398	339-468	14
		8	159	130-190	4.2
3M AFFF[c]	ADD50 (mg kg body wt^{-1} d^{-1})	5	130	103-164	3.8
		8	39	28-53	2.8
	LC50 (mg kg feed^{-1})	5	467	390-559	4.8
		8	109	78-154	2.6

[a] Insufficient data to calculate
[b] Concentration and dose are expressed as the sum of PFOS and PFOA.
[c] Concentration and dose are based on PFOS concentration in the diet (analysis indicated that 3M AFFF was 91% PFOS).
ADD50 = average daily dose resulting in 50% lethality; AFFF = aqueous film-forming foam; CI = confidence interval; LC50 = dietary concentration resulting in 50% lethality; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonate.

Table 5. LT50 in Japanese quail (Coturnix japonica) chicks exposed to dietary PFOS, PFOA, PFOS + PFOA, or AFFF for 5 d

Analyzed dietary concentration (mg kg feed^{-1})	ADD (mg kg body wt^{-1} d^{-1})	Day 5 LT50	95% CI	Slope	Day 8 LT50	95% CI	Slope
PFOS							
0	0	--[a]	--	--	--	--	--
62	11	--	--	--	--	--	--
91	17	--	--	--	--	--	--
216	36	6.4	5.2-7.8	8.3	6.1	5.6-6.8	9.2
471	57	4.6	4.3-5.0	20	4.6	4.3-5.0	20
654	49	4.1	3.7-4.5	13	4.1	3.7-4.5	13
866	58	4.0	3.6-4.4	15	4.0	3.6-4.4	15
920	45	3.8	3.5-4.3	13	3.8	3.5-4.2	13
1955	102	3.4	2.9-3.9	7.7	3.4	2.9-3.8	7.7
PFOA							
0	0	--	--	--	--	--	--
162	30	--	--	--	--	--	--
262	52	--	--	--	--	--	--
368	66	--	--	--	6.3	5.7-6.9	10
447	59	5.0	4.3-5.8	8.69	5.1	4.5-5.7	7.9
590	77	4.6	4.3-5.0	21	4.6	4.3-5.0	21
814	95	4.2	3.8-4.6	13	4.2	3.8-4.6	11
926	74	3.8	3.4-4.2	12	3.8	3.4-4.2	12
1208	--	3.8	3.5-4.1	17	3.8	3.5-4.1	17
PFOS + PFOA[b]							
0/0	0 + 0	--	--	--	--	--	--
43/45	8.5 + 8.7	--	--	--	--	--	--

58/62	11 + 12	--	--	--	--	--	--
74/79	15 + 16	--	--	--	--	--	--
92/90	22 + 22	--	--	--	--	--	--
104/102	20 + 19	--	--	--	7.5	6.7-8.3	11
134/145	20 + 22	--	--	--	6.1	5.7-6.6	15
155/164	25 + 27	--	--	--	6.4	5.9-7.0	13
296/292	32 + 31	4.0	3.6-4.4	15	4.0	3.6-4.4	15
3M AFFF[c]							
0	0	--	--	--	--	--	--
73	15	--	--	--	--	--	--
164	38	--	--	--	7.7	7.1-8.3	16
213	50	--	--	--	6.8	6.2-7.5	10
325	75	--	--	--	5.9	5.4-6.5	10
465	125	--	--	--	5.5	5.0-6.0	11
499	156	4.9	4.3-5.5	10	4.7	4.2-5.3	9.0
634	186	4.3	4.0-4.7	20	4.3	4.0-4.7	20
1399	--	4.4	3.6-5.3	5.5	4.4	3.8-5.1	5.5

[a] Insufficient data to calculate.
[b] Concentration and dose are expressed as the sum of PFOS plus PFOA.
[c] Concentration and dose are based on PFOS concentration in the diet (analysis indicated that 3M AFFF was 91% PFOS).
ADD = average daily dose (mg kg body wt^{-1} d^{-1}); AFFF = aqueous film-forming foam; CI = confidence interval; LT50 = time (days) at which 50% of the subjects died

4.1.3 Feed intake and body weight

Consumption of treated feed in the PFOS, PFOA, and PFOS + PFOA trials decreased numerically in a dose-related manner as did body weights while in the AFFF trials, feed intake was numerically less in birds exposed to AFFF PFOS, but not related to dose and only marginally reduced at higher feed concentrations of 6:2 FtTAoS provided by Ansul AFFF (Table 6).

4.1.4 Feed intake during the 5-d exposure period.

For the 5 d that birds were on treated feed, intake ranged from 90 to 11% of control intake for birds exposed to PFOS, from 94 to 15% for birds fed diets containing PFOA, 99 to 18% for birds fed diets containing PFOS + PFOA, 86 to 51% for birds exposed to AFFF PFOS and 103 to 86% for fed diets containing 6:2 FtTAoS provided by Ansul AFFF (Table 6).

4.1.5 Feed intake from d 6 to d 23.

After birds were provided control feed at the end of d 5, feed intake increased in surviving birds from d 6 to d 23 but generally continued to be less than intake of the control group in a dose-related manner in the PFOS, PFOA and PFOS + PFOA trials, less than control intake in the 3M AFFF trial, but not related to dose, and generally greater than control intake in the Ansul AFFF trial. In the PFOS trial, feed intake from d 6 to d 23 ranged from 90 to 64% of control intake, 98 to 59% for the PFOA trial, 102 to 61% for the PFOS + PFOA trial, 96 to 73% for the 3M AFFF trial and 118 to 99% for the Ansul AFFF trial (Table 6).

4.1.6 Body weights.

Mean body weights were significantly lower compared to controls at the end of d 5 beginning at feed concentrations of 62 mg PFOS kg feed^{-1}, 262 mg PFOA kg feed^{-1}, 43 + 45 mg PFOS + PFOA kg feed^{-1} and 73 mg AFFF PFOS kg^{-1}. Body weights were not significantly affected by consumption of feed containing 6:2 FtTAoS provided by Ansul AFFF. On d 23, body weights of surviving birds were significantly lower compared to controls at 91 mg PFOS kg feed^{-1} and greater, 262 mg PFOA kg feed^{-1} and greater, 155 + 164 mg PFOS + PFOA kg feed^{-1} and 164 mg AFFF PFOS kg feed^{-1} and greater (Table 6).

4.1.7 Liver weight

Exposure to PFOS and/or PFOA and AFFF PFOS induced changes in absolute and relative liver weights while exposure to 6:2 FtTAoS provided by Ansul AFFF had no effect on liver weight (Table 6).

Table 6. Feed intake and mean body and liver weight of Japanese quail (*Coturnix japonica*) chicks exposed to dietary PFOS, PFOA, PFOS + PFOA, 3M AFFF or Ansul AFFF

Analyzed dietary concentration (mg kg^{-1})	ADD (d 5) (mg kg bw^{-1} d^{-1})	Treated feed intake[a] (g bird^{-1} d^{-1}) d 0 - 5	Untreated feed intake[a] (g bird^{-1} d^{-1}) d 6 - 23	Mean body weight[b] (g) day 8 (SE)	Mean body weight[b] (g) d 23 (SE)	Liver weight (g) d8 (95%CI)	Liver weight (g) d23 (95%CI)	Relative Liver weight (g) d8 (95%CI)	Relative Liver weight (g) d23 (95%CI)
PFOS									
0	0	11.4	16.5	80 (1.14)	129 (3.53)	2.05 (1.90-2.20)	3.03 (2.68-3.38)	2.59 (2.48-2.70)	2.35 (2.15-2.55)
62	11	10.3	14.9	70** (1.12)	125 (3.81)	1.70**(1.52-1.89)	2.92 (2.56-3.29)	2.48 (2.31-2.65)	2.32 (2.17-2.47)
91	17	8.31	13.8	53** (1.79)	116** (0.875)	1.31**(1.25-1.37)	2.80 (2.57-3.03)	2.41 (2.17-2.65)	2.42 (2.23-2.61)
216	36	4.55	10.5	33** (1.67)	102** (3.21)	--	2.38 (1.43-3.33)	--	2.32 (1.51-3.13)
471	57	2.76	0	--	--	--	--	--	--
654	49	1.75	0	--	--	--	--	--	--
866	58	1.56	0	--	--	--	--	--	--
920	45	1.23	0	--	--	--	--	--	--
1955	102	1.21	0	--	--	--	--	--	--
	NOAEL			--	--	--	36	17	36
	LOAEL			11	17	11	--	--	--
PFOA									
0	0	11.3	17.0	78 (3.18)	127 (2.43)	2.06 (1.92–2.21)	2.99 (2.52–3.46)	2.68 (2.56–2.79)	2.34 (2.02–2.65)
162	30	10.6	16.7	73 (3.18)	121 (2.27)	2.19 (2.00–2.38)	2.81 (2.62–3.00)	2.97 (2.75–3.20)	2.33 (2.22–2.44)
262	52	9.34	14.6	53** (3.26)	115* (2.77)	2.46 (1.99–2.93)	2.70 (2.34–3.06)	4.20** (3.80–4.60)	2.35 (2.01–2.69)
368	66	6.07	13.5	49** (5.03)	112* (7.51)	2.11 (1.38–2.85)	3.16 (2.28–6.10)	4.53** (2.30–6.76)	2.79 (0.905–4.68)
447	59	3.78	8.69[d]	54* (8.21)	--	2.33 (0.867–3.80)	--	4.28** (3.55–5.02)	--
590	77	3.10	0	50 (14.2)	--	--	--	--	--
814	95	3.12	10.0[d]	34** (14.2)	--	1.78	--	3.57	--
926	74	1.78	0	--	--	--	--	4.49	--
1208	---	1.68[e]	0	--	--	--	--	--	--
	NOAEL			30	30	66	66	30	66
	LOAEL			52	52	--	--	52	--
PFOS + PFOA[f]									
0/0	0 + 0	11.1	16.3	77 (1.66)	123 (1.86)	2.01 (1.88–2.14)	2.75 (2.35–3.16)	2.63 (2.52–2.74)	2.23 (1.92–2.53)
43/45	8.5 + 8.7	11.0	16.6	70* (1.66)	123 (1.98)	1.80 (1.68–1.92)	2.92 (2.64–3.20)	2.62 (2.55–2.69)	2.37 (2.15–2.59)
58/62	11 + 12	9.77	15.6	66** (1.66)	121 (3.44)	1.74 (1.56–1.92)	2.82 (2.46–3.17)	2.64 (2.44–2.85)	2.31 (2.12–2.51)
74/79	15 + 16	8.43	14.5	54** (1.66)	116 (3.34)	1.56** (1.36-1.76)	2.79 (2.62–2.96)	3.03 (2.63–3.43)	2.40 (2.29–2.51)
92/90	22 + 22	8.75	13.3	48** (1.75)	118 (2.51)	1.46** (1.19-1.73)	2.99 (2.71–3.27)	3.22* (2.64–3.80)	2.54 (2.31–2.77)
104/102	20 + 19	5.96	12.7	43** (2.06)	114 (1.25)	1.57* (1.36–1.78)	2.64 (2.34–2.94)	3.78** (3.17–4.39)	2.32 (2.10–2.55)
134/145	20 + 22	3.91	12.5	48** (5.25)	111 (2.85)	—	2.59 (0.00–7.14)	—	2.33 (0.00–5.67)
155/164	25 + 27	4.24	10.0	39** (3.71)	103* (8.45)	1.80 (0.000–6.17)	2.66 (0.080–5.24)	4.03** (3.05–5.00)	2.58 (2.39–2.77)
296/292	32 + 31	2.02	0	--	0	--	--	--	--
	NOAEL			--	20 + 22	11 + 12	25 + 27	15 + 16	25 + 27
	LOAEL			8.5 + 8.7	25 + 27	15 + 16	--	22 + 22	--
3M AFFF[g]									
0	0	12.5	17.4	81 (1.59)	128 (2.59)	2.02 (0.109)[j]	2.74 (0.141)[h]	2.49 (2.21–2.78)	2.13 (1.83–2.43)
73	15	10.8	16.7	67** (1.59)	125 (2.73)	1.77 (0.109)	2.99 (0.149)	2.64 (2.39–2.89)	2.39 (2.19–2.59)
164	38	8.22	14.3	50** (1.97)	116* (3.10)	1.14	2.46 (0.169)	2.77	2.11 (1.98–2.24)

213	50	7.09	13.6	39** (2.69)	109** (4.10)	1.30* (0.198)	2.20 (0.223)	3.41** (3.29–3.52)	2.03 (1.65–2.40)
325	75	6.31	16.2	31** (3.56)	99.5** (5.80)	1.05** (0.243)	2.24 (0.316)	3.40* (0.00–8.29)	2.27 (0.00–4.87)
465	125	6.51	12.7	33** (5.03)	96.7** (8.20)	1.18	2.23	3.22	2.31
499	156	7.09	13.2	26** (7.11)	98.5* (8.20)	--	2.66	--	2.70
634	186	6.54	--	22.4** (2.94)	--	--	--	--	--
1399	---	5.52	--	--	--	--	--	--	--
	NOAEL			--	15	15	75	15	75
	LOAEL			15	38	50	--	50	--
Ansul AFFF[h]									
0	0	12.4	15.7	79 (1.26)	121 (3.49)	2.03 (1.89–2.17)	2.50 (2.07–2.93)	2.53 (2.41–2.64)	2.06 (1.78–2.34)
9	1.9	12.8	16.4	78 (1.26)	130 (3.49)	1.89 (1.74–2.04)	3.15 (2.64–3.66)	2.46 (2.34–2.58)	2.41 (2.08–2.74)
18	3.3	12.0	16.4	80 (1.26)	129 (3.49)	2.00 (1.86–2.14)	3.00 (2.18–3.82)	2.57 (2.41–2.72)	2.30 (1.73–2.87)
35	6.8	12.0	16.2	77 (1.26)	122 (3.49)	2.01 (1.87–2.14)	2.47 (2.26–2.67)	2.62 (2.49–2.74)	2.02 (1.86–2.18)
73	14	11.9	18.6	79 (1.26)	124 (3.49)	2.13 (1.95–2.32)	2.81 (2.42–3.20)	2.64 (2.55–2.74)	2.26 (2.00–2.51)
140	27	11.9	16.5	78 (1.26)	126 (3.49)	2.15 (2.00–2.31)	3.05 (2.23–3.86)	2.73 (2.53–2.92)	2.38 (1.85–2.90)
279	52	11.8	16.8	80 (1.26)	133 (3.49)	2.17 (1.99–2.35)	2.62 (2.20–3.03)	2.75 (2.46–3.04)	1.97 (1.69–2.25)
559	100	11.3	15.5	79 (1.26)	127 (3.49)	2.14 (1.98–2.31)	2.90 (2.37–3.43)	2.72 (2.58–2.85)	2.25 (1.97–2.54)
1118	192	10.7	15.7	79 (1.26)	127 (3.49)	2.18 (2.02–2.35)	2.85 (2.48–3.22)	2.76 (2.61–2.90)	2.24 (2.02–2.46)
	NOAEL			192	192	192	192	192	192
	LOAEL			--	--	--	--	--	--

[a]No statistical analysis for feed intake because n = 1 (all birds in a treatment were housed in a single pen and were eating from the same feeder).
[b]Data expressed as mean with standard error below in parentheses.
[c]Half of the surviving birds were euthanized on d 8 for serum and liver analysis.
[d]Feed consumption through day 8 when all chicks had died.
[e]All birds were dead by day 5 at the morning check.
[f]Dietary concentrations and ADDs are for PFOS/PFOA.
[g]Dietary concentrations and ADDs are based on PFOS concentration in the diet (analysis indicated that 3M AFFF was 91% PFOS).
[h]Dietary concentrations and ADDs are based on nominal 6:2 FtTAoS concentrations.
[j]Absolute liver weights in the 3M AFFF were normally distributed; thus, data are expressed as mean and SE (in parentheses)
*$p < 0.05$.
** $p < 0.01$.

4.1.8 PFOS trial.

Absolute liver weight of birds exposed to PFOS that were necropsied on d 8 were significantly less than liver weights of controls beginning at 62 mg kg feed^{-1}, but there were no differences in relative liver weights. Because of mortalities, no birds were necropsied at d 8 in dose groups exposed to greater concentrations of PFOS. There were no differences in absolute or relative liver weights in birds that were necropsied on d 23.

4.1.9 PFOA trial.

In birds exposed to PFOA, there were no differences in absolute liver weight at d 8 or d 23. Relative liver weights were significantly greater compared to controls at dietary concentrations of beginning at 262 mg kg feed^{-1} in birds necropsied on d 8 but there were no differences in birds necropsied on d 23.

4.1.10 PFOS + PFOS trial.

Absolute and relative liver weights in birds exposed to a combination of PFOS and PFOA were different compared to controls only at d 8. Absolute liver weights were significantly less compared to controls beginning at dietary concentrations of 74 + 79 mg PFOS + PFOA kg feed^{-1} (ADD = 15 + 16 mg kg body weight^{-1} d^{-1}), and relative liver weights were significantly greater compared to controls beginning at dietary concentrations of 92 + 90 mg PFOS + PFOA kg feed^{-1}.

4.1.11 3M AFFF trial.

In birds exposed to AFFF PFOS, absolute liver weights were significantly less, and relative weights were significantly greater compared to controls beginning at dietary concentrations of 213 mg kg^{-1} on d 8. There were no differences in absolute or relative liver weights on d 23.

4.1.12 Serum and liver concentrations

Serum and hepatic concentrations of PFOS, AFFF PFOS and PFOA in birds necropsied on d 8 generally tended to increase with feed concentration to a point, while d 23 concentrations, with some exceptions, were considerably less compared to d 8 concentrations and did not appear to be related to initial feed concentration (Figure 2).

4.1.13 PFOS trial.

In the PFOS trial, the average concentration of PFOS at d 23 in serum and liver was approximately 21% of the concentration at d 8 (Table 7 and Figure 2). The serum to liver ratio of PFOS was approximately 2.2:1 at both d 8 and d 23. The hepatic concentration of PFOS in mortalities averaged 233 ± 11 mg kg^{-1} wet weight.

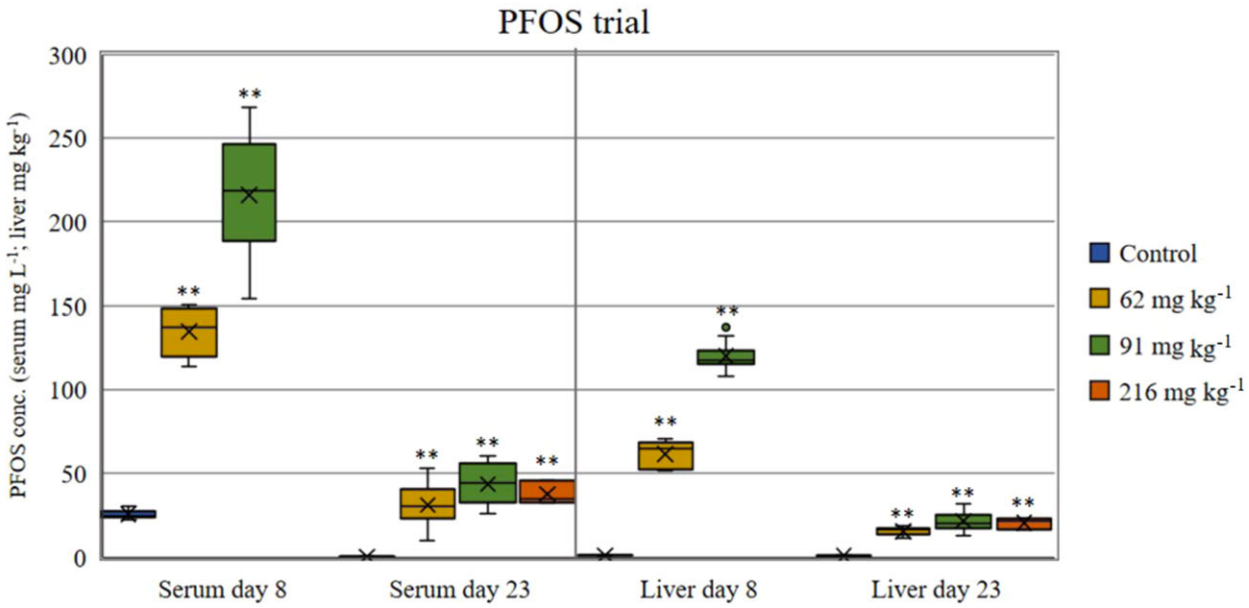


Figure 2. Effect of Dietary PFOS exposure on Serum and Liver Concenrations
The solid line within each box is the median value of the data points. The x within the box locates the mean. The bottom line of the box is the median of the first quartile of the data, whereas the top line of the box is the median of the third quartile. Whiskers extend to the minimum and maximum values. Circles outside of whiskers represent outliers.

Table 7. Day 23 serum of liver concentration as a percent of day 8 concentration and serum to liver raio on days 8 and 23.

	Day 23 concentration as % of day 8 concentration		Serum to liver concentration ratio	
Study	Serum (range)	Liver (range)	Day 8 (range)	Day 23 (range)
PFOS	22 (20–23)	22 (20–25)	2.0 (1.8–2.2)	1.9 (1.8–2.0)
PFOA	1.9 (0.75-3.7)	3.5 (0.59-8.3)	2.1 (1.4-3.6)	1.2 (0.017-1.8)
PFOS + PFOA				
PFOS	19 (14-23)	15 (10-22)	1.3 (1.0-1.4)	1.6 (1.4-1.9)
PFOA	1.9 (0.30-5.0)	1.6 (0.20-5.6)	3.1 (0.30-8.3)	2.2 (1.7-3.4)
3M AFFF[a]	21 (18-24)	15 (7.9-19)	1.3 (0.9-1.6)	2.0 (1.4-2.7)

[a]Serum and liver concentrations are PFOS (analysis indicated that 3M AFFF was 91% PFOS).

4.1.14 PFOA trial.

In the PFOA trial, there was considerable variability in both serum and liver concentrations. On d 8, serum concentrations of PFOA were the same across feed concentrations except for a 2-fold increase at 368 mg kg feed^{-1}and hepatic concentrations of PFOA increased with feed concentration until a decrease at 447 mg kg^{-1} feed. On d 23, serum concentrations ranged from 365% of d 8 concentrations at 162 mg kg^{-1} feed to 76% at 368 mg kg^{-1} feed. Hepatic concentrations varied from 0.6% to 8.3% of d 8 concentrations over the same range of feed concentrations (Figure 3). Similarly, the serum to liver ratio of PFOA varied by feed

concentration at both d 8 (1.4:1 to 3.6:1) and d 23 (1.7:1 to 159:1). The hepatic concentration of PFOA in mortalities averaged 442 ± 15 mg kg^{-1} wet weight.

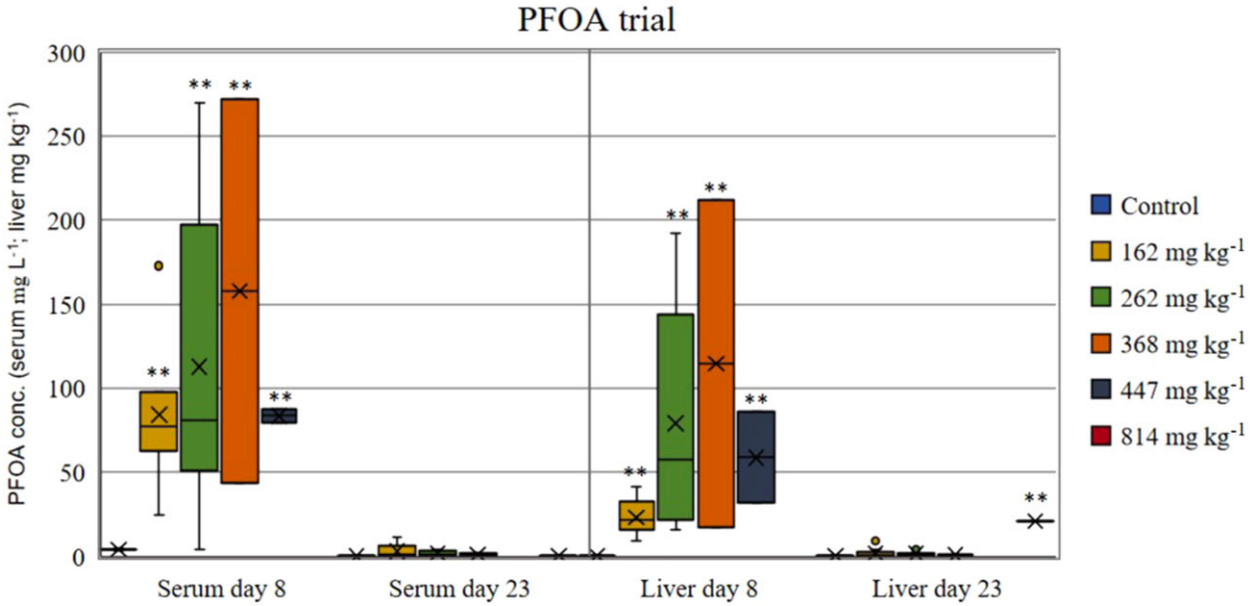


Figure 3. Effect of dietary PFOA exposure on serum and liver PFOA concentrations
The solid line within each box is the median value of the data points. The x within the box locates the mean. The bottom line of the box is the median of the first quartile of the data, whereas the top line of the box is the median of the third quartile. Whiskers extend to the minimum and maximum values. Circles outside of whiskers represent outliers.

4.1.15 PFOS + PFOA trial.

In the PFOS + PFOA trial, serum and hepatic concentrations of both PFOS and PFOA generally increased with increasing feed concentration at d 8 with considerably lower concentrations at d 23 that were not related to feed concentration (Figure 4). On d 23, serum concentrations of PFOS were 19% of d 8 concentrations and hepatic concentrations were 15% of d 8 concentrations while serum and hepatic concentrations of PFOA at d 23 were 1.9 and 1.6% of d 8 concentrations (Table 7 and Figures 4 and 5). The average ratio of PFOS to PFOA in serum at d 8 was 2.3:1 and 45:1 at d 23. In the liver, the ratio of PFOS to PFOA at d 8 was 4.6:1 and 60:1 at d 23. The serum to liver ratio of PFOS at d 8 was 1.3:1 and 1.6:1 at d 23. The serum to liver ratio for PFOA was 3.1:1 at d 8 and 2.2:1 at d 23. The hepatic concentration of PFOS + PFOA in mortalities average 206 ± 12.1 + 120 ± 5.7 mg kg^{-1} wet weight.

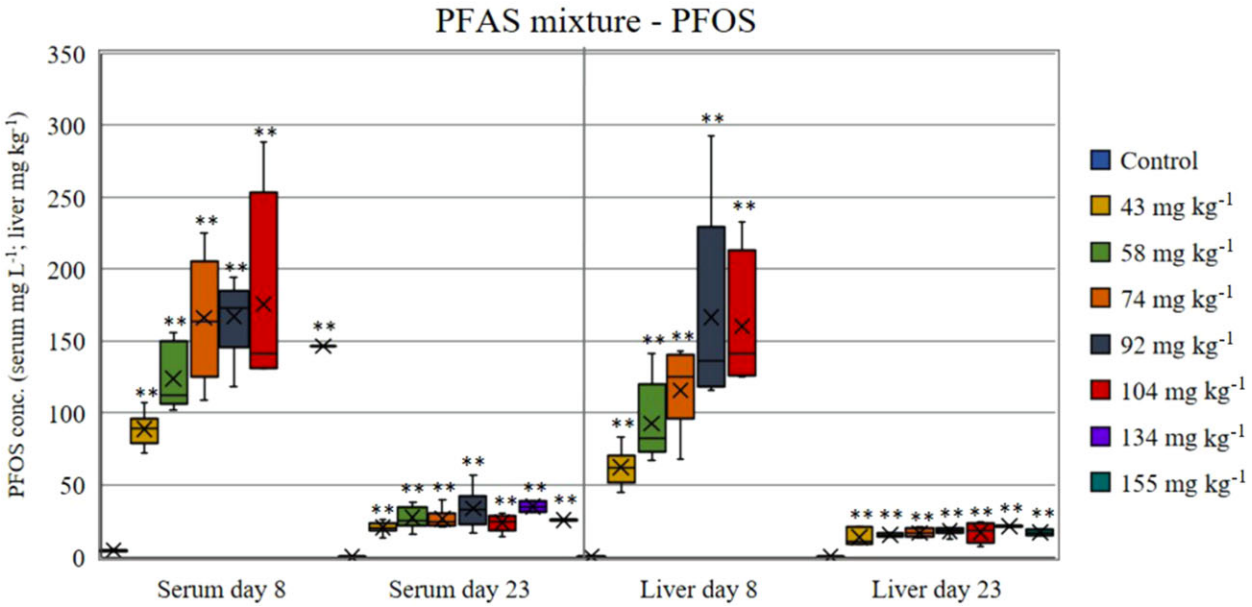


Figure 4. Effect of dietary PFOS + PFOA exposure on serum and liver PFOS concentrations

The solid line within each box is the median value of the data points. The x within the box locates the mean. The bottom line of the box is the median of the first quartile of the data, whereas the top line of the box is the median of the third quartile. Whiskers extend to the minimum and maximum values. Circles outside of whiskers represent outliers. PFAS = poly- and perfluoroalkyl substances.

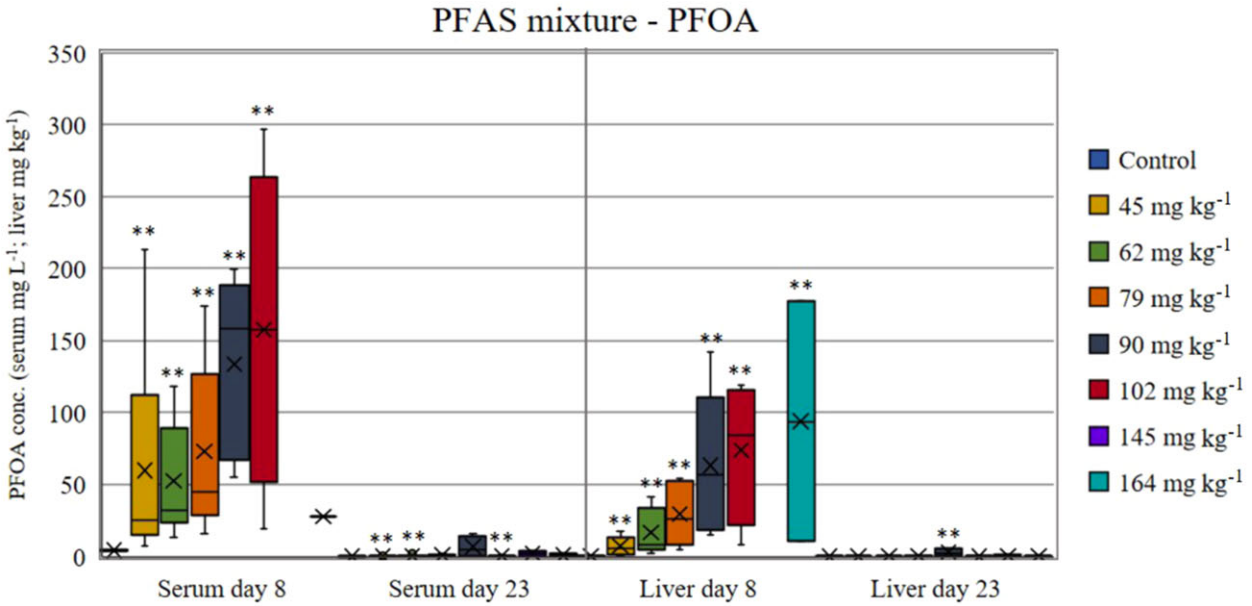


Figure 5. Effect of dietary PFOS + PFOA exposure on serum and liver PFOA concentrations

The solid line within each box is the median value of the data points. The x within the box locates the mean. The bottom line of the box is the median of the first quartile of the data, whereas the top line of the box is the median of the third quartile. Whiskers extend to the

minimum and maximum values. Circles outside of whiskers represent outliers. PFAS = poly- and perfluoroalkyl substances.

4.1.16 3M AFFF Trial.

In the 3M AFFF trial, the concentration of PFOS at d 23 was 21% of the concentration at d 8 in the serum and 15% in the liver (Table 7 and Figure 6). The serum to liver ratio of PFOS was 1.3 at d 8 and 2.0 at d 23. The hepatic concentrations of PFOS in mortalities average 169 ± 7.7 mg kg^{-1} wet weight.

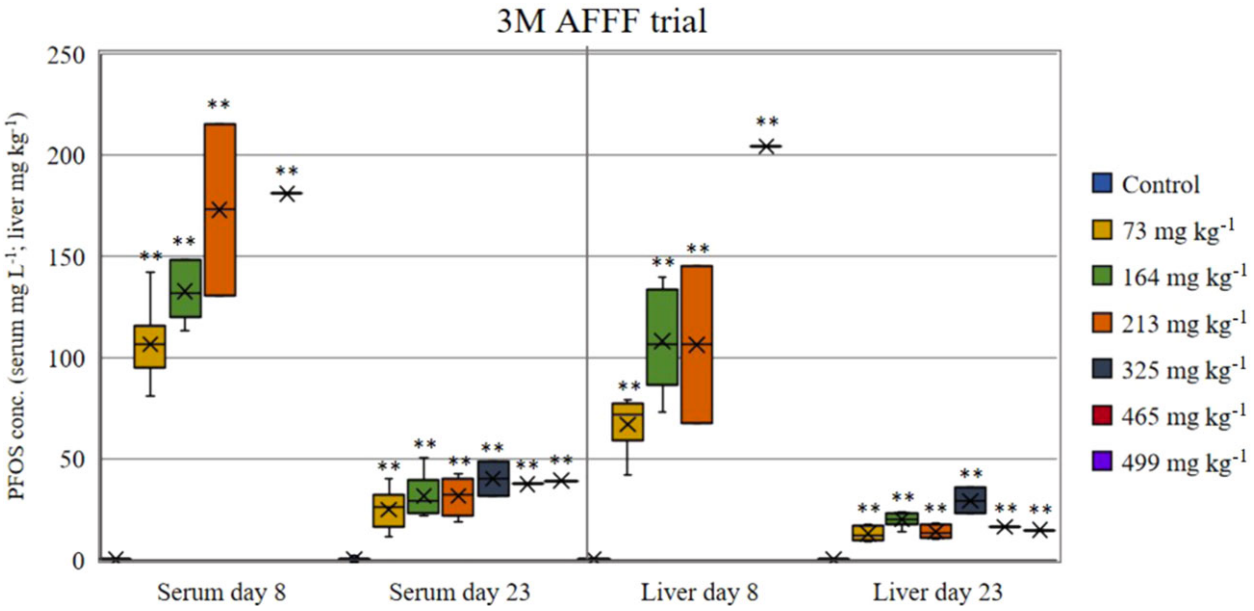


Figure 6. Effect of dietary 3M AFFF exposure on serum and liver PFOS concentrations The solid line within each box is the median value of the data points. The x within the box locates the mean. The bottom line of the box is the median of the first quartile of the data, whereas the top line of the box is the median of the third quartile. Whiskers extend to the minimum and maximum values. Circles outside of whiskers represent outliers. PFAS = poly- and perfluoroalkyl substances.

4.1.17 Discussion

The design of the present study is similar to the USEPA and OECD avian dietary toxicity test guidelines OCSPP 850.2200 (USEPA 2012) and OECD 205 (1991) and mimics the design of the Newsted et al. (2006) study because the latter was the only published study assessing the subacute effects of PFOS in birds that could be used for comparative purposes. The pri- mary difference between the present study design and that of Newsted et al. (2006) is the use of Japanese quail rather than the northern bobwhite and mallard. The OCSPP guidelines state use of the mallard and northern bobwhite is preferred, but the Japanese quail is one of the additional species that may be used (USEPA 2012). The OECD 205 guidelines indicate Japanese quail as one of the recommended species (OECD 1991). We chose to use the Japanese quail because of the existing breeding colony at Michigan State University and because we have had experience using this species in other avian toxicity studies (Cohen-Barnhouse, Zwiernik et al. 2011, Cohen-Barnhouse, Zwiernik et al. 2011). The design of the present study and of the study by Newsted et al. (2006) differs from the OCSPP 850.2200 and OECD 205 guidelines by extending the duration

of the test period to 23 d (5 d ex- posure plus 18 d on clean feed). The extended period on clean feed allowed assessment of reversals in feed con- sumption and body weight change. In addition, in both the present study and the study by Newsted et al. (2006), sam- ples of birds were necropsied on days 8 and 23 and tissues were taken for chemical analysis that allowed assessment of accumulation and depuration of the test compounds. In a recent evaluation of the avian acute oral and subacute dietary toxicity tests for use in regulatory decisions, Hilton et al. (2019) report that results from the subacute dietary toxicity test for 119 pesticides registered with the USEPA between 1998 and 2017 did not identify risks not identified by the acute test, bringing into question its utility. However, the authors state that the subacute test does have utility for compounds with a high potential to bioaccumulate, as is the case for PFOS and, to a lesser extent, PFOA.

Examination of ADD50s and LC50s and other endpoints related to mortality for PFOS, PFOA, and PFOS + PFOA indicated that PFOS was more subacutely toxic than PFOA to Japanese quail. In addition, the toxicities of PFOS and PFOA appeared to be additive; AFFF PFOS was generally less toxic than PFOS and PFOA based on dietary concentrations, and 6:2 FtTAoS provided by Ansul AFFF was not toxic at the concentrations fed. Based on day 5 ADD50 values for PFOS and PFOA (38 mg kg body wt^{-1} d^{-1} for PFOS and 68 mg kg body wt^{-1} d^{-1} for PFOA), the relative potency of PFOA compared to PFOS is 0.56 (Table 4). The day 5 ADD50 value for PFOS + PFOA was approximately 28 mg PFOS + 28 mg PFOA kg body $weight^{-1}$ d^{-1}. If it is assumed that the relative toxicity of PFOA is approximately half of PFOS in Japanese quail, then the combination of 28 mg PFOS and 28 mg PFOA kg body $weight^{-1}$ d^{-1} would be equivalent to 28 + 14 or 42 mg PFOS kg body $weight^{-1}$ d^{-1}, which is close to the PFOS day 5 ADD50 value of 38 mg kg body $weight^{-1}$ d^{-1}. The day 5 LC50 for PFOS (351 mg kg $feed^{-1}$) was 0.71 the value for PFOA (496 mg kg $feed^{-1}$; Table 4). The day 5 LC50 value for PFOS + PFOA was 199 mg PFOS + 199 mg PFOA kg body $weight^{-1}$ d^{-1}. If the concentration of PFOA is multiplied by 0.7, the combination of PFOS + PFOA would be equivalent to 199 + 139 or 338 mg PFOS kg $feed^{-1}$, which approximates the day 5 LC50 value for PFOS of 351 mg kg $feed^{-1}$. The ADDs for the greatest dietary concentrations resulting in no mortalities show the same rela- tionships: 17 mg PFOS, 30 mg PFOA, and 15 + 16 mg PFOS + PFOA kg body $weight^{-1}$ d^{-1} (equivalent to approximately 23 mg PFOS kg body wt^{-1} d^{-1}). Comparing the toxicity of PFOS, PFOA, and AFFF PFOS, the ADDs for the greatest concentrations re- sulting in no mortalities were equivalent for PFOS and AFFF PFOS (17 and 15 mg kg body wt^{-1} d^{-1}) and less than the ADD for PFOA (30 mg PFOA kg body wt^{-1} d^{-1}). However, the day 5 ADD50 for AFFF PFOS (130 mg kg body wt^{-1} d^{-1}) was >3-fold greater than the day 5 ADD50 for PFOS (38 mg kg body wt^{-1} d^{-1}) and approximately 2-fold greater than the ADD50 for PFOA (68 mg kg body wt^{-1} d^{-1}). Although these data suggest that AFFF PFOS is less toxic than PFOS as well as PFOA, these discrepancies in doses are in part due to the greater feed consumption of 3M AFFF birds compared with PFOS and PFOA birds at similar di- etary concentrations. If average daily feed consumption at the concentrations resulting in mortality for the 3 trials through day 5 is compared, birds exposed to AFFF PFOS had an average daily feed intake that was >3-fold greater compared to PFOS birds and approximately 2-fold greater compared to PFOA birds (2.2, 4.1, and 7.0 g d^{-1}). Feed palatability may have been a factor contributing to differences in feed intake between the 3 trials.

As with mortality endpoints, comparison of ADDs corresponding to changes in feed consumption and body weight indicated PFOS was more toxic than PFOA, the toxicities of PFOS and PFOA were additive, AFFF PFOS was less toxic than PFOS and 6:2 FtTAoS provided by Ansul AFFF was not toxic at the concentrations fed (Table 3). The decreases in feed consumption and body weight caused by exposure to PFOS and PFOA were dose-related while the decreases caused by exposure to AFFF PFOS were not. The lowest observed adverse effect levels (LOAELs) for decrease in body weight at d 5 were lowest for PFOS (62 mg kg feed^{-1}; ADD = 11 mg kg body weight^{-1} d^{-1}), greatest for PFOA (262 mg kg feed^{-1}; ADD = 52 mg kg body weight^{-1} d^{-1}) and intermediate for the combination of PFOS and PFOA (43 + 45 mg kg feed^{-1}; ADD = 8.5 + 8.7 mg kg body weight^{-1} d^{-1}) and AFFF PFOS (73 mg kg feed^{-1}; ADD = 15 mg kg body weight^{-1} d^{-1}). Based on body weight on d 23, birds in the PFOS trial continued to be more adversely affected compared to birds exposed to PFOA while birds in the combination and 3M AFFF trials had LOAELs that were intermediate. The LOAEL for PFOS was 91 mg kg feed^{-1} (ADD = 17 mg kg body weight^{-1} d^{-1}), 262 mg kg feed^{-1} (ADD = 52 mg kg body weight^{-1} d^{-1}) for PFOA, 155 + 164 mg kg feed^{-1} (ADD = 25 + 27 mg kg body weight^{-1} d^{-1}) for PFOS + PFOA and 164 mg kg feed^{-1} (ADD = 38 mg kg body weight^{-1} d^{-1}) for AFFF PFOS. As with mortality endpoints, average feed consumption at concentrations that resulted in significant decreases in body weight in birds exposed to PFOS or PFOA was approximately half of that of birds exposed to PFOS + PFOA or AFFF PFOS.

There were decreases in absolute liver weight and increases in relative liver weight in Japanese quail exposed to PFOS, AFFF PFOS, and/or PFOA only at day 8 (Table 6). Although the range in percentage decreases and increases in weight (in excess of 10%) suggests biological significance, because the tissues were not examined for pathology, a definitive statement is not possible. As with other endpoints, PFOS was most potent at inducing the decrease in absolute liver weight (LOAEL = 11 mg kg body wt^{-1} d^{-1}) compared to PFOS + PFOA (LOAEL = 15 + 16 mg kg body wt^{-1} d^{-1}) and AFFF PFOS (LOAEL = 50 mg kg body wt^{-1} d^{-1}). Dietary concentrations of PFOA were not sufficient to cause a decrease in absolute liver weights. Exposure to PFOS did not cause an increase in relative liver weights as did the other treatments, indicating that the weight loss induced by PFOS was severe enough to cause a proportional decrease in liver weight. The lowest ADD associated with an increase in relative liver weight was 22 mg PFOS + 22 mg PFOA kg body weight^{-1} d^{-1}. The ADDs for increased relative liver weight were equivalent for PFOA and AFFF PFOS (52 and 50 mg kg body wt^{-1} d^{-1}).

In general, both PFOS (PFOS trial and AFFF PFOS) and PFOA accumulated in both serum and liver of exposed Japanese quail as feed concentration increased through d 5 (Figures 2 – 6). It has been shown that the tissue distribution of both PFOS and PFOA is determined by their ability to bind to proteins and that serum/plasma, kidney and liver are the predominant sites of accumulation in a variety of species (Han, Snow et al. 2003, Jones, Hu et al. 2003, Kennedy, Butenhoff et al. 2004, Conder, Hoke et al. 2008, Yeung, Loi et al. 2009, Yoo, Guruge et al. 2009, Huang, Dzierlenga et al. 2019). From d 8 to d 23, PFOS and AFFF PFOS concentrations in both serum and liver decreased by approximately 80% and PFOA concentrations decreased by 97%, with the exception of serum PFOA concentrations in the PFOA trial that generally increased during depuration (Figures 2, 3, and 6). It is not surprising that the general decline in PFOA concentrations from d 8 to d 23 (98%) was greater than the decline in PFOS (80%) over the same time period in that there are numerous reports of greater retention of PFOS compared to PFOA in both avian and mammalian species (Butenhoff, Kennedy et al. 2004, Lau, Butenhoff et al. 2004,

Conder, Hoke et al. 2008, Yeung, Loi et al. 2009, Yoo, Guruge et al. 2009). Examination of serum and liver PFOS and PFOA concentrations from the PFOS + PFOA trial in the present study support the greater retention of PFOS compared to PFOA in that despite the equal concentrations of the two chemicals in the feed, concentrations of PFOS at d 8 were nearly double the concentration of PFOA in the serum and almost 5-fold greater in the liver. At d 23, the ratios of PFOS to PFOA in the serum and liver were 44:1 and 64:1. The concentrations of PFOS in the serum was consistently greater than hepatic concentrations resulting in serum to liver ratios ranging from 1.2:1 to 2.1:1. The serum to liver ratios for PFOA were quite variable and generally greater than serum to liver ratios for PFOS, indicating preferential accumulation of PFOA in the serum compared to the liver. Yeung et al (2009) reported that PFOA accumulated more in blood and kidney compared to the liver during both the exposure and depuration periods in chickens dosed with a mixture of PFOS, perfluorodecanoate (PFDA) and PFOA.

In a study very similar to the present study, Newsted et al. (2006) evaluated the acute oral toxicity of a 3M production lot of potassium perfluorooctane sulfonate PFOS (86.9%) in juvenile mallards (*Anus platyrhynchos*) and northern bobwhites (*Colinus virginianus*). Ten-d-old birds were provided feed containing PFOS (8.7 to 1125 mg PFOS kg feed^{-1}) for 5 d and then untreated feed for 17 d. The LD50 was determined to be 150 mg kg body weight^{-1} d^{-1} (cumulative dose of 750 mg kg body weight^{-1} over the 5-d period) for mallards and 61 mg kg body weight^{-1} d^{-1} (cumulative dose of 305 mg kg body weight^{-1}) for bobwhites. The LD50s for PFOS and AFFF PFOS in Japanese quail in the present study (37.6 and 149 mg kg body weight^{-1} d^{-1}; cumulative doses of 188 and 745 mg kg body weight^{-1}) are approximately 0.6 and 2.4-fold the LD50 reported for northern bobwhites. While dependent on experimental design, the greatest dietary concentrations of PFOS and AFFF PFOS) that resulted in no mortalities were similar for Japanese quail (91 and 73 mg kg feed^{-1}; cumulative doses of 85 and 75 mg kg body weight^{-1}) and northern bobwhites (70 mg PFOS kg feed^{-1}; cumulative dose of 119 mg kg body weight^{-1}). The d 5 LC50 reported by Newsted et al. (2006) for mallards was 1002 mg PFOS kg feed^{-1} and 319 mg PFOS kg feed^{-1} for northern bobwhites, which is similar to the LC50 values of 389 mg PFOS and 506 mg AFFF PFOS kg feed^{-1} calculated for Japanese quail. The lowest LT50 values reported by Newsted at al. (2006) for mallards and northern bobwhites were 4.97 and 3.06 d at a dietary concentration of 1125 mg PFOS kg feed^{-1}. In the present study, LT50s for PFOS at dietary concentrations of 920 and 1955 mg kg feed^{-1}, which bracket the greatest concentration used by Newsted et al. (2006) were 3.83 and 3.35 d. For AFFF PFOS, LT50 values at dietary concentrations of 634 and 1399 mg kg feed^{-1} were 4.32 and 4.36 d. The LOAELs for significant decreases in body weight of mallards and northern bobwhites at d 5 occurred at dietary concentrations of 281 mg PFOS kg feed^{-1} (74.2 mg kg body weight^{-1} d^{-1}) and 141 mg PFOS kg feed^{-1} (44.7 mg kg body weight^{-1} d^{-1}). In the present study, the PFOS LOAEL based on 5-d body weight was 62 mg kg feed^{-1} (11 mg kg body weight^{-1} d^{-1}) and the LOAEL for PFOS- 3M was 73 mg kg feed^{-1} (15 mg kg body weight^{-1} d^{-1}). These comparisons suggest that the Japanese quail and northern bobwhite are similar in sensitivity to PFOS.

As has been demonstrated for mallards (Newsted et al. 2006), the concentrations of PFOS and AFFF PFOS at both 8 and 23 d were greater in the serum compared to the liver in Japanese quail with serum to liver ratios of ranging from 1.3:1 to 2.3:1. The ratios of serum to liver concentrations in mallards averaged 1.7:1. In northern bobwhites, hepatic PFOS concentrations were greater than serum concentrations at d 8, but less than serum concentrations on d 22 with

serum to liver ratios of 0.52 and 2.4. In the present study, decreases in serum and liver PFOS and AFFF PFOS from d 8 to d 23 in Japanese quail averaged 80%. In comparison, Newsted et al. (2006) reported liver and serum half-lives of 17.5 and 6.86 days for PFOS in mallards and a liver half-life of 12.8 days for northern bobwhites. Hepatic concentrations associated with mortality in mallards and northern bobwhites approximated 166 and 159 mg PFOS kg^{-1} wet weight while in the present study, the average concentrations of PFOS and AFFF PFOS in mortalities was 240 and 169 mg kg^{-1} wet weight. The similarity in average hepatic concentrations of PFOS in mortalities across three different avian species is striking and suggests that this endpoint is a more consistent indicator of toxicity than endpoints related to dietary concentration and dose.

4.2 Chronic Exposure Study

4.2.1 Dietary concentrations and average daily dose

Analyzed dietary concentrations in the PFOS trial were 0, 2.1, 4.0, 8.6, 14 and 18 mg kg $feed^{-1}$ and analyzed concentrations of AFFF PFOS in the 3M AFFF trial were 0, 2.1, 5.0, 11, 19 and 27 mg kg $feed^{-1}$. Components of the 3M AFFF and their relative proportions were PFOS (91%), PFHxS (7%), perfluorobutane sulfonate (1%) and PFOA (1%). Analyzed concentrations of PFOS were on average 87% of nominal concentrations for the PFOS trial (range 80 to 93%) and 111% (range 84 to 135%) for the 3M AFFF trial. The ADDs for the PFOS trial were 0, 0.28, 0.55, 1.1, 1.8 and 2.4 mg PFOS kg body $weight^{-1}$ d^{-1} and 0, 0.27, 0.66, 1.4, 2.5 and 3.4 mg AFFF PFOS kg body $weight^{-1}$ d^{-1} for the 3M AFFF trial

4.2.2 Adult mortality and clinical observations

One adult male and 6 adult females died or were euthanized during the course of the 20-week PFOS trial. The male was in the control group and died accidentally. Two females in the 8.7 mg kg $feed^{-1}$, 1 in the 14 mg kg $feed^{-1}$, and 3 in the 18 mg kg $feed^{-1}$ treatment groups died or were euthanized due to excessive aggression (pecking) by their male mate. In the AFFF PFOS trial 5 males and 10 females died or were euthanized. Distribution of mortalities was 4 in the control group and 2 in the 2.1 mg kg $feed^{-1}$, 3 in the 5.0 mg kg $feed^{-1}$, 3 in the 11 mg kg $feed^{-1}$, 2 in the 19 mg kg $feed^{-1}$ and 1 in the 27 mg kg $feed^{-1}$ groups. None of these mortalities was considered to be treatment related but rather due to accidental injury or aggression between specific breeding pairs. There were no clinical signs observed in the adult birds suggestive of exposure to PFOS.

4.2.3 Adult feed consumption, body weight gain and body weights at necropsy

Feed consumption of adult female birds from 0 to 20 weeks of age was unaffected by exposure to PFOS or AFFF PFOS, but total feed consumed by males in the 18 mg PFOS kg $feed^{-1}$ (ADD = 2.4 mg kg body $weight^{-1}$ d^{-1}) and 27 mg AFFF PFOS kg $feed^{-1}$ (ADD = 3.4 mg kg body $weight^{-1}$ d^{-1}) groups was significantly less compared to controls (Table 8). Body weight gains of adult female quail from 4 to 20 weeks of age were not significantly affected by exposure to PFOS or AFFF PFOS but there were significant effects on body weight gains of adult males. The dietary concentration of 19 mg AFFF PFOS kg $feed^{-1}$ (ADD = 2.5 mg kg body $weight^{-1}$ d^{-1}) resulted in a significantly lower body weight gain when compared to controls (Table 9). Similarly, body weights of females at necropsy were not affected by exposure to PFOS or AFFF PFOS but male body weights were significantly less compared to controls at 14 mg PFOS kg

feed^{-1} (ADD = 1.8 mg kg body weight^{-1} d^{-1}) and greater and 11 mg AFFF PFOS kg feed^{-1} (ADD = 1.4 mg kg body weight^{-1} d^{-1}) and greater (Table 9).

Table 8. Total feed consumed and cumulative PFOS intake in female and male Japanese quail fed PFOS or 3M AFFF from 0 to 20 weeks of age

Dietary concentration (mg kg^{-1} feed)	ADD (mg kg bw^{-1} d^{-1})	Total feed consumption (g) from 0 to 20 weeks of age		Cumulative PFOS intake (mg)	
		Female	Male	Female	Male
PFOS					
0	0	2704 (51)[a]	2252 (48)	0 (1.2)	0 (0.4)
2.1	0.28	2549 (51)	2185 (47)	5.4* (1.2)	4.6** (0.4)
4.1	0.55	2715 (51)	2198 (47)	11** (1.2)	9.0** (0.4)
8.7	1.1	2602 (55)	2118(47)	23** (1.2)	18** (0.4)
14	1.8	2665 (53)	2086 (47)	37** (1.2)	29** (0.4)
18	2.4	2616 (57)	2062* (47)	47** (1.2)	37** (0.4)
AFFF PFOS					
0	0	2714 (87)	2166 (51)	0 (1.4)	0 (0.7)
2.1	0.27	2627 (84)	2188 (55)	5.6* (1.4)	4.6** (0.7)
5.0	0.66	2671 (84)	2070 (55)	13** (1.4)	10** (0.7)
11	1.4	2528 (84)	2133 (53)	28** (1.3)	24** (0.7)
19	2.5	2678 (84)	2001 (53)	51** (1.3)	38** (0.7)
27	3.4	2532 (81)	1979* (51)	68** (1.3)	53** (0.6)

Data are presented as mean with standard error in parentheses.
*Means within column are significantly different from control mean ($p < 0.05$).
**Means within column are significantly different from control mean ($p < 0.01$).

Table 9. Body weight gain and body weight at necropsy of female and male Japanese quail fed PFOS or 3M AFFF from 0 to 20 weeks of age

Dietary concentration (mg kg^{-1} feed)	ADD (mg kg bw^{-1} d^{-1})	Mean body weight gain (g)		Mean body weights at necropsy (g)	
		Female	Male	Female	Male
PFOS					
0	0	68.8 (3.5)	46.5 (2.3)	173 (4)	150 (3)
2.1	0.28	62.4 (3.5)	50.1 (2.4)	161 (4)	146 (3)
4.1	0.55	62.9 (3.5)	38.4 (2.4)	168 (4)	142 (3)
8.7	1.1	67.6 (3.7)	41.1 (2.4)	172 (4)	142 (3)
14	1.8	66.5 (3.6)	38.3 (2.4)	170 (4)	136** (3)
18	2.4	59.8 (3.9)	36.0 (2.4)	161 (4)	137** (3)
AFFF PFOS					
0	0	61.5 (2.7)	34.4 (1.9)	173 (3)	144 (2)
2.1	0.27	60.0 (2.5)	38.0 (1.9)	170 (3)	145 (2)

5.0	0.66	63.0 (2.5)	35.1 (1.9)	175 (3)	143 (2)
11	1.4	59.8 (2.7)	33.1 (1.8)	167 (3)	135* (2)
19	2.5	57.7 (2.5)	26.9* (1.9)	164 (3)	129** (2)
27	3.4	57.6 (2.5)	27.9 (1.8)	166 (3)	131** (2)

Data are presented as mean with standard error in parentheses.
*Means within column are significantly different from control mean ($p < 0.05$).
**Means within column are significantly different from control mean ($p < 0.01$).

4.2.4 Egg production and hatchability

Exposure to PFOS or AFFF PFOS did not have a significant effect on the mean number of viable eggs laid per hen but exposure to PFOS resulted in a significant decrease in hatchability at a dietary concentration of 18 mg kg feed^{-1} (ADD = 2.4 mg kg body weight^{-1} d^{-1}) (Table 10).

Table 10. Number of viable eggs laid by female Japanese quail and percent chicks hatched during weeks 10 to 20 of dietary exposure to PFOS or 3M AFFF

Dietary concentration (mg kg^{-1} feed)	ADD (mg kg bw^{-1} d^{-1})	Mean viable eggs laid per hen[a]	95% CI	% Chicks hatched from viable eggs	SE
PFOS					
0	0	49	45 – 54	72.9	4.2
2.1	0.28	42	32 – 52	77.4	3.7
4.1	0.55	45	40 – 49	71.7	3.3
8.7	1.1	38	29 – 47	68.9	4.0
14	1.8	43	35 – 52	66.3	4.9
18	2.4	40	33 - 47	57.5*	6.0
AFFF PFOS					
0	0	49	39 – 59	88.9	0.8
2.1	0.27	48	40 – 56	84.8	1.8
5.0	0.66	43	33 – 52	84.4	1.2
11	1.4	53	49 – 58	88.3	2.4
19	2.5	45	37 – 53	78.5	8.4
27	3.4	45	39 – 52	62.8	7.1

[a]Viable eggs laid per hen were not normally distributed so data were log transformed for statistical analysis.
*Means within column are significantly different from control mean ($p < 0.05$).
PFOS = perfluorooctane sulfonate; AFFF = aqueous film forming foam; ADD = average daily dose; bw = body weight; CI = confidence interval; SE = standard error

4.2.5 Embryo mortality

In general, there was a dose-related increase in embryo mortality for both PFOS and AFFF PFOS (Figure 7). In the PFOS trial, the greatest mortality occurred after day 14 of incubation across all groups with the proportions being similar (31.7 - 38.8%). In the 3M AFFF trial, embryo mortality occurred primarily in the first 7 d of incubation across treatment groups with exception of the greatest feed concentration that had the greatest proportion of embryos dying after 14 d of incubation. There was a significant increase in the number of embryos dying

after day 14, dead pips and live pips at 27 mg AFFF PFOS kg feed^{-1} (ADD = 3.4 mg kg body weight^{-1} d^{-1}) compared to controls (Table 11).

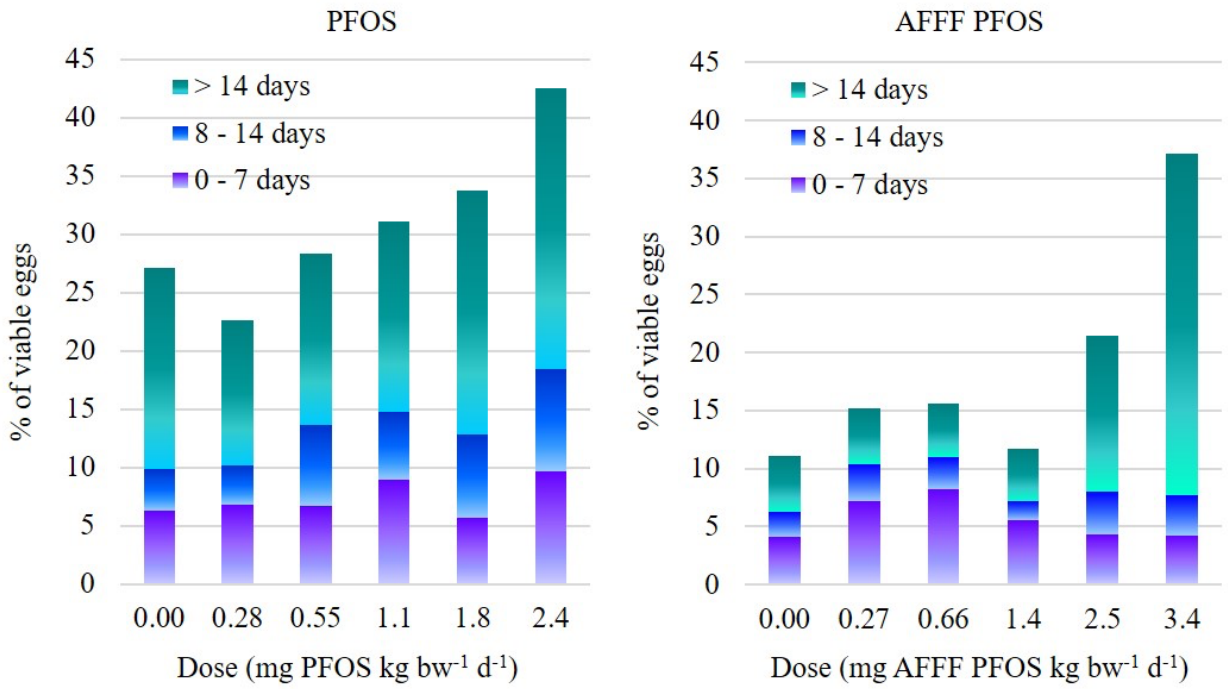


Figure 7. Percent of viable eggs that experienced embryo mortality

Table 11. Effect of dietary exposure of adult Japanese quail to PFOS or 3M AFFF on stage of embryo mortality and incidence of deformities in their offspring

	Stage of embryo mortality[a]										# of deformities	# viable eggs	# of live hatches[b]
	0 – 7 d	SE	8 – 14 d	SE	>14 d	SE	Dead pip	SE	Live pip	SE			
ADD							PFOS						
0	5.0 (23.4)	1.2	2.8 (13.1)	1.0	8.2 (38.3)	1.7	2.3 (10.7)	0.5	3.1 (14.5)	0.9	6	789	575 (72.9)
0.28	4.6 (30.3)	1.2	2.2 (14.5)	1.0	5.9 (38.8)	1.7	0.5 (3.29)	0.5	2.0 (13.2)	0.9	1	672	520 (77.4)
0.55	4.8 (23.6)	1.2	5.0 (24.6)	1.0	6.6 (32.5)	1.7	1.0 (4.93)	0.5	2.9 (14.3)	0.9	4	717	514 (71.7)
1.1	4.8 (28.7)	1.2	3.1 (18.6)	1.0	5.7 (34.1)	1.7	0.8 (4.79)	0.5	2.3 (13.8)	0.9	6	537	370 (68.9)
1.8	3.7 (16.7)	1.2	4.7 (21.3)	1.0	8.2 (37.1)	1.7	2.0 (9.05)	0.5	3.5 (15.8)	0.9	4	655	434 (66.3)
2.4	5.0 (22.6)	1.2	4.6 (20.8)	1.0	7.0 (31.7)	1.7	1.8 (8.14)	0.5	3.7 (16.7)	0.9	1	520	299 (57.5)*
							AFFF PFOS						
0	2.7 (37.0)	0.9	1.4 (19.2)	0.5	1.6 (21.9)	0.8	0.8 (11.0)	0.6	0.8 (11.0)	0.8	0	655	582 (88.9)
0.27	5.2 (47.3)	0.9	2.3 (20.9)	0.5	2.0 (18.2)	0.8	0.5 (4.55)	0.6	1.0 (9.09)	0.8	1	723	613 (84.8)
0.66	5.4 (52.9)	0.9	1.8 (17.6)	0.5	1.7 (16.7)	0.8	0.2 (1.96)	0.6	1.1 (10.8)	0.8	2	654	552 (84.4)
1.4	4.1 (47.7)	0.9	1.2 (14.0)	0.5	1.4 (16.3)	0.8	0.6 (7.0)	0.6	1.3 (15.1)	0.8	6	736	650 (88.3)
2.5	2.8 (20.0)	0.9	2.4 (17.1)	0.5	3.8 (27.1)	0.8	1.5 (10.7)	0.6	3.5 (25.0)	0.8	3	651	511 (78.5)
3.4	2.9 (11.4)	0.9	2.4 (9.45)	0.5	6.9** (27.2)	0.8	5.4** (21.3)	0.6	7.8** (30.7)	0.8	1	683	429 (62.8)

[a]Data presented as mean and standard error. Numbers in parentheses are percent of embryos at a particular stage of development.
[b]Numbers in parentheses refer to percent hatchability.
*Means within column are significantly different from control mean ($p < 0.05$).
**Means within column are significantly different from control mean ($p < 0.01$).

4.2.6 Chick survivability through 7 and 14 d of age

Survivability of chicks raised on clean feed was affected by in ovo exposure to PFOS or AFFF PFOS (Table 12). Chicks in the PFOS trial had significantly reduced survivability through the first 7 d at feed concentrations of 8.7 and 18 mg kg feed^{-1} (ADDs = 1.1 and 2.4 mg kg body weight^{-1} d^{-1}) but not 14 mg kg feed^{-1} (ADD = 1.8 mg kg body weight^{-1} d^{-1}). Chicks in the 3M AFFF trial had a dose-dependent decrease in survivability that was significant beginning at feed concentrations of 11 mg AFFF PFOS kg feed (ADDs ≥ 1.4 mg kg body weight^{-1} d^{-1}) over the same period.. At 7 d of age, the number of chicks was reduced to 50 in those groups that exceeded that number and survivability was assessed over the subsequent 7 d. There were no significant differences in chick survivability compared to controls for both PFOS and AFFF PFOS. Percent survivability during this period ranged from 95.3 to 99.4 % in the PFOS trial and from 85.1 to 98.8 % in the 3M AFFF trial.

Table 12. Effect of dietary exposure of adult Japanese quail to PFOS or 3M AFFF on offspring survivability from 0 to 7 and 8 to 14 d of age

Dietary concentration (mg kg^{-1} feed)	ADD (mg kg bw^{-1} d^{-1})	% Survivability[a] day 0 – 7	95% CI	% Survivability[a] day 8 – 14	95% CI
PFOS					
0	0	93.9	91.9 - 95.8	98.0	96.2 - 99.8
2.1	0.28	87.4	80.6 - 94.1	96.8	91.0 - 100
4.1	0.55	90.4	86.9 - 93.9	95.3	90.5 - 100
8.7	1.1	72.9**	56.6 - 89.1	97.6	95.2 - 100
14	1.8	84.3	79.1 - 89.4	99.4	98.4 - 100
18	2.4	79.0**	71.2 - 86.8	96.0	92.5 - 99.5
AFFF PFOS					
0	0	95.6	93.3 - 97.8	98.8	97.0 - 100
2.1	0.27	90.5	86.0 - 94.9	93.6	84.2 - 100
5.0	0.66	91.3	86.3 - 96.4	98.5	96.5 - 100
11	1.4	71.6**	61.9 - 81.3	96.8	94.2 - 99.4
19	2.5	57.2**	42.1 - 72.3	85.1	63.6 - 100
27	3.4	44.3**	30.2 - 58.5	86.0	64.0 - 100

[a]Percent survivability data were arc sin transformed for statistical analysis and then back-transformed for presentation.

*Means within column are significantly different from control mean ($p < 0.05$).

**Means within column are significantly different from control mean ($p < 0.01$).

4.2.7 Chick body weights

Chick body weights at hatch and 7 and 14 d of age were variably affected by in ovo exposure to PFOS or AFFF PFOS (Table 13). Body weight of chicks in the PFOS group were significantly less compared to controls at adult feed concentrations as low as 2.1 mg kg feed^{-1} (ADDs ≥ 0.28 mg kg body weight^{-1} d^{-1}) at hatch, 4.1 mg kg feed^{-1} (ADD = 0.55 mg kg body weight^{-1} d^{-1}) at 7 d of age and 8.7 mg kg feed^{-1} (ADD = 1.1 mg kg body weight^{-1} d^{-1}) at 14 d of age. Chicks in the 3M AFFF trial had significantly lower body weights compared to controls at adult feed concentrations as low as 2.1 mg AFFF PFOS kg feed^{-1} (ADDs ≥ 0.27 mg kg body

weight^{-1} d^{-1}) at hatch, At 7 d of age, only chicks in the 27 mg AFFF PFOS kg feed^{-1} (ADD = 3.4 mg kg body weight^{-1} d^{-1}) group had significantly lower body weights compared to controls and body weights were not significantly affected at 14 d of age.

Table 13. Effect of dietary exposure of adult Japanese quail to PFOS or 3M AFFF on offspring body weight from 0 to 14 d of age

Dietary concentration (mg kg^{-1} feed)	ADD (mg kg bw^{-1} d^{-1})	Chick body weights (g)					
		Hatch	SE	day 7	SE	day 14	SE
PFOS							
0	0	6.9	0.02	17.5	0.2	47.6	0.3
2.1	0.28	6.5**	0.03	17.4	0.2	46.7	0.3
4.1	0.55	6.8	0.03	18.6**	0.2	47.2	0.3
8.7	1.1	6.9	0.03	18.8**	0.2	49.0*	0.4
14	1.8	6.7**	0.03	16.7**	0.2	46.1**	0.3
18	2.4	6.7*	0.03	16.6**	0.2	45.9**	0.4
AFFF PFOS							
0	0	6.9	0.1	19.4	0.4	48.8	0.8
2.1	0.27	6.5**	0.1	18.8	0.4	48.3	0.8
5.0	0.66	6.6**	0.1	18.1	0.4	47.7	0.8
11	1.4	6.8	0.1	18.8	0.4	48.5	0.8
19	2.5	6.8	0.1	17.7	0.5	47.8	0.9
27	3.4	6.5**	0.1	16.3**	0.5	45.7	0.9

*Means within column are significantly different from control mean ($p < 0.05$).
**Means within column are significantly different from control mean ($p < 0.01$).

4.2.8 Liver weights

Exposure to PFOS or AFFF PFOS had a variable effect on absolute and relative liver weights. There was no effect on absolute or relative liver weight in adult quail exposed to PFOS (Table 14), but 14-d-old chicks (males and females combined) had increased absolute liver weight at 18 mg kg feed^{-1} (ADD = 2.4 mg kg body weight^{-1} d^{-1}) and increased relative weight at 14 and 18 mg kg feed^{-1} (ADDs = 1.8 and 2.4 mg kg body weight^{-1} d^{-1}) compared to controls (Table 14). In adult birds fed diets containing 3M AFFF, the only significant effect was an increase in absolute liver weight in adult females at 27 mg AFFF PFOS kg feed^{-1} (ADD = 3.4 mg kg body weight^{-1} d^{-1}) (Table 14). There were no significant changes in absolute or relative liver weights in 14-d-old chicks in the 3M AFFF trial (Table 14).

Table 14. Effect of dietary exposure of adult Japanese quail to PFOS or 3M AFFF on chick (females and males combined) liver weight and liver weight relative to body weight at 14-d of age

Dietary concentration (mg kg^{-1} feed)	ADD (mg kg bw^{-1} d^{-1})	Chick liver weight (g)	SE	Chick liver weight relative to bw (%)[a]	95% CI

PFOS					
0	0	1.47	0.02	2.94	2.84 - 3.03
2.1	0.28	1.39	0.02	2.94	2.82 - 3.06
4.1	0.55	1.47	0.02	2.97	2.87 - 3.08
8.7	1.1	1.54	0.03	2.97	2.85 - 3.08
14	1.8	1.50	0.02	3.10*	3.01 - 3.19
18	2.4	1.58**	0.03	3.22**	3.11 - 3.33
AFFF PFOS					
0	0	1.56	0.02	3.07	2.99 - 3.16
2.1	0.27	1.61	0.02	3.13	3.05 - 3.21
5.0	0.66	1.59	0.02	3.14	3.04 - 3.21
11	1.4	1.65	0.02	3.15	3.07 - 3.24
19	2.5	1.71	0.02	3.35	3.27 - 3.44
27	3.4	1.70	0.03	3.42	3.34 - 3.51

[a]Relative liver weights were arc sin transformed for data analysis. Means presented are back-transformed means with 95% confidence interval.
*Means within column are significantly different from control mean ($p < 0.05$).
**Means within column are significantly different from control mean ($p < 0.01$).

4.2.9 Pathology

The same pathologist was not available to evaluate tissues from both trials. As a result, some of the evaluation criteria are slightly different between the two trials. A summary of the significant results for each trial by sex is provided in Table 15. A summary of all the lesions by sex in each trial is in the Appendix.

4.2.10 Liver.

The severity of extramedullary hematopoesis was significant for males in the PFOS trial and females in the AFFF PFOS trial. Twenty-five percent of the males in the 18 mg PFOS kg feed^{-1} (ADD = 2.4 mg PFOS kg body weight^{-1} d^{-1}) group had the lesion whereas in the AFFF PFOS trial, the lesion occurred in 25 % of the males and 25, 56 and 81% of the females in the 3 greatest trearment groups. Canalicular cholestasis, myofibroblast proliferation and heterophilic inflammation were assessed in the AFFF PFOS trial only and lesion severity was significant for males, males and females, and males, respectively. However, neither the percentage of birds having the lesion or the severity of the lesion corresponded to dose.

4.2.11 Kidney.

Tubular regeneration was assessed in PFOS trial and severity was significant for females with 25% of the birds in the 14 mg kg feed^{-1} (ADD = 1.8 mg kg body weight^{-1} d^{-1}) group having the lesion. Glomerulopathy was also assessed only in the PFOS trial with severity for both sexes being significant. With the exception of one control female, the lesion was restricted to the 2.1 mg kg feed^{-1} (ADD = 0.28 mg kg body weight^{-1} d^{-1}) group. Tubular degeneration was assessed in the AFFF PFOS trial only and severity was significant for females. Lesions occurred in the two greatest treatment groups, although the incidence and severity did not correspond to dose.

Table 15. Effect of dietary exposure of adult Japanese quail to PFOS or 3M AFFF from 0 to 20 weeks of age on liver and kidney histological lesion scores by sex[a]

LIVER

Extramedullary hematopoiesis

PFOS lesion scores					AFFF PFOS lesion scores				
Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected[b]	Score (KW *p*-value = 0.4159)	% of males affected	Score (KW *p*-value = 0.0066)	Dose (mg PFOS kg bw^{-1} day^{-1})	% of female affected	Score (KW *p*-value = 0.0002)	% of males affected	Score (KW *p*-value = 0.0847)
0.00	0	0.00	0	0.00	0.00	19	0.31	0	0.00
0.28	0	0.00	0	0.00	0.27	44	0.56	13	0.13
0.55	0	0.00	6.3	0.06	0.66	19	0.19	0	0.00
1.1	0	0.00	0	0.00	1.4	25	0.31	25	0.31
1.8	0	0.00	0	0.00	2.5	56	0.94	25	0.31
2.4	6.3	0.06	25	0.25	3.4	81	1.44	25	0.31

Canalicular cholestasis

PFOS lesion scores[c]					AFFF PFOS lesion scores				
Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected[b]	Score	% of males affected	Score	Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected	Score (KW *p*-value = 0.3096)	% of males affected	Score (KW *p*-value = 0.0237)
0.00	---	---	---	---	0.00	6.3	0.06	6.3	0.06
0.28	---	---	---	---	0.27	0	0.00	38	0.44
0.55	---	---	---	---	0.66	13	0.13	38	0.50
1.1	---	---	---	---	1.4	19	0.19	56	0.56
1.8	---	---	---	---	2.5	13	0.19	44	0.75
2.4	---	---	---	---	3.4	0	0.00	13	0.13

Myofibroblast proliferation

PFOS lesion scores[c]					AFFF PFOS lesion scores				
Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected[b]	Score	% of males affected	Score	Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected	Score (KW *p*-value = 0.0400)	% of males affected	Score (KW *p*-value = 0.0119)
0.00	---	---	---	---	0.00	31	0.38	19	0.19
0.28	---	---	---	---	0.27	69	0.88	63	0.63
0.55	---	---	---	---	0.66	56	0.56	25	0.31

1.1	---	---	---	---	1.4	75	0.81	44	0.50
1.8	---	---	---	---	2.5	81	1.06	63	0.81
2.4	---	---	---	---	3.4	69	0.94	75	0.75

Heterophilic inflammation

PFOS lesion scores[c]					AFFF PFOS lesion scores				
Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected[b]	Score	% of males affected	Score	Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected	Score (KW *p*-value = 0.2330)	% of males affected	Score (KW *p*-value = 0.0426)
0.00	---	---	---	---	0.00	31	0.31	0	0.00
0.28	---	---	---	---	0.27	31	0.38	31	0.31
0.55	---	---	---	---	0.66	6.3	0.06	6.3	0.06
1.1	---	---	---	---	1.4	19	0.19	13	0.13
1.8	---	---	---	---	2.5	31	0.31	19	0.19
2.4	---	---	---	---	3.4	6.3	0.13	0	0.00

KIDNEY

Tubular regeneration

PFOS lesion scores					AFFF PFOS lesion scores[d]				
Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected[b]	Score (KW *p*-value = 0.0082)	% of males affected	Score (KW *p*-value = 0.1200)	Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected	Score	% of males affected	Score
0.00	6.3	0.06	13	0.19	0.00	---	---	---	---
0.28	6.3	0.06	13	0.25	0.27	---	---	---	---
0.55	6.3	0.13	13	0.13	0.66	---	---	---	---
1.1	6.3	0.06	6.3	0.25	1.4	---	---	---	---
1.8	25	0.50	6.3	0.06	2.5	---	---	---	---
2.4	0	0.00	31	0.50	3.4	---	---	---	---

Glomerulopathy

PFOS lesion scores					AFFF PFOS lesion scores[d]				
Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected[b]	Score (KW *p*-value = 0.0067)	% of males affected	Score (KW *p*-value = 0.0009)	Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected	Score	% of males affected	Score
0.00	6.3	0.13	0	0.00	0.00	---	---	---	---
0.28	13	0.50	25	0.38	0.27	---	---	---	---

0.55	0	0.00	0	0.00	0.66	---	---	---	---
1.1	0	0.00	0	0.00	1.4	---	---	---	---
1.8	0	0.00	0	0.00	2.5	---	---	---	---
2.4	0	0.00	0	0.00	3.4	---	---	---	---

Tubular degeneration

PFOS lesion scores[c]					AFFF PFOS lesion scores				
Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected[b]	Score	% of males affected	Score	Dose (mg PFOS kg bw^{-1} day^{-1})	% of females affected	Score (KW *p*-value = 0.0003)	% of males affected	Score (KW *p*-value = 0.6900)
0.00	---	---	---	---	0.00	0	0.00	0	0.00
0.28	---	---	---	---	0.27	0	0.00	0	0.00
0.55	---	---	---	---	0.66	0	0.00	6.3	0.06
1.1	---	---	---	---	1.4	0	0.00	6.3	0.06
1.8	---	---	---	---	2.5	38	0.38	6.3	0.06
2.4	---	---	---	---	3.4	25	0.31	0	0.00

[a]Lesions were graded on a severity scale of 0 to 4 according to the following criteria: 0 = no lesion recognized; 1 = minimal lesions, 1 to 3 foci or small foci of a few cells; 2 = mild lesions, increased number of foci or more of the lesion; 3 = moderate lesions, more lesions (2-3 per 10x field of vision); 4 = severe lesions, the majority of cells and/or extensive regions involved with the lesions

[b]n for all dose groups was 16 birds.

[c]Not assessed in the PFOS trial.

[d]Not assessed in the PFOS AFFF trial.

4.2.12 Serum, liver and egg concentrations

In general, there was a dose-related increase in serum and liver concentrations of PFOS or AFFF PFOS in adult quail exposed to PFOS or 3M AFFF via the feed (Table 16 Figures 8-10) and in chicks exposed in ovo (Figure 11). Adults in the 3M AFFF trial also had dose-related increases in serum and liver PFHxS and PFOA (Table 16 Figures 12,13), but the chicks showed an increase in PFHxS until the 20 ug/g experimental group where there was a drop off (Figure 14). In both the PFOS and 3M AFFF trials, the concentrations of serum and liver PFOS in males exceeded concentrations in females. In the PFOS trial serum concentrations were on average 6.6-fold greater and hepatic concentrations were 5.5-fold greater compared to females, whereas in the 3M AFFF trial serum and liver PFOS concentrations were 8.8- and 4.2-fold greater in males compared to females. Serum to liver ratios for PFOS were 3.4:1 for males and 2.9:1 for females and for males and females in the 3M AFFF trial 1.9:1 and 0.95:1. In chicks, serum and hepatic concentrations of PFOS and AFFF PFOS approximated 37% of adult female concentrations. The chick serum to liver ratio was 1.7:1 for both PFOS and AFFF PFOS. In the 3M AFFF trial, serum and liver PFHxS concentrations were 11 and 5% of PFOS concentrations in adult males, 37 and 12% in adult females and 15 and 5.3% in chicks. Concentrations of PFOS and AFFF PFOS generally increased in the yolks of eggs with increases in feed concentrations (Appendix). Concentrations of PFOS and AFFF PFOS also increased in the albumen with increasing feed concentrations (data not presented), but were only 0.29 and 0.045% of corresponding yolk concentrations. Concentrations of PFOS in eggs laid during week 10 were on average 41% of concentrations in eggs laid during weeks 1 and 2 and AFFF PFOS concentrations at week 10 were 64% of week 1 and 2 concentrations. The ratio of PFOS in the yolk of eggs laid during week 10 to the concentration of PFOS in the serum of adult females at necropsy was 1.4:1 and for AFFF PFOS the ratio was 3.41:1.

Table 16. Effect of dietary PFOS or 3M AFFF on adult Japanese quail serum and liver PFOS and PFHxS concentrations after 20 wk of exposure

Dietary concentration (mg kg^{-1} feed)	ADD (mg kg bw^{-1} d^{-1})	Serum PFOS[a] (mg L^{-1})	95% CI	Serum PFHxS[a] (mg L^{-1})	95% CI	Liver PFOS[a] (mg kg^{-1})	95% CI	Liver PFHxS[a] (mg kg^{-1})	95% CI
				PFOS					
		Females							
0	0	0.40	0.28-0.53	---	---	0.16	0.00-0.44	---	---
2.1	0.28	14.06**	0.00-42.74	---	---	7.12**	0.00-14.95	---	---
4.1	0.55	25.43**	1.04-49.81	---	---	6.29**	2.90-9.68	---	---
8.7	1.1	48.96**	0.00-114.28	---	---	12.07**	7.25-16.89	---	---
14	1.8	28.66**	14.56-42.76	---	---	12.07**	9.96-14.18	---	---
18	2.4	35.51**	3.74-67.28	---	---	16.85**	12.29-21.41	---	---
		Males							
0	0	0.57	0.33-0.80	---	---	0.14	0.00-0.29	---	---
2.1	0.28	54.16**	31.09-77.23	---	---	18.23**	15.54-20.92	---	---
4.1	0.55	178.72**	78.60-278.84	---	---	36.45**	27.80-45.09	---	---
8.7	1.1	223.76**	117.28-330.25	---	---	65.35**	54.15-76.54	---	---
14	1.8	244.02**	151.01-337.02	---	---	95.52**	72.48-118.56	---	---
18	2.4	322.14**	215.72-428.56	---	---	98.87**	57.75-140.00	---	---
				AFFF PFOS					
		Females							
0	0	0.10	0.09-0.10	0.03	0.03-0.04	0.24	0.12-0.37	0.02	0.01-0.04
2.1	0.27	6.41**	1.46-11.35	2.46**	1.36-3.55	8.23**	3.19-13.27	1.04**	0.51-1.56
5.0	0.66	9.67**	5.84-13.49	4.25**	3.21-5.28	12.97**	5.06-20.89	1.43**	0.44-2.42
11	1.4	21.42**	12.71-30.12	8.35**	5.81-10.88	24.42**	19.24-29.60	2.78**	1.99-3.56
19	2.5	44.70**	26.97-62.43	13.95**	11.67-16.23	41.08**	14.47-67.68	4.84**	3.61-6.06
27	3.4	52.41**	31.29-73.53	16.23**	11.30-21.17	42.28**	30.55-54.02	5.05**	3.47-6.64
		Males							
0	0	0.33	0.16-0.50	0.05	0.03-0.06	0.32	0.16-0.47	0.02	0.00-0.05
2.1	0.27	61.74**	52.53-70.96	5.51**	4.52-6.50	24.70**	19.12-30.28	0.87**	0.66-1.07

5.0	0.66	141.99**	124.61-159.37	13.44**	8.35-18.53	52.05**	40.03-64.07	2.27**	1.09-3.44
11	1.4	189.76**	153.31-226.21	16.28**	8.29-24.27	113.00**	58.93-167.07	2.50**	0.92-4.08
19	2.5	237.43**	185.48-289.38	30.91**	10.67-51.16	156.37**	108.11-204.64	9.52**	0.00-20.39
27	3.4	278.93**	235.46-322.41	42.67**	25.07-60.27	230.51**	178.17-282.84	16.39**	10.98-21.81

[a]PFOS and PFHxS concentrations in serum and liver were not normally distributed. The data were log-transformed for analysis and the results shown are back-transformed means with 95% confidence intervals.

**Means within columns are significantly different from control mean ($p < 0.01$).

PFOS = perfluorooctane sulfonate; AFFF = aqueous film forming foam; PFHxS = perfluorohexanesulfonate; ADD = average daily dose; bw = body weight; CI = confidence interval

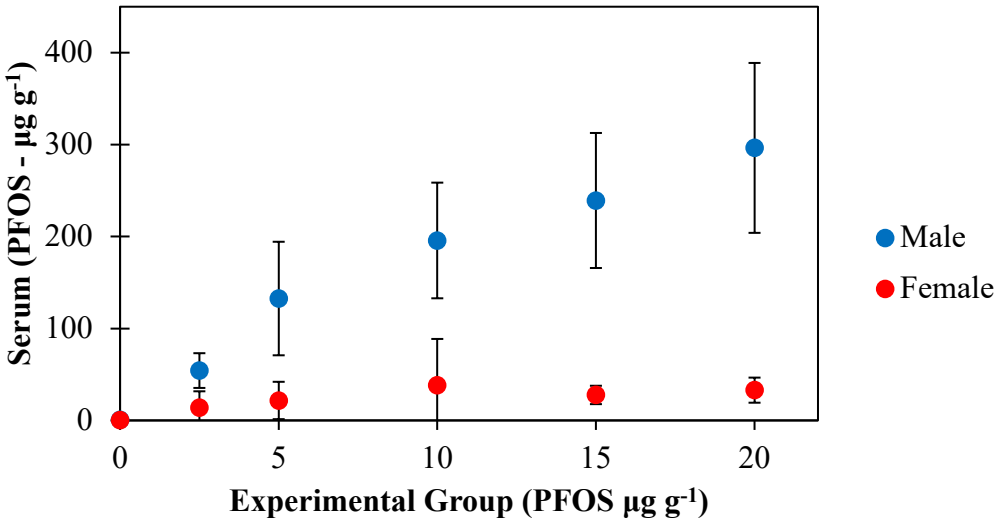


Figure 8. Serum PFOS Concentration in Adult Quail from Chronic PFOS Trial

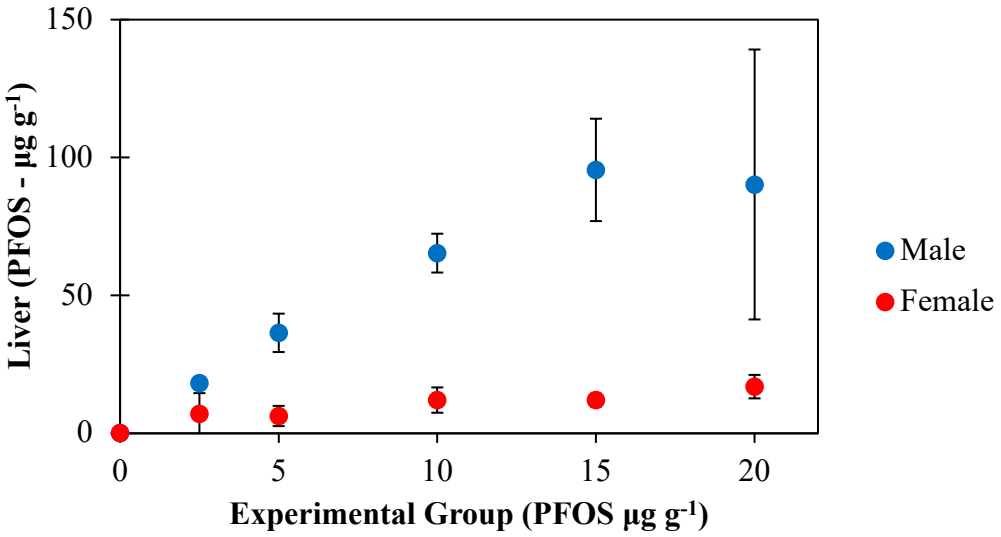


Figure 9. Liver PFOS Concentrations in Adult Quail from Chronic PFOS Trial

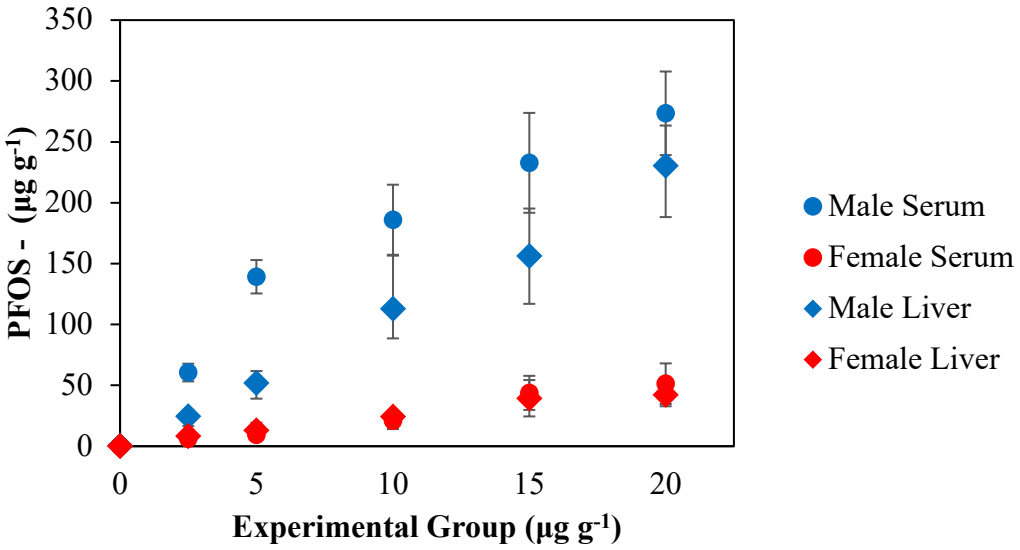


Figure 10. Liver and Serum PFOS Concentrations in Adult Quail from 3M AFFF Chronic Trial

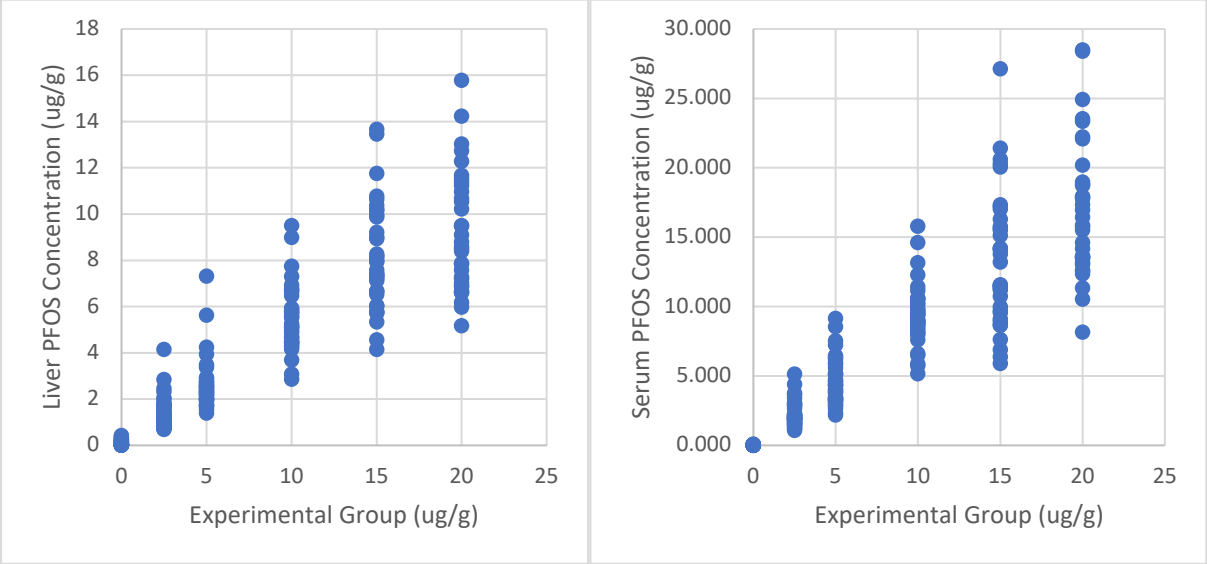


Figure 11. Liver and Serum PFOS Concentrations in Quail Chicks from PFOS Chronic Trial

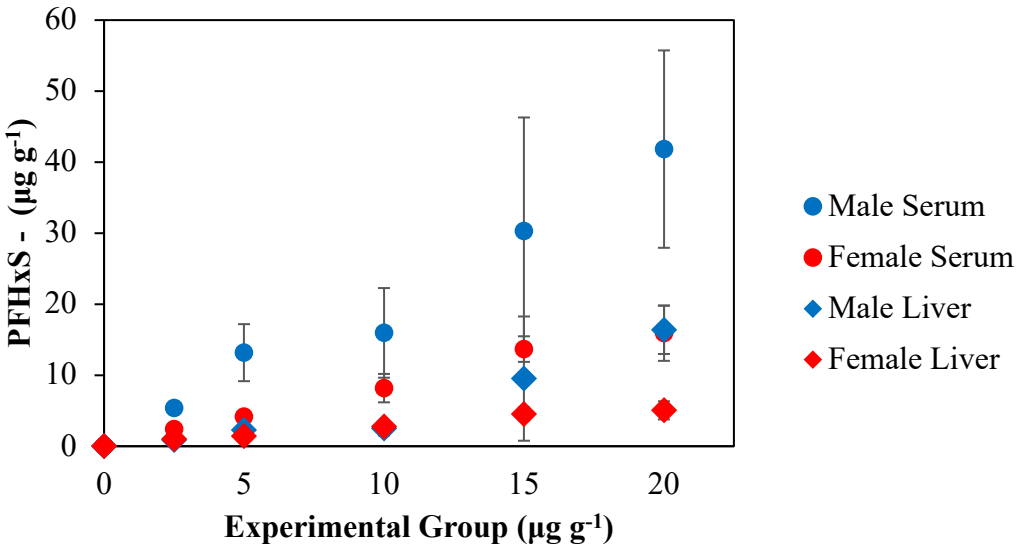


Figure 12. Liver and Serum PFHxS Concentrations in Adult Quail from 3M AFFF Chronic Trial

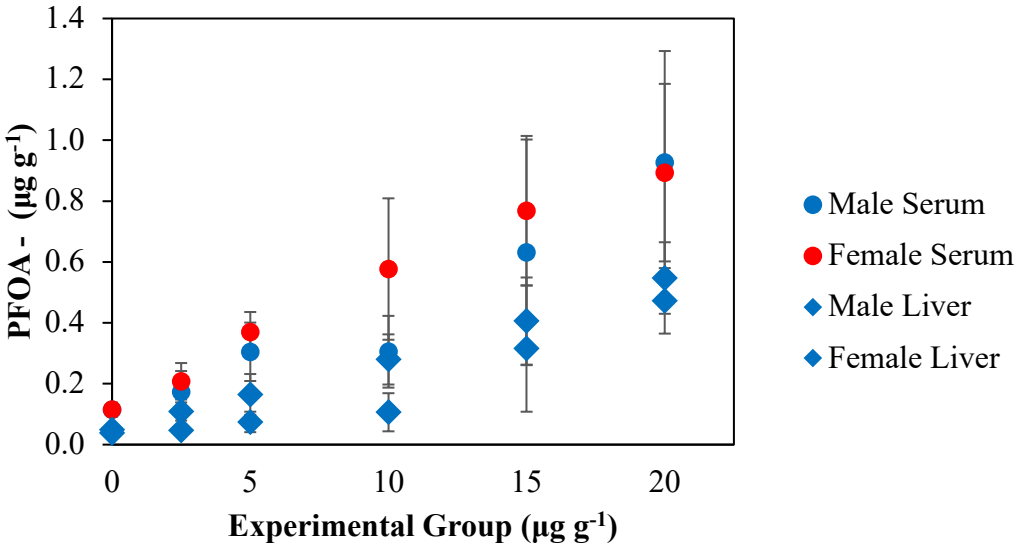


Figure 13. Liver and Serum PFOA Concentrations in Adult Quail from 3M AFFF Chronic Trial

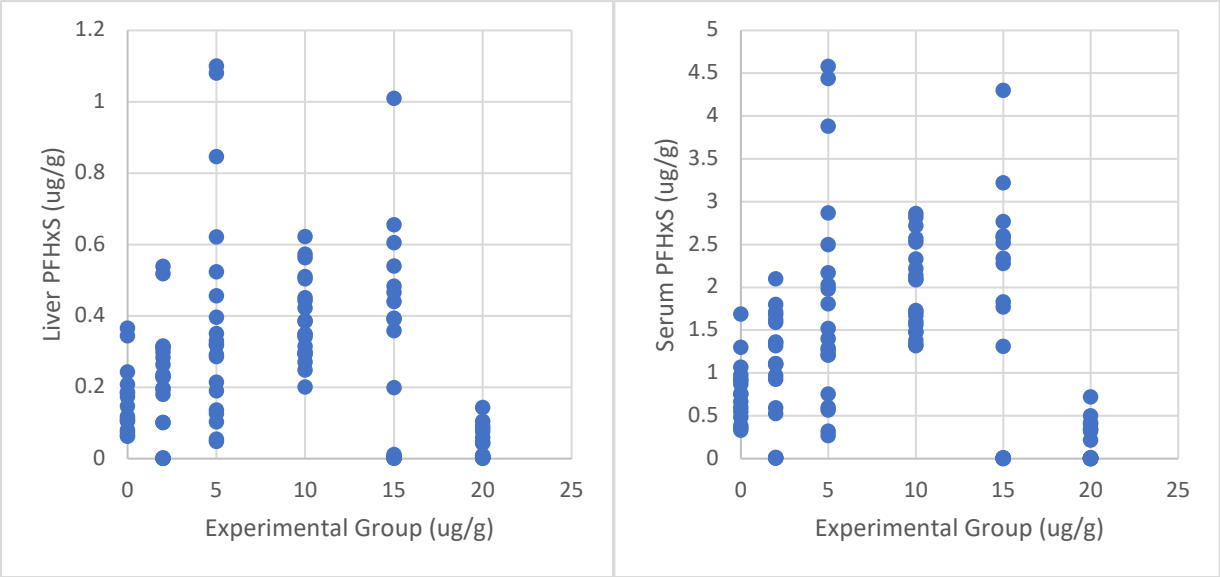


Figure 14. Liver and Serum PFHxS Concentrations in Quail Chicks from 3M AFFF Chronic Trial

4.2.13 Discussion

4.2.13.1 Adult mortality and clinical observations

There were no mortalities or clinical signs in adult Japanese quail that were considered related to exposure to PFOS or AFFF PFOS. The 7 adults (0.96% mortality) in the PFOS trial and 15 adults (7.8% mortality) in the 3M AFFF trial that died or were euthanized during the 20-weeks trials experienced accidental injuries or injuries as a result of aggression between breeding pairs during the egg-laying phase despite the relatively low light intensity. Typically, feathers were stripped from the head and neck region of the female by the male during copulation and in some cases, sores developed that were treated twice daily with antibiotic ointment. If treatment of the wound did not promote healing and severity increased, the bird was euthanized. In some cases, the female was the aggressor.

In a study very similar to the present study, Newsted et al. (2007) evaluated the chronic toxicity of a 3M production lot of potassium PFOS (86.9%) in mallards (*Anus platyrhynchos*) and northern bobwhites (*Colinus virginianus*) at feed concentrations ranging from 10 to 150 mg kg feed^{-1}. In that study, treatment-related mortalities of adult quail and mallards occurred in the 50 (ADD = 2.6 mg kg body weight^{-1} day^{-1}) and 150 mg kg feed^{-1} (ADD = 7.3 mg kg body weight^{-1} day^{-1}) groups. Clinical signs of toxicity were commonly observed and included reduced reaction stimuli, wing droop, loss of coordination, thin appearance, lacrimation, loss of righting reflex, lower limb rigidity, convulsions, shallow and rapid respiration, ruffled appearance, lower limb weakness, lethargy, gasping, prostrate posture and spasms. In the 50 mg kg feed^{-1} group there were 5 treatment-related deaths and 3 incidental deaths among the northern bobwhites. No mention was made of injuries resulting from aggressive behavior between breeding pairs. The ADD for the northern bobwhites in the 50 mg kg feed^{-1} group (2.6 mg kg body weight^{-1} day^{-1}) approximated ADDs for Japanese quail in the greater treatment groups in the present study that did not result in treatment-related deaths or clinical signs of toxicity. The difference in the

incidence of adult mortality and clinical signs between the two studies could be due to species differences between the northern bobwhite and Japanese quail and/or the age of the birds at the beginning of exposure (24-week-old in the Newsted et al. [2007] study and 1-d-old in the present study). Newsted et al. (2007) reported that adult northern bobwhites in the 10 mg kg feed^{-1} (ADD = 0.77 mg kg body weight^{-1} day^{-1}) group did not experience mortality but they did display clinical signs attributed to PFOS that included reduced reaction to external stimuli, ruffled appearance and lethargy beginning at 5 weeks of exposure.

In another avian reproductive toxicity study that involved exposure of northern bobwhites to PFOS at drinking water concentrations ranging from 0.216 to 18.7 ng mL^{-1} (ADDs = 2.99 x 10^{-5} – 2.45 x 10^{-3} mg kg body weight^{-1} day^{-1}) or a 1.2:1 mixture of PFOS:PFHxS at concentrations ranging from 0.375 to 22.9 ng mL^{-1} (ADDs = 5.04 x 10^{-5} – 3.10 x 10^{-3} mg kg body weight^{-1} day^{-1}), Dennis et al. (2020) stated that adult survival was 92% over the course of the 90-d study and only 1 death of 4 could possibly be attributed to PFOS exposure (0.596 ng mL water^{-1}) It was observed that birds became increasingly aggressive during photostimulation. During the egg laying phase of the trial feather stripping and sores that required treatment were common.

4.2.13.2 Adult feed consumption, body weight gain and body weights at necropsy

In the present study only males had significantly depressed feed consumption and that was at the greatest feed concentrations of PFOS and AFFF PFOS. Similarly, body weight gain of males only was significantly depressed (23%) and only in the 19 mg AFFF PFOS kg feed^{-1} group (ADD = 2.5 mg AFFF PFOS kg body weight^{-1} d^{-1}). Despite the lack of significance, body weight gains of adult males exposed to AFFF PFOS at 27 mg kg feed^{-1} (ADD = 3.4 mg kg body weight^{-1} day^{-1}) and PFOS beginning at a feed concentration of 4.1 mg kg feed^{-1} (ADD = 0.55 mg kg body weight^{-1} day^{-1}) ranged from 12 to 23% less than controls. Adult females in the 18 mg PFOS kg feed^{-1} group (ADD = 2.4 mg PFOS kg body weight^{-1} d^{-1}) had an average body weight gain that was 13% less compared to controls whereas body weight gain in the group exposed to the greatest concentration of AFFF PFOS (27 mg kg feed^{-1}; ADD = 3.4 mg kg body weight^{-1} day^{-1}) was only 6% less compared to controls. As with feed consumption and body weight gain, body weight at necropsy was significantly lower compared to controls in males only, but these difference were less than 10 %. The lesser effect of PFOS and AFFF PFOS on female body weight gain and body weights at necropsy compared to males could be due to compartmentalization and periodic elimination of the chemical via the egg by the laying female.

Newsted et al. (2007) reported no significant effect of 10 mg PFOS kg feed^{-1} (ADD = 0.77 mg kg body weight^{-1} day-1) on feed consumption or body weights of northern bobwhites or mallards but exposure to 50 (quail ADD = 2.6 mg kg body weight^{-1} day^{-1}) and 150 mg PFOS kg feed^{-1} (quail ADD = 7.3 mg kg body weight^{-1} day^{-1}) resulted in significant decreases in feed consumption and body weights beginning at 1 and 2 weeks of exposure, respectively. The ADD of 2.6 mg kg body weight^{-1} day^{-1} approximates the ADDs for Japanese quail in the greater treatment groups in the present study that resulted in significant or numerical decreases in adult male body weight gain. Body weight gain of northern bobwhite females exposed to 22.9 ng PFOS:PFHxS mL water^{-1} (ADD = 3.10 x 10^{-3} mg kg body weight^{-1} day^{-1}) was significantly

reduced at the end of the 90-d trial (Dennis, Karnjanapiboonwong et al. 2020). This ADD is more than a factor of 3 less than the ADDs in the present and Newsted et al. (2007) studies, suggesting greater bioavailability of PFOS in drinking water compared to feed.

4.2.13.3 Egg production and hatchability

Exposure of Japanese quail to PFOS or AFFF PFOS had no significant effect on egg production but there was an effect on hatchability. Exposure to 18 mg PFOS kg feed^{-1} (ADD = 2.4 mg kg body weight^{-1} day^{-1}) resulted in a significant decrease in hatchability (21% less than control hatchability) and exposure to 19 and 27 mg AFFF PFOS kg feed^{-1} (ADDs = 2.5 and 3.4 mg kg body weight^{-1} day^{-1}) resulted in numerical decreases in hatchability ranging from 12 to 29% less than control hatchability. These results are similar to those reported by Newsted et al. (2007) in that northern bobwhites exposed to 10 mg PFOS kg feed^{-1} (ADD = 0.77 mg kg body weight^{-1} day^{-1}) had no significant decreases in egg production or hatchability, although there was a numerical decrease in hatchability (13%). In the Newsted et al. (2007) study, birds in the 50 and 150 mg kg feed^{-1} groups were removed from the trial at weeks 6 and 4 prior to egg laying because of excessive treatment-related mortality. In northern bobwhites exposed to PFOS or PFOS:PFHxS via the drinking water there were no significant differences in egg production among treatment groups. There was, however, an increase in hatchability compared to controls in the 0.958 (ADD = 1.31 x 10^{-4} mg kg body weight^{-1} day^{-1}) and 22.9 (ADD = 3.10 x 10^{-3} mg kg body weight^{-1} day^{-1}) ng PFOS/PFHxS mL water^{-1} groups. The only numerical decrease in hatchability compared to controls (13%) occurred in the 0.596 ng PFOS mL water^{-1} (ADD = 8.50 x 10^{-5} mg kg body weight^{-1} day^{-1}) group.

4.2.13.4 Embryo mortality

In the present study, the greatest proportion of Japanese quail embryos that died prior to hatching, which was generally dose-dependent, did so after 14 d of incubation in the PFOS trial regardless of feed concentration, whereas in the 3M AFFF trial, embryo mortality occurred predominantly during the first 7 d of incubation with the exception of the greatest feed concentration that had a significant increase in the number of embryos dying after day 14, dead pips and live pips. Newsted et al. (2007) did not comment on embryo mortality, but data show a 2% decrease in northern bobwhite hatchlings compared to live 3-week embryos for controls and a 10% decrease for the 10 mg PFOS kg feed^{-1} group (ADD = 0.77 mg kg body weight^{-1} day^{-1}), which implies late mortality of embryos related to exposure to PFOS. Dennis et al. (2020) analyzed arrested embryo development in northern bobwhites exposed to PFOS and PFOS/PFHxS in drinking water. They reported earlier arrested embryo development in the 0.596 ng PFOS mL water^{-1} (ADD = 8.50 x 10^{-5} mg kg body weight^{-1} day^{-1}) group and later arrested development in the 18.7 ng PFOS mL water^{-1} group (ADD = 2.45 x 10^{-3} mg kg body weight^{-1} d^{-1}) compared to controls. Furthermore, the proportion of pipped eggs that did not hatch was significantly greater in the 18.7 ng PFOS mL water^{-1} group (ADD = 2.45 x 10^{-3} mg kg body weight^{-1} d^{-1}) compared to controls. The inability of an egg to hatch once pipping begins implies that the potential hatchling is too weak to complete the process.

4.2.13.5 Chick survivability through 7 and 14 d of age

Survivability of chicks raised on clean feed through 7 d of age was affected by in ovo exposure to PFOS at feed concentrations of 8.7 and 18 mg kg feed^{-1} (ADDs =1.1 and 2.4 mg kg body weight^{-1} d^{-1}) but not 14 mg kg feed^{-1} (ADD = 1.8 mg kg body weight^{-1} d^{-1}) and at feed concentrations of AFFF PFOS beginning at 11 mg kg feed^{-1} (ADDs ≥ 1.4 mg kg body weight^{-1} d^{-1}). The percent decrease in survivability was not related to PFOS dose and ranged from 4 to 22% less than control hatchability. There was a dose-related decrease in survivabilty for AFFF PFOS, which ranged from 5 to 54% less than control survivability. Although there were no significant differences in chick survivability from day 8 to 14, survivability at the two greatest doses of AFFF PFOS was approximately 14 % lower than control survivability. Similar to the results of the present study, Newsted et al. (2007) reported a 17% decrease in survivability of 14-d-old northern bobwhite chicks exposed in ovo to 10 mg PFOS kg feed^{-1} (ADD = 0.77 mg kg body weight^{-1} day^{-1}). Survivability of northern bobwhite chicks through 21 d of age was not adversely affected by in ovo exposure to drinking water concentrations as high as 18.7 ng PFOS mL water^{-1} (ADD = 2.45 x 10^{-3} mg kg body weight^{-1} d^{-1}) and 22.9 ng PFOS/PFHxS mL water^{-1} (ADD = 3.10 x 10^{-3} mg kg body weight^{-1} d^{-1}).

4.2.13.6 Chick body weights

In ovo exposure to PFOS or AFFF PFOS had a variable effect on chick body weights at hatch and 7 and 14 d of age. With one exception the differences between body weight of exposed chicks compared to controls (less or greater) did not exceed 7%. The exception was average body weight of 7-d-old chicks in the 27 mg AFFF PFOS kg feed^{-1} group (ADD = 3.4 mg kg body weight^{-1} day^{-1}) that was 16% less than average control body weight and that difference decreased to 6% at 14 d of age. Newsted et a. (2007) did not report body weights of northern bobwhites chicks exposed in ovo to 10 mg PFOS kg feed^{-1} (ADD = 0.77 mg kg body weight^{-1} day^{-1}). The effect of PFOS or PFOS/PFHxS in drinking water on body weights of northern bobwhite chicks exposed in ovo (Dennis et al. 2020) was similar to the results of the present study in that weights were either less or greater than control body weights with no relation to dose.

4.2.13.7 Liver weights

Exposure to PFOS or AFFF PFOS caused an increase in absolute and/or relative liver weights in adult and/or juvenile Japanese quail at greater feed concentrations. In adults, there was a significant increase (26%) in absolute liver weight of females exposed to 27 mg AFFF PFOS kg feed^{-1} (ADD = 3.4 mg kg^{-1} d^{-1}). Although not significant, the increase in relative liver weight in the same group was 25% compared to controls. In ovo exposure to 18 mg PFOS kg feed^{-1} (ADD = 2.4 mg kg body weight^{-1} d^{-1}) caused a significant increase in absolute (7%) and relative (10%) liver weights of 14-d-old chicks. Newsted et al. (2007) reported an increase in adult female absolute and relative liver weights that occurred at a concentration of 10 mg PFOS kg feed^{-1} (ADD = 0.77 mg kg^{-1} d^{-1}). The authors stated that the increase in liver weight was considered to be adaptive because there was an absence of liver pathology. In contrast, in the

present study there was hepatic pathology in adults exposed to AFFF PFOS that suggests that the increase in liver weights of adult females in the 27 mg AFFF PFOS kg feed^{-1} (ADD = 3.4 mg kg^{-1} d^{-1}) group reflects a toxic response.

4.2.13.8 Histopathology

The only significant pathology that could be related to exposure to PFOS or AFFF PFOS was hepatic extramedullary hematopoiesis that was most predominant in females in the AFFF PFOS trial at dietary concentrations of 11, 19 and 27 mg kg feed^{-1} (ADDs = 1.4, 2.5 and 3.4 mg kg body weight^{-1} d^{-1} with the incidence increasing with dose from 25 to 81% and average severity scores increasing from 0.31 to 1.44. The lesion also occurred in 25% of the males in the same treatment groups. Hematopoiesis occurs in the bone marrow of healthy adult birds. Extramedullary hematopoiesis after reproductive activity is the result of pathology in the animal and is induced by specific cytokines and growth factors when decreased production in the bone marrow does not meet the need for circulating erythrocytes and/or leukocytes. In contrast to the present study, Newsted et al. (2007) did not detect pathological changes in the liver or kidneys of adult northern bobwhites exposed to 10 mg PFOS kg feed^{-1} (ADD = 0.77 mg kg^{-1} d^{-1}).

4.2.13.9 Serum, liver and egg concentrations

Serum and liver concentrations of PFOS in adult and juvenile quail exposed to PFOS or AFFF PFOS generally increased with dose as did serum and liver PFHxS in adults and juveniles in the 3M AFFF trial. It has been shown that the tissue distribution of PFOS is determined by its ability to bind to proteins and that serum/plasma, kidney and liver are the predominant sites of accumulation in a variety of species (Han et al. 2003, Jones et al. 2003, Conder et al. 2008, Yeung et al. 2009, Yoo et al. 2009, Huang et al. 2019). The greater concentrations of PFOS, AFFF PFOS and PFHxS in males compared to females is due to deposition of the chemicals into the egg by the female. In the present study, serum and liver concentrations of PFOS and AFFF PFOS in males were approximately 6-fold greater compared to females. Newsted et al. (2007) also reported sex differences in serum and hepatic PFOS concentrations in adult northern bobwhites and mallards with concentrations being approximately 17-fold greater in male northern bobwhites and 6-fold greater in male mallards compared to females. Newsted et al. (2007) stated that despite the sex differences in serum and liver PFOS concentrations, serum:liver PFOS ratios were similar between sexes (approximately 1.7:1 for northern bobwhites and 1.5:1 for mallards). In the present study, serum:liver ratios for PFOS and AFFF PFOS were also similar between sexes (approximately 3.2:1 for PFOS and 1.4:1 for AFFF PFOS).

Serum and liver concentrations of PFOS and AFFF PFOS in 14-d-old Japanese quail chicks were approximately 37% of adult female concentrations. In the Newsted et al. study (2007) serum and liver concentrations in northern bobwhite chicks approximated adult female concentrations.

Adult females transferred PFOS and AFFF PFOS consumed via the feed into the yolk of eggs in a generally dosed-related manner with greater concentrations occurring during the first 2

weeks of the 10-week egg laying period compared to the last week. Concentrations of PFOS and AFFF PFOS in week 10 eggs were 41 and 64% of week 1 and 2 eggs. A similar phenomenon has been reported for the great tit (*Parus major*) and Audouin's gull (*Larus audouinii*) environmentally exposed to PFAS in that egg yolk PFOS concentrations decreased with the laying order of the clutch (Vicente, Sanpera et al. 2015, Lasters, Groffen et al. 2019). The concentration of PFOS in northern bobwhite eggs at a dietary concentration of 10 mg kg feed (Newsted et al. 2007) is similar to the average concentration of PFOS and AFFF PFOs at equivalent ADDs in the present study (62 versus 64 mg kg^{-1}).

4.2.13.10 Critical effect, NOAELs, ADDs, tissue concentrations

Of the endpoints examined in the present study that were significantly different compared to controls, chick survivability was considered to be the most ecologically relevant to inform development of TRVs. Benchmark modeling using the US Environmental Protection Agency benchmark dose (BMD) software (version 3.1.1; http://www.epa.gov/ncea/bmds) was attempted to derive the lower 95% confidence limit of the BMD (BMDL) for a 20% decrease in chick survivability for PFOA and AFFF PFOS. However, all models were judged by the BMD software as "questionable". For this reason, it was decided that the no adverse effect levels (NOAELs) for the critical effect of chick survivability would be used.

The chick survivability NOAEL for PFOS is 4.1 mg kg $feed^{-1}$ (ADD = 0.55 mg kg body $weight^{-1}$ d^{-1}) and 5.0 mg kg $feed^{-1}$ (ADD = 0.66 mg kg body $weight^{-1}$ d^{-1}) for AFFF PFOS. These values are close to the chick survivability LOAEL (no NOAEL) reported by Newsted et al. (2007) of 10 mg kg $feed^{-1}$ (ADD = 0.77 mg kg body $weight^{-1}$ d^{-1}) for PFOS in northern bobwhite. Serum, liver and egg concentrations corresponding to the chick survivability NOAELs (values for PFOS and AFFF PFOS are averaged to provide single values) in the present study as well PFOS concentrations in the same tissues corresponding to the LOAEL for northern bobwhite chick survivability in the Newsted et al. (2007) study are given in Table 17. Values between the two studies, particularly the ADDs, are in good agreement.

In the study by Dennis et al. (2020), northern bobwhites showed some effects of exposure to PFOS and PFOS:PFHxS by drinking water. The authors reported an adverse effect on adult female body weight gain at an ADD of 3.1 x 10^{-3} mg PFOS:PFHxS kg body $weight^{-1}$ d^{-1} and the inability of pipped chicks to hatch at an ADD of 2.45 x 10^{-3} mg PFOS kg body $weight^{-1}$ d^{-1}. Of the two effects, it would seem that the effect on hatching is more ecologically relevant in terms of a population effect and should be considered the key effect. The corresponding NOAEL is 8.50 x 10^{-5} mg PFOS kg body $weight^{-1}$ d^{-1} (Table 17). This ADD based on drinking water exposure is approximately 8000-fold less than the ADDs based on exposure to PFOS via the feed. Dennis et al. (2020) state that PFAS in water are likely 100% bioaccessible in water compared to food, which may account for the almost 4-factor difference in NOAELs between the two feeding studies and the drinking water study. It will be important to determine the bioavailability of PFOS in water compared to feed to inform which type of ADD should be used for risk assessment purposes. Determination of tissue concentrations of PFOS resulting from exposure via drinking water should help answer this question. It would also be valuable to

establish the concentration of PFOS in drinking water that results in a significant effect on chick survivability for better comparison to feed exposure studies.

4.2.13.11 Toxicity reference values

Avian TRVs for PFOS were published by Newsted et al. (2005) using the chick survivability LOAEL reported by Newsted et al. (2007) and dividing by a total uncertainty factor of 36 based on US Environmental Protection Agency Great Lakes Initiative methodology (US Environmental Protection Agency, 1995). The total uncertainty factor of 36 was comprised of a factor of 6 for interspecies extrapolation, a factor of 6 associated with exposure duration and a factor of 2 to account for use of a LOAEL instead of a NOAEL. In the present study, the total uncertainty factor would be 18 because a NOAEL is available. Using the average of the PFOS and AFFF PFOS NOAELs for chick survivability, this uncertainty factor results in TRVs of 0.26 mg kg $feed^{-1}$ and 0.034 mg kg bw^{-1} d^{-1}. TRVs based on serum and liver concentrations in adult females and chicks and yolk concentrations at the NOAEL for chick survivability are given in Table 17 as are the TRVs from the Newsted et al. (2005) report.

Table 17. NOAELs, LOAELs and TRVs for Japanese quail and northern bobwhites based on avian reproduction studies assessing the effects of PFOS administered in the feed or drinking water

Measure of PFOS exposure	Japanese quail – dietary[a]			Northern bobwhite – dietary[b]			Northern bobwhite – drinking water[c]	
	NOAEL[d]	LOAEL[d]	TRV[d,e]	NOAEL	LOAEL	TRV[f]	NOAEL	LOAEL
Feed or water concentration (mg kg $^{-1}$ or mg L^{-1})	4.6	9.9	0.26	-	10	0.27	18.7 x 10^{-3}	5.96 x 10^{-4}
ADD (mg kg bw^{-1} d^{-1}	0.61	1.3	0.034	-	0.77	0.021	8.50 x 10^{-5}	2.45 x 10^{-3}
Adult female serum (mg L^{-1})	18	35	1.0	-	8.7	0.24	-	-
Adult female liver (mg kg^{-1})	10	18	0.56	-	4.9	0.14	-	-
Chick serum (mg L^{-1})	5.3	13	0.30	-	-	-	-	-
Chick liver (mg kg^{-1})	3.1	6.0	0.17	-	-	-	-	-
Egg (mg kg^{-1})[g]	33	63	1.8	-	62	1.7	-	-

[a]Present study.
[b]Newsted et al. (2005, 2007).
[c]Dennis et al. (2020).
[d]Values are based on the average of PFOS and AFFF PFOS values.
[d]TRVs calculated by dividing NOAEL values by a total uncertainty factor of 18.
[f]TRVs calculated by dividing LOAEL values by a total uncertainty factor of 36.
[g]Based on concentrations in eggs layed during week 10.
NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level; TRV = toxicity reference value; PFOS = perfluorooctane sulfonate; AFFF = aqueous film forming foam; PFHxS = perfluorohexanesulfonate; ADD = average daily dose; bw = body weight

5. Internal Dose Investigation

Because of the apparent reduced toxicity of PFOS+PFOA and AFFF exposure when considering the feed concentrations and subsequent calculated doses, further work was performed on the data collected during the acute trials, looking at internal concentrations and comparing for many of the outcomes. This included calculating the average liver PFOS concentration of quail that died and comparing liver concentrations for the first necropsy to lethality within exposure groups. We limited our analysis to the first necropsy because there was significant depuration of PFOS by the second necropsy (Figure 15). The PFOS liver concentrations for first necropsy and dead quail were then also used to calculate a residue liver concentration resulting in 50% lethality (LR50). The LR50 is calculated in the same manner as the LD50 and LT50 where a probit is calculated and regressed against the log of the concentration.

During the chronic trials quail were exposed through feed to either PFOS or 3M AFFF at PFOS concentrations of 0, 2.1, 4.0, 8.6, 14 and 18 mg PFOS kg feed^{-1} and 0, 2.1, 5.0, 11, 19 and 27 mg AFFF PFOS kg feed^{-1} for a total of 4 weeks. At 4 weeks, quail were sorted by sex and randomly paired (male/female) and tagged. Egg laying began at 10 weeks of age and eggs were collected for the next 10 weeks. Endpoints of exposure observed were feed intake, adult and chick body weight and weight gain, egg production, hatchability, embryo mortality, liver weight and liver and kidney pathology.

5.1 Results

While LC50s and ADD50s resulted in greatest apparent toxicity of PFOS alone, when one looks at the liver concentrations of PFOS in quail exposed to PFOS, PFOS+PFOA and 3M AFFF, there is little variability in the level of PFOS and the lethality of the internal dose (Figure 15). Furthermore, the PFOS liver concentrations are much lower than the PFOA liver concentrations, indicating that PFOS is much more toxic than PFOA to Japanese quail. The PFOS concentrations from the PFOS+PFOA and 3M AFFF exposures are lower than the PFOS alone, indicating that both PFOA and the other PFAS present in AFFF contribute to toxicity. The average liver concentration of PFOS in dead quail are shown in Table 18 and show no significant difference among doses within each trial with one exception. The exception is that the liver concentration of the 1399 µg/g dose of AFFF is significantly lower than for the 164 , 499, and 634 µg/g doses ($\alpha<0.05$). However, when we consider the different trials, the PFOS liver concentration in dead quail from the PFOS+PFOA trial is significantly lower than the liver concentration from the PFOS trial ($p = 0.04$) and the PFOS liver concentration in the AFFF trial is significantly lower than both the PFOS+PFOA trial ($p = 0.009$) and the PFOS trial ($p = 1.8 \times 10^{-6}$).

Table 18. PFOS Liver Concentrations of Dead Quail

PFOS Acute Trial			PFOS + PFOA Trial			AFFF Trial		
PFOS Dose (mg kg feed^{-1})	Liver PFOS conc. (µg/g)	Std. Dev.	PFOS Dose (mg kg feed^{-1})	Liver PFOS conc. (µg/g)	Std. Dev.	AFFF Dose (mg kg feed^{-1})	Liver PFOS conc. (µg/g)	Std. Dev.
216	205	68	92	230	NA*	164	208	33
471	274	81	104	210	90	213	170	75
654	234	107	134	181	32	325	132	28
866	236	85	155	210	86	465	185	81
920	248	101	296	232	107	499	188	79
1955	258	104				634	198	65
						1399	120	36

*Only two values

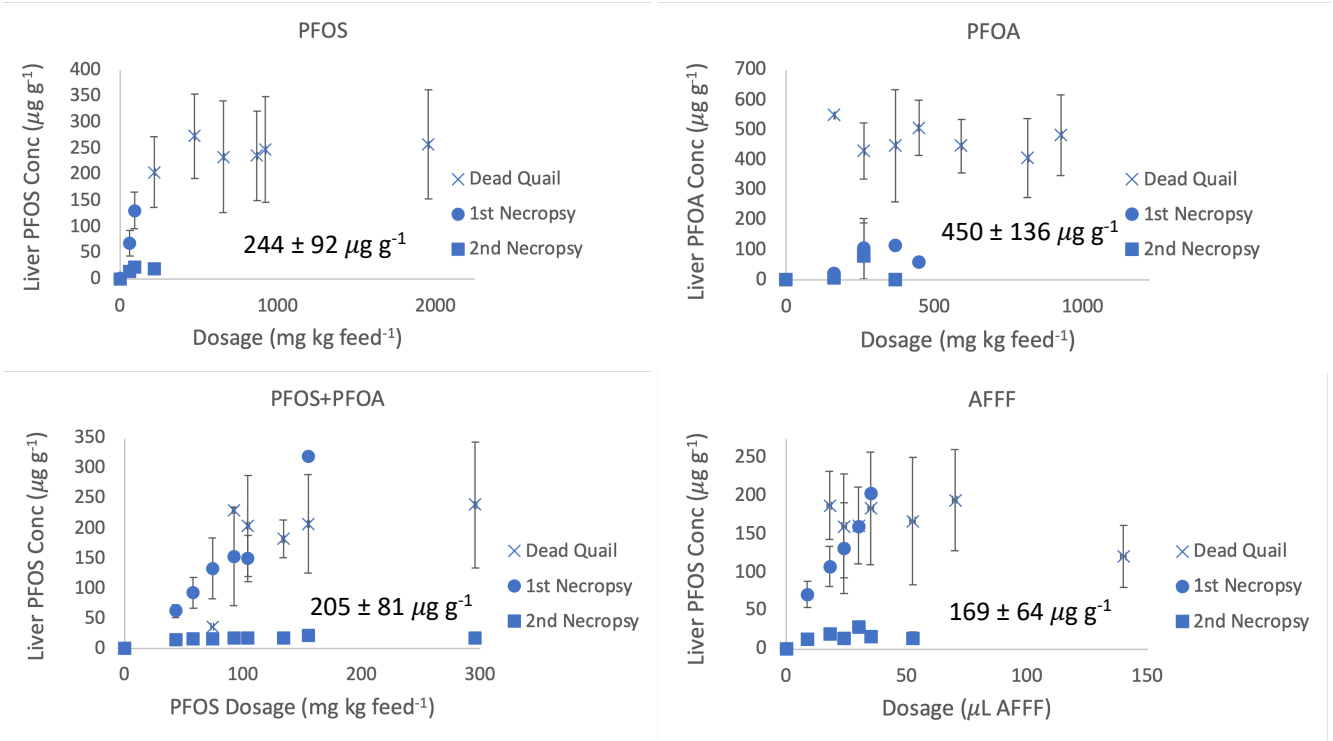


Figure 15. PFOS or PFOA liver concentrations from acute exposures (values are average concentrations of PFAS in dead quail livers)

The LR50 values from these data also show a different interpretation of toxicity than the feed-based LC50s and ADD50s (Table 2). The lowest LR50 is for 3M AFFF, consistent with the liver concentrations from dead quail, but none of the LR50s are statistically different. This lack of significance could be a result of the low numbers of probit values we have in each of the

LR50 plots. Given more data points would allow for more statistical power. Unfortunately, the jump from no death to 100% die off appears to be quite steep causing the low power.

Table 19. LR50 values in Japanese quail from acute exposure to PFAS

Exposure	LR50 (µg/g)	95% C.I.	r^2
PFOS	155	143-169	0.217
PFOA	279	230-338	0.766
PFOS+PFOA*	149	132-168	0.500
3MAFFF*	140	130-152	0.862

*vales are for PFOS

As with the dead quail liver concentrations and the LC50s and ADD50s, the LR50s for PFOA are much higher than PFOS, supporting the argument that PFOA is less toxic in Japanese quail than PFOS.

Of the endpoints for the chronic trial, the only significant effect on reproduction was chick survivability. The survivability was significantly decreased at a PFOS feed concentration of 8.7 mg kg feed^{-1} and AFFF PFOS of 11 mg kg feed^{-1}. These were translated into Toxic Reference Values (TRVs) of 0.25 mg kg feed^{-1} and 0.034 mg kg bw^{-1} d^{-1}. The ability to combine the PFOS and AFFF exposure data into single TRVs was an indication that the differences seen in the acute exposure between PFOS and AFFF exposure was not indicative of differences in internal doses. Unfortunately, the dead chicks were not labeled, so their individual livers could not be analyzed as unique. However, the chicks that were sacrificed at day 14 and weighed were labeled and their livers analyzed for PFOS. Ignoring feed concentration and plotting 14-day body weight vs. liver PFOS concentration indicates a significant negative correlation at liver concentrations greater than 5.5 µg/g (slope = -0.58; CI = -0.81 – -0.35; p = 1.73 x 10^{-6} Figure 16).

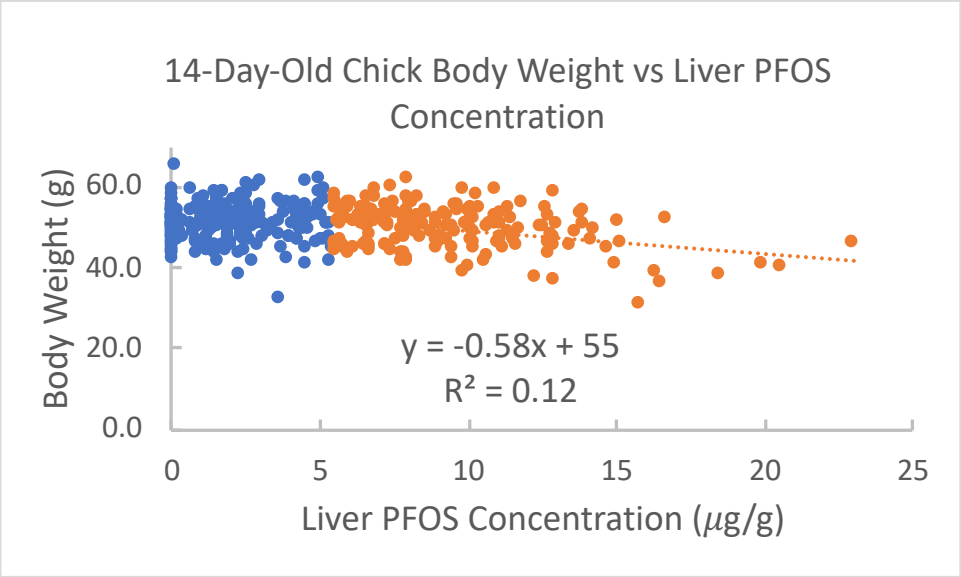


Figure 16. 14-Day old Japanese quail chick body weight as a function of liver PFOS concentration. Blue symbols indicate liver concentrations less than 5.5 µg/g and showed no correlation of body weight to concentration. Red symbols indicate liver concentrations greater than 5.5 µg/g and are included in the regression.

The egg yolk PFOS concentrations from the chronic trials also showed great variability in within and between doses. While, as mentioned above, they generally increased with increasing dose, there were certainly outliers and the variability increased with increasing dose (Figure 17).

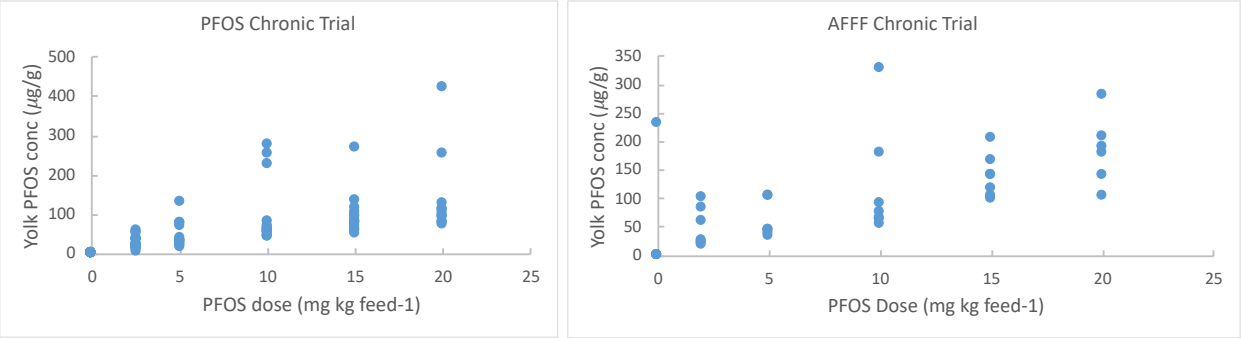


Figure 17. Yolk PFOS Concentrations (µg/g) from PFOS and AFFF Chronic Trials

The AFFF chronic trial values appear to be lower than for the PFOS chronic trial for similar doses. However, only the 15 mg kg feed^{-1} dose is significant ($p = 0.049$). This is certainly because of the great variability within each dose. What is notable is that the vales for yolk concentrations are comparable to the values observed in dead quail liver from the acute trials. This indicates that the hen passes a considerable burden of PFOS to their offspring.

5.2 Conclusions

The data presented on health effects of PFAS on outcomes in both the acute and chronic trials when correlated with feed concentraitons and/or doses does not tell the complete picture of the effects observed in Japanese quail. There is evidence from both the acute and chronic exposures that the internal dose removes some variability seen when considering feed concentration and resulting average daily dose. Furthermore, it appears that PFOS is the dominant PFAS in AFFF with respect to toxicity and that PFOA is much less toxic. Future toxicity studies should prioritize internal doses when considering PFAS toxicity.

6. Conclusions and Implications for Future Research

6.1 Acute Exposure Study

Based on dietary concentrations related to mortality, feed consumption and body and organ weight endpoints, the results of the present study indicate that PFOS is acutely more toxic to Japanese quail than PFOA. Results also suggest that the acute toxicities of PFOS and PFOA are additive. AFFF PFOS was less toxic than PFOS and PFOA based on dietary concentrations and 6:2 FtTAoS provided by Ansul AFFF was not toxic at concentrations fed. However, examination of hepatic concentrations of PFOS, AFFF PFOS and PFOA in birds that died on trial suggests there is a tissue threshold for mortality and the threshold for AFFF PFOS is less than the thresholds for PFOS and PFOA and equivalent to PFOS + PFOA.

6.2 Chronic Exposure Study

Examination of the effects of dietary PFOS and a legacy AFFF containing PFOS AFFF PFOS on reproduction and chick survivability and growth in Japanese quail determined that the

NOAELs associated with chick survivability, which is considered the critical effect, were 4.1 mg PFOS kg feed^{-1} (0.55 mg PFOS kg body weight^{-1} d^{-1}) and 5.0 mg AFFF PFOS kg feed^{-1} (0.66 mg AFFF PFOS kg body weight^{-1} d^{-1}). Toxicity reference values were calculated by averaging the PFOS and AFFF PFOS values and dividing by a total uncertainty factor of 18. Resulting TRVs are 0.25 mg kg feed^{-1} and 0.034 mg kg bw^{-1} d^{-1}, which are similar to TRVs reported by Newsted et al. (2005) for northern bobwhites.

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8. Appendices

8.1 Supporting Data

All effects data is housed in a single Excel Spreadsheet, as that is a much more easily digestible form.

8.1.1 Acute PFOS Exposure Feed Concentration Analysis

Nominal Dose ($\mu g\ g^{-1}$)	Measured Concentration ($\mu g\ g^{-1}$)	Nominal Dose ($\mu g\ g^{-1}$)	Measured Concentration ($\mu g\ g^{-1}$)	Nominal Dose ($\mu g\ g^{-1}$)	Measured Concentration ($\mu g\ g^{-1}$)
Control	0.20	281	216	1125	852
Control	0.18	281	175	1125	946
Control	0.22	281	257	1125	801
70	66.7	561	390	1687	1039
70	67.2	561	357	1687	617
70	52.5	561	666	1687	1104
140	95.4	841	608	2250	1632
140	97.6	841	715	2250	2137
140	80.4	841	640	2250	2096

8.1.2 Acute PFOS Exposure Concentrations

Control Quail				62 $\mu g\ g^{-1}$ Feed Concentration			
Quail #	Liver Conc ($\mu g\ g^{-1}$)	Serum Conc ($\mu g\ g^{-1}$)	Necropsy	Quail #	Liver Conc ($\mu g\ g^{-1}$)	Serum Conc ($\mu g\ g^{-1}$)	Necropsy
21	0.69	0.24	1st	1341	13.1	31.5	2nd
22	0.70	0.41	2nd	1342	16.7	52.3	2nd
23	0.72	0.35	2nd	1343	132	148	1st
24	0.65	0.47	2nd	1344	13.4	30.0	2nd
25	1.17	0.32	2nd	1345	17.9	9.91	2nd
26	0.88	0.36	2nd	1346	11.5	27.1	2nd
27	1.00		1st	1347	65.0	114	1st
28	1.28	0.30	1st	1348		139	1st
29		0.27	1st	1350	52.6	112	1st
30	0.78	0.32	2nd	1351	13.7	21.6	2nd
31	1.14	0.22	1st	1352	52.3	59.1	1st
32		0.34	1st	1353	17.2		2nd
33	0.87		1st	1354	65.2	146	1st
34	0.63	0.48	2nd	1355	69.1	131	1st
35	3.83	0.25	1st	1356	18.5	42.8	2nd
36	1.05	0.35	2nd	1357	64.9	126	1st
37	1.07	0.24	1st	1358	51.3	142	1st
38	1.10	0.24	1st	1359	70.5		1st
39	0.62	0.32	2nd	1360	17.3	30.3	2nd

91 μg g^{-1} Feed Concentration				216 μg g^{-1} Feed Concentration			
Quail #	Liver Conc (μg g^{-1})	Serum Conc (μg g^{-1})	Necropsy	Quail #	Liver Conc (μg g^{-1})	Serum Conc (μg g^{-1})	Necropsy
2741	38.4	54.8	2nd	4141	121		Dead
2742	31.9		2nd	4142	120		Dead
2743	24.6	48.5	2nd	4145	327		Dead
2744	15.4	54.6	2nd	4146	21.4	34.0	2nd
2745	22.4	37.9	2nd	4147	214		Dead
2746	222	600	1st	4150	209		Dead
2747	132	236	1st	4153	230		Dead
2748	118	214	1st	4154	176		Dead
2749	112	247	1st	4155	23.2	44.9	2nd
2750	13.0	25.3	2nd	4159	241		Dead
2751	108		1st	4160	16.9	31.7	2nd
2753	28.6	59.2	2nd				
2754	18.5	33.4	2nd				
2755	137	263	1st				
2756	120	214	1st				
2757	18.0	27.7	2nd				
2758	117	151	1st				
2760	116	156	1st				

*Serum could not be collected from dead quail.

471 μg g^{-1}		654 μg g^{-1}		866 μg g^{-1}		920 μg g^{-1}		1955 μg g^{-1}	
Quail #	Liver Conc (μg g^{-1})	Quail #	Liver Conc (μg g^{-1})	Quail #	Liver Conc (μg g^{-1})	Quail #	Liver Conc (μg g^{-1})	Quail #	Liver Conc (μg g^{-1})
5521	261	6821	472	1361	336	2761	203	4146	386
5527	299	6824	315	1362	289	2763	259	4163	379
5528	486	6825	374	1363	183	2769	95.1	4164	84.4
5530	240	6829	223	1364	135	2773	306	4165	89.4
5531	173	6833	196	1365	257	2774	178	4167	354
5532	264	6835	138	1366	209	2775	185	4168	239
5533	237	6836	138	1367	278	2776	160	4169	298
5535	205	6838	179	1368	198	2777	320	4172	194
5536	253	6839	189	1369	131	2778	306	4176	254
5537	302	6840	171	1375	404	2779	468	4177	314
5540	293	6843	180	1376	178	2780	247	4180	243

*Serum could not be collected from dead quail.

8.1.3 Acute PFOA Exposure Feed Concentration Analysis

Nominal Dose (μg g^{-1})	Measured Concentration (μg g^{-1})	Nominal Dose (μg g^{-1})	Measured Concentration (μg g^{-1})	Nominal Dose (μg g^{-1})	Measured Concentration (μg g^{-1})
Control	4.29	500	424	1250	1267
Control	1.74	500	431	1250	1064
Control	1.91	500	370	1250	1284
200	154	750	452	1500	1236
200	163	750	649	1500	1504
200	163	750	525	1500	1446
350	282	1000	612		
350	235	1000	660		
350	143	1000	769		

8.1.4 Acute PFOA Exposure Concentrations

Control Quail				162 μg g^{-1} Feed Concentration			
Quail #	Liver Conc (μg g^{-1})	Serum Conc (μg g^{-1})	Necropsy	Quail #	Liver Conc (μg g^{-1})	Serum Conc (μg g^{-1})	Necropsy
41	0.60	3.67	1st	1381	41.1	173	1st
42	0.36	0.09	2nd	1383	25.3	97.5	1st
43	0.40	0.24	2nd	1384	18.2	70.2	
44	0.39	0.09	2nd	1385	0.56	1.25	2nd
45	0.41	3.96	1st	1386	25.2	77.1	1st
46	0.38	4.54	1st	1387	0.52	0.62	2nd
47	0.41	0.20	2nd	1388	0.85	1.05	2nd
48	0.48	0.14	2nd	1389	35.4	85.8	1st
49	0.69	5.44	1st	1390	4.13	11.5	2nd
50	0.48	0.06	2nd	1391	0.40	0.86	2nd
51	0.47	4.16	1st	1392	37.3	7.72	2nd
52	0.44	3.46	1st	1393	17.3		1st
53	0.53	4.22	1st	1394	0.70	0.49	2nd
54	0.45	0.21	2nd	1395	0.56	0.84	2nd
55	0.44	0.19	2nd	1396	16.0	62.4	1st
56	0.60	0.09	2nd	1397	15.6	374	1st
57	0.21	4.28	1st	1398	9.06	5.66	2nd
58	0.74	0.27	2nd	1399	8.86	24.9	1st
59	0.50	4.32	1st	1400	0.54	0.79	2nd
60	0.49	3.99	1st				

262 μg g^{-1} Feed Concentration				368 μg g^{-1} Feed Concentration			
Quail #	Liver Conc (μg g^{-1})	Serum Conc (μg g^{-1})	Necropsy	Quail #	Liver Conc (μg g^{-1})	Serum Conc (μg g^{-1})	Necropsy
2781	1.62	3.58	2nd	4181	212	272	1st
2784	26.8	68.6	1st	4182	488		Dead
2785	549		1st	4184	0.40	0.64	2nd
2787	15.8	66.7	1st	4186	461		Dead
2788	4.27	10.4	2nd	4187	320		Dead
2789	284	270	1st	4190	476		Dead
2790	0.81	0.42	2nd	4191	527		Dead
2791	128	370	1st	4192	0.65	1.03	2nd
2792	128	1.39	2nd	4195	304		Dead
2793	192	173	1st	4196	16.9	43.8	1st
2794	0.69	0.68	2nd	4199	0.95	1.91	2nd
2795	88.4	3.86	1st				
2796	1.49	3.98	2nd				
2798	23.9	93.1	1st				
2799	0.47	0.88	2nd				
2800	0.63	1.58	2nd				

447 μg g^{-1} Feed Concentration				590 μg g^{-1}		814 μg g^{-1}	
Quail #	Liver Conc (μg g^{-1})	Serum Conc (μg g^{-1})	Necropsy	Quail #	Liver Conc (μg g^{-1})	Quail #	Liver Conc (μg g^{-1})
5542	369		Dead	6841	496	1504	620
5546	314		Dead	6842	491	1505	383
5547	359		Dead	6843	481	1506	441
5549	348		Dead	6846	504	1507	584
5550	582		Dead	6847	464	1509	392
5551	328		Dead	6850	739	1512	21
5554	894		Dead	6851	439	1514	387
5555	455		Dead	6852	607	1515	385
5556	380		Dead	6856	425	1518	395
5557	31.6	79.6	1st	6857	428	1519	387
5558	85.7	87.5	1st	6860	503	1520	488

926 μg g^{-1}		1208 μg g^{-1}	
Quail #	Liver Conc (μg g^{-1})	Quail #	Liver Conc (μg g^{-1})
2901	665	4301	303
2902	401	4302	544
2903	538	4303	669
2904	354	4304	657
2905	181	4305	529
2908	292	4306	296
2909	301	4307	459
2912	409	4309	634
2913	381	4310	451
2916	443	4311	397
2917	505	4315	378

8.1.5 Acute PFOS + PFOA Exposure Feed Concentration Analysis

Nominal Dose Each (μg g^{-1})	Measured PFOS Concentration (μg g^{-1})	Measured PFOA Concentration (μg g^{-1})	Nominal Dose Each (μg g^{-1})	Measured PFOS Concentration (μg g^{-1})	Measured PFOA Concentration (μg g^{-1})
Control	0.71	1.14	150	113	95.78
Control	0.70	1.20	150	65.0	58.35
Control	0.73	1.25	150	256	191.09
50	42.8	36.22	175	194	114.16
50	41.5	36.72	175	148	132.97
50	38.4	33.70	175	153	156.09
75	62.1	49.12	200	188	166.23
75	60.9	50.45	200	174	140.41
75	70.0	60.51	200	178	146.30
100	64.5	56.75	400	330	241.75
100	120	101.34	400	325	266.68
100	76.7	65.83	400	291	238.01
135	97.2	91.75			
135	116	98.58			
135	113	102.72			

8.1.6 Acute PFOS + PFOA Exposure Liver and Serum Concentration Analysis

Control Quail					
Quail #	Necropsy	Liver PFOS Conc (μg g^{-1})	Liver PFOA Conc (μg g^{-1})	Serum PFOS Conc (μg g^{-1})	Serum PFOA Conc (μg g^{-1})
61	2nd			0.060	0.061
62	2nd			0.075	0.083
63	2nd			0.069	0.161
64	2nd	0.281	0.091	0.076	0.075
65	2nd			0.059	0.041
66	1st	0.323	0.033	5.081	4.475
67	1st	0.637	0.838	4.929	4.065
68	1st	0.276	0.330	5.485	3.795
69	2nd			0.055	0.116
70	1st	0.363	0.193	4.07	3.823
71	1st	0.320	0.314	4.24	4.355
72	1st	0.586	0.687	3.66	3.925
73	1st	0.383	0.514	4.63	4.493
75	2nd	0.775	0.092	0.05	0.038
76	2nd	0.343	0.110	0.06	0.278
78	2nd	0.323	0.064	0.25	0.048
79	1st	0.882	0.918	4.51	4.116
80	2nd			0.07	0.165

43/45 μg g^{-1} Feed Concentration					
Quail #	Necropsy	Liver PFOS Conc (μg g^{-1})	Liver PFOA Conc (μg g^{-1})	Serum PFOS Conc (μg g^{-1})	Serum PFOA Conc (μg g^{-1})
1401	2nd			19.7	0.115
1402	1st	83.5	17.2	435	213
1403	2nd	9.38	0.101	13.6	0.110
1404	2nd	20.6	0.059	26.1	0.081
1405	1st	49.9	0.578	429	14.7
1406	2nd	11.1	0.107	21.3	0.073
1407	2nd		5.40	42.4	0.073
1408	2nd		2.62	21.9	0.977
1409	1st	58.2	5.08	98.1	27.2
1411	1st	62.3	0.672	88.6	22.8
1412	1st	44.8	11.3	107	7.641
1413	1st		1.06	16.7	0.096
1414	1st	61.1	3.57	91.7	45.1
1415	2nd		14.2	20.1	0.487
1417	2nd	59.1	17.2	72.0	14.8
1418	2nd	71.8	0.101	24.8	0.286
1419	1st	67.7	0.059	76.2	134.4
1420	2nd			19.9	0.050

58/62 μg g^{-1} Feed Concentration					
Quail #	Necropsy	Liver PFOS Conc (μg g^{-1})	Liver PFOA Conc (μg g^{-1})	Serum PFOS Conc (μg g^{-1})	Serum PFOA Conc (μg g^{-1})
2801	2nd			35.6	4.49
2802	2nd			25.5	0.060
2803	1st	86.6	4.51	154	26.0
2804	2nd	14.2	0.310	23.8	0.290
2806	1st	82.2	26.7	109	83.6
2807	2nd			38.2	0.659
2808	2nd			84.1	0.261
2809	1st	67.0	35.8	116	119
2810	1st	141.6	41.2	109	91.05
2812	2nd			30.7	0.072
2813	1st	71.1	1.95	139	13.7
2814	2nd	14.9	0.449	20.2	0.511
2815	2nd		8.49	33.4	2.43
2816	1st	82.8	7.93	105	33.0
2817	1st	131	6.02	102	22.6
2818	1st	78.0		156	31.4
2819	2nd			15.6	0.086
2820	2nd	16.6	0.095	22.6	0.079

74/79 μg g^{-1} Feed Concentration					
Quail #	Necropsy	Liver PFOS Conc (μg g^{-1})	Liver PFOA Conc (μg g^{-1})	Serum PFOS Conc (μg g^{-1})	Serum PFOA Conc (μg g^{-1})
4201	2nd	0.140	21.3	39.9	0.190
4202	1st	13.8	108	138	48.94
4203	2nd	0.600	19.3	25.1	1.73
4204	1st	52.2	95.7	169	26.76
4205	1st	38.8	130	225	173.5
4206	1st	54.0	250	203	111.4
4207	2nd			33.0	0.451
4209	2nd	0.560	17.7	24.8	1.52
4210	2nd	0.261	15.4	21.2	0.834
4212	2nd			22.8	0.177
4214	2nd	0.658	13.0	23.3	2.45
4215	2nd	0.344	14.0	21.4	0.957
4216	1st	10.1	67.9	109	34.67
4217	1st	4.98	141	159	15.81
4218	1st	7.89	125	206	41.18
4219	1st	52.6	143	120	131.9
4220	2nd			27.3	0.086

92/90 µg g^{-1} Feed Concentration					
Quail #	Necropsy	Liver PFOS Conc (µg g^{-1})	Liver PFOA Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})	Serum PFOA Conc (µg g^{-1})
5561	1st	111	9.42		
5562	2nd	17.7	6.02	21.1	16.2
5563	Died	263	143		
5564	2nd	20.2	0.27	57.3	0.787
5565	1st	120	14.9	195	55.2
5566	2nd	18.8	1.35	29.0	5.08
5567	1st	355	177	132	158
5568	Died	197	154		
5570	2nd	12.2	3.50	16.8	11.0
5571	2nd	17.9	5.03	34.3	15.3
5572	1st	116	22.0	175	79.5
5573	2nd			32.4	0.166
5574	2nd			35.7	0.091
5575	1st	114	63.1		
5576	1st	182	80.3		
5577	1st	167	57.0	173	177
5578	2nd	20.3	1.11	44.6	5.09
5579	1st	76.3	32.5		
5580	1st	137	79.7	118	199

104/102 μg g^{-1} Feed Concentration					
Quail #	Necropsy	Liver PFOS Conc (μg g^{-1})	Liver PFOA Conc (μg g^{-1})	Serum PFOS Conc (μg g^{-1})	Serum PFOA Conc (μg g^{-1})
6861	1st	129	63.7	150	165
6862	Died	364	171.4		
6863	Died			28.4	1.13
6864	2nd	24.6	0.164	30.8	0.318
6865	2nd	7.11	0.118	14.0	0.120
6866	1st	233	119	288	297
6867	1st			19.9	0.148
6868	1st	117	7.34		
6869	Died	171	135		
6870	2nd	16.7	0.360	24.0	0.374
6871	Died	163	95.9		
6872	2nd	19.7	0.336	25.5	0.796
6873	Died	275	125		
6874	1st	125	105	133	150
6875	1st	141	81.2		
6876	Died	155	124		
6877	Died	135	90.0		
6878	Died	166	94.4		
6879	1st	154	8.00	131	19.3
6880	1st	149	53.4		

134/145 μg g^{-1} Feed Concentration					
Quail #	Necropsy	Liver PFOS Conc (μg g^{-1})	Liver PFOA Conc (μg g^{-1})	Serum PFOS Conc (μg g^{-1})	Serum PFOA Conc (μg g^{-1})
1521	2nd	21.4	1.03	31.0	3.92
1522	Died	159	79.5		
1523	Died	224	167.1		
1524	Died	137	140.8		
1525	Died	160	90.7		
1526	Died	182	97.4		
1527	Died	153	65.7		
1528	Died	212	116.6		
1529	Died	221	146.1		
1530	Died	208	122.6		
1531	Died	188	78.4		
1532	Died	165	108.5		
1534	Died	217	120.1		
1535	Died	143	77.2		
1539	2nd	21.8	0.152	39.0	0.336

155/164 μg g^{-1} Feed Concentration					
Quail #	Necropsy	Liver PFOS Conc (μg g^{-1})	Liver PFOA Conc (μg g^{-1})	Serum PFOS Conc (μg g^{-1})	Serum PFOA Conc (μg g^{-1})
2921	Died	286	134		
2922	Died	218	106		
2923	Died	174	93.6		
2924	Died	214	111		
2925	Died	133	106		
2926	Died	159	96.9		
2927	2nd	19.4	0.469	26.4	1.77
2928	Died	158	131		
2929	Died	384	242		
2930	1st	159	10.7	147	28.0
2931	Died	141	108		
2932	Died	114	96.6		
2936	Died	188	118		
2938	Died	287	195		
2939	2nd	14.7	0.121	25.0	0.183
2940	Died	319	177		

296/292 μg g^{-1} Feed Concentration			
Quail #	Necropsy	Liver PFOS Conc (μg g^{-1})	Liver PFOA Conc (μg g^{-1})
4321	Died	377	173
4322	Died	165	75.3
4323	Died	257	180
4324	Died	408	180
4325	Died	124	112
4326	Died	242	109
4327	Died	134	133
4331	Died	209	167
4336	Died	239	175
4337	Died	103	83.2
4338	Died	144	95.5
4339	Died	325	205
4340	Died	379	190

8.1.7 Acute 3M AFFF Exposure Liver and Serum PFOS Concentration Analysis

Control			
Quail #	Necropsy	Liver PFOS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})
81	1st	0.239	0.145
82	2nd	0.097	0.133
83	1st	0.331	
84	1st	0.099	0.134
85	2nd	0.152	0.094
86	2nd	0.128	0.080
87	2nd		0.086
88	2nd	0.145	0.103
89	1st	0.160	0.275
90	1st	0.168	0.161
91	1st	0.248	0.099
92	2nd	0.267	0.076
93	2nd		0.922
94	2nd	0.166	0.094
95	1st		0.121
96	2nd		0.089
97	1st	0.383	0.141
98	1st	0.290	0.122
99	1st	0.267	0.151
100	2nd		0.092

8.8 µL g^{-1}			
Quail #	Necropsy	Liver PFOS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})
2821	2nd	9.86	35.6
2822	1st	41.6	96.1
2823	1st	73.4	80.9
2824	2nd	9.68	25.8
2825	2nd	16.8	40.0
2826	1st	61.3	94.8
2827	1st	79.1	142
2829	2nd	8.98	27.9
2830	2nd	14.0	22.2
2831	2nd	17.6	27.1
2832	2nd		16.5
2833	1st	106	108
2834	1st	71.1	107
2835	1st	78.7	118
2836	1st	88.8	
2837	1st	72.4	
2838			11.3
2839			15.9
2840	1st	57.9	106

18 µL g^{-1}			
Quail #	Necropsy	Liver PFOS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})
1422	Died	243	
1423	1st	139	113
1424	Died	176	
1425	2nd	19.4	21.6
1428	Died	122	
1429	Died	192	
1430	Died	206	
1431	2nd	20.4	23.3
1432	2nd	22.8	27.4
1433	2nd	13.9	35.6
1434	1st	73.0	148
1435	1st	93.7	122
1436	1st	23.4	50.6
1437	1st	90.8	131
1438	1st	120	132
1439	1st	131	148
1440	2nd	18.9	30.9

24 µL g^{-1}			
Quail #	Necropsy	Liver PFOS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})
4221	Died	138	
4223	2nd	18.2	31.3
4224	Died	133	
4225	2nd	14.2	42.3
4226	Died	223	
4227	Died	121	
4228	1st	67.4	130
4229	Died	184	
4230	1st	145	215
4234	Died	76.6	
4235	2nd	12.7	33.4
4236	Died	142	
4237	Died	233	
4238	Died	279	
4239	1st	103	
4240	2nd	10.3	18.6

30 µL g^{-1}			
Quail #	Necropsy	Liver PFOS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})
5581	Died	190	
5582	2nd	22.6	31.6
5583	Died	208	
5585	Died	114	
5586	Died	176	
5587	Died	143	
5590	2nd	35.6	48.6
5591	Died	109	
5594	Died	95.5	
5595	Died	178	
5596	Died	153	
5597	Died	118	
5598	Died	252	
5599	Died	234	
5600	Died	126	

35 µL g^{-1}			
Quail #	Necropsy	Liver PFOS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})
2941	Died	134	
2944	Died	186	
2945	1st	204	181
2948	Died	160	
2949	Died	126	
2952	2nd	16.1	37.3
2953	Died	239	
2954	Died	117	
2955	Died	178	
2956	Died	283	
2957	Died	138	
2958	Died	124	
2959	Died	340	

52.5 µL g^{-1}			
Quail #	Necropsy	Liver PFOS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})
1541	Died	258	
1542	Died	98.6	
1545	Died	220	
1546	Died	76.4	
1547	2nd	14.6	39.0
1550	Died	301	
1551	Died	133	
1553	Died	205	
1554	Died	221	
1555	Died	199	
1558	Died	187	
1559	Died	98.2	

70 µL g^{-1}			140 µL g^{-1}		
Quail #	Necropsy	Liver PFOS Conc (µg g^{-1})	Quail #	Necropsy	Liver PFOS Conc (µg g^{-1})
4341	Died	259	5601	Died	89.5
4342	Died	257	5602	Died	115
4344	Died	129	5603	Died	184
4345	Died	199	5609	Died	135
4346	Died	153	5610	Died	78.3
4347	Died	277	5611	Died	65.8
4348	Died	106	5613	Died	114
4349	Died	210	5615	Died	110
4350	Died	271	5617	Died	145
4351	Died	182	5619	Died	105
4353	Died	99.8	5620	Died	192

8.1.8 3M AFFF Analysis

Sample:	1	4	7
Analyte	Concentration (µg/mL)	Concentration (µg/mL)	Concentration (µg/mL)
PFBS	212	225	244
PFHxS	647	681	734
PFOS	9464	9985	9754
PFHpA	65	55	52
PFOA	344	377	324
PFNA	19	20	17

8.1.9 Ansul AFFF Analysis

Analyte	Concentration (µg/mL)	Analyte	Concentration (µg/mL)
PFBA	0.697	PFHpS	0.084
PFPeA	0.737	PFDA	0.292
4:2 FTS	0.032	PFOS	0.125
PFHxA	0.000	PFUnA	0.036
PFBS	0.131	PFNS	0.105
PFHpA	0.295	PFDoA	0.117
PFPeS	0.028	NMeFOSAA	0.055
6:2 FTS	7.334	PFDS	0.090
PFOA	5.507	NEtFOSAA	0.021
PFHxS	0.040	PFTriA	0.045
PFNA	0.081	PFTetA	0.070
8:2 FTS	4.276	FOSA	0.050

8.1.10 Chronic PFOS Exposure Feed Concentration Analysis

PFOS Starter Diet - mixed 13 Oct 2017		PFOS Second Starter Diet - mixed 16 Jan 2018		PFOS Layer Diet - mixed 2 Feb 2018	
Nominal Concentraiton ($\mu g\ g^{-1}$)	PFOS Concentration ($\mu g\ g^{-1}$)	Nominal Concentraiton ($\mu g\ g^{-1}$)	PFOS Concentration ($\mu g\ g^{-1}$)	Nominal Concentraiton ($\mu g\ g^{-1}$)	PFOS Concentration ($\mu g\ g^{-1}$)
Control	0	Control	0	Control	0
Control	0	Control	0	Control	0
Control	0	Control	0	Control	0
2.5	1.75	2.5	1.91	2.5	2.33
2.5	2.07	2.5	2.40	2.5	3.98
2.5	2.26	2.5	2.14	2.5	4.29
5	3.61	5	4.79	5	8.21
5	3.94	5	4.76	5	8.31
5	3.68	5	5.23	5	9.12
10	7.53	10	9.55	10	11.4
10	7.62	10	11.1	10	13.9
10	9.5	10	9.61	10	11.3
15	12.6	15	12.8	15	16.9
15	18.4	15	14.3	15	15.3
15	13.6	15	13.8	15	15.8
20	17.7	20	17.0	20	0
20	19.0	20	19.9	20	
20	16.5	20	23.7	20	

8.1.11 Chronic AFFF Exposure Feed Concentration Analysis

June 6, 2018 Feed		August 2, 2018 Feed	
Nominal Concentraiton ($\mu g\ g^{-1}$)	PFOS Concentration ($\mu g\ g^{-1}$)	Nominal Concentraiton ($\mu g\ g^{-1}$)	PFOS Concentration ($\mu g\ g^{-1}$)
Control	0.011	Control	0.006
Control	0.008	Control	0.004
Control	0.008	Control	0.000
2.5	1.71	2.5	2.51
2.5	2.37	2.5	2.43
2.5	2.24	2.5	1.61
5	3.70	5	4.83
5	11.5	5	2.07
5	4.84	5	3.06
10	9.66	10	10.9
10	11.2	10	9.88
10	12.8	10	10.0
15	11.3	15	16.0
15	19.6	15	21.8
15	26.0	15	16.8
20	20.8	20	21.9
20	37.9	20	23.8
20	26.3	20	28.4

8.1.12 Chronic PFOS Adult Serum and Liver Concentrations

Control			
Quail	Gender	Serum Conc ($\mu g\ g^{-1}$)	Liver Conc ($\mu g\ g^{-1}$)
153	Male	0.407	0.026
154	Female	0.335	0.011
155	Male	0.354	0.063
156	Female	0.421	0.107
157	Male	0.621	0.354
158	Female	0.582	0.693
167	Male	0.809	0.124
168	Female	0.342	0.093
169	Male	0.692	0.120
170	Female	0.391	0.030
172	Female	0.043	0.027

2.5 µg g^{-1}			
Quail	Gender	Serum Conc (µg g^{-1})	Liver Conc (µg g^{-1})
4381	Male	61.2	17.1
4382	Female	7.16	
4383	Male	41.5	
4385	Male	32.0	18.1
4387	Male	93.6	
4388	Female	4.70	2.64
4389	Male	57.8	
4390	Female	41.8	17.4
4392	Female	3.34	1.78
4394	Female	4.00	2.32
4398	Female	3.68	
4399	Male	78.5	21.6
4401	Male	57.5	18.6
4405	Male	45.0	16.1
4406	Female	46.8	15.8
4409	Male	40.7	2.54
4410	Female	3.96	17.1

5 µg g^{-1}			
Quail	Gender	Serum Conc (µg g^{-1})	Liver Conc (µg g^{-1})
2941	Male	110	
2961	Male	254	
2962	Female	43.5	6.49
2963	Male	99.3	28.2
2964	Female	11.7	4.09
2965	Male	200	
2966	Female	14.8	3.41
2967	Male	176	44.6
2968	Female	11.0	4.71
2969	Male	93.2	39.1
2970	Female	7.59	4.15
2973	Male	100	40.2
2974	Female	17.5	7.16
2975	Male	71.3	30.3
2976	Female	8.30	
2978	Female	66.4	14.0

10 µg g^{-1}			
Quail	Gender	Serum Conc (µg g^{-1})	Liver Conc (µg g^{-1})
1582	Female	179	20.9
1583	Male	207	67.2
1584	Female	18.4	
1586	Female	34.6	
1587	Male	141	74.5
1588	Female	18.8	
1589	Male	277	
1596	Female	25.7	8.69
1597	Male	288	60.0
1598	Female	23.2	12.4
1599	Male	164	59.6
1600	Female	24.9	10.5
1602	Female	16.9	11.4
1604	Female	15.2	8.50
1605	Male	145	

15 µg g^{-1}			
Quail	Gender	Serum Conc (µg g^{-1})	Liver Conc (µg g^{-1})
6933	Male	182	89.8
6934	Female	29.2	12.8
6935	Male	377	103
6936	Female	20.7	12.5
6937	Male	242	65.8
6940	Female	18.9	10.9
6941	Male	242	112
6942	Female	29.2	9.1
6950	Female	48.1	9.9
6951	Male	201	108
6958	Female	26.9	13.4

20 µg g^{-1}			
Quail	Gender	Serum Conc (µg g^{-1})	Liver Conc (µg g^{-1})
5673	Male	235	87.4
5674	Female	56.0	22.4
5675	Male	369	144
5678	Female	46.3	14.7
5679	Male	302	90.8
5681	Male	519	
5682	Female	33.7	15.5
5683	Male	270	
5690	Female	26.8	13.3
5691	Male	221	56.0
5692	Female	8.90	
5693	Male	277	
5694	Female	37.8	22.2
5695	Male	275	116
5696	Female	26.1	12.9
5698	Female	38.5	

8.1.13 Chronic PFOS Chick Serum and Liver Concentrations

Hatch	Dose	Serum Conc ($\mu g\ g^{-1}$)	Liver Conc ($\mu g\ g^{-1}$)
1	0	0.056	0.26
1	0	0.097	0.21
1	2.5	5.15	4.15
2	0	0.011	0.15
2	0	0.014	0.12
2	0	0.011	0.11
2	0	0.012	0.17
2	2.5	2.85	2.06
2	2.5	1.70	1.29
2	2.5	2.98	2.47
2	2.5	1.62	1.20
2	5	9.14	7.31
2	5	4.35	3.93
2	5	5.16	4.25
2	5	3.37	2.74
2	10	9.60	5.77
2	10	15.8	9.50
2	15	6.89	6.66
2	15	8.70	5.34
2	15	8.95	4.56
2	15	9.57	6.52
2	20	13.6	9.49
2	20	17.9	7.27
3	0	0.013	0.42
3	0	0.012	0.26
3	0	0.020	0.25
3	0	0.013	0.14
3	2.5	1.83	1.47
3	2.5	1.59	1.74
3	2.5	1.91	0.88
3	2.5	2.11	1.83
3	5	8.55	5.63
3	5	3.21	2.48
3	5	2.20	1.83
3	5	2.65	1.71
3	10	9.42	4.68
3	10	5.87	3.70
3	10	6.50	4.45
3	10	10.3	4.87
3	15	9.98	7.42
3	15	6.39	6.66

Hatch	Dose	Serum Conc ($\mu g\ g^{-1}$)	Liver Conc ($\mu g\ g^{-1}$)
3	15	9.70	9.21
3	15	14.2	10.20
3	20	15.5	7.58
3	20	12.6	8.59
3	20	17.0	7.86
3	20	13.2	7.02
4	0	0.020	0.03
4	0	0.009	0.03
4	0	0.007	0.02
4	0	0.024	0.02
4	2.5	1.23	0.94
4	2.5	2.08	1.22
4	2.5	2.91	1.97
4	2.5	3.75	2.85
4	5	2.32	1.72
4	5	3.22	2.23
4	5	3.24	2.36
4	5	4.36	2.14
4	10	8.72	7.30
4	10	5.17	3.08
4	10	7.62	4.95
4	10	8.97	6.64
4	15	5.90	4.15
4	15	11.5	8.14
4	15	11.5	9.87
4	15	9.10	7.94
4	20	15.7	10.68
4	20	10.5	6.66
4	20	12.4	8.50
4	20	22.1	12.74
5	0	0.010	0.03
5	0	0.007	0.02
5	0	0.022	0.02
5	0	0.010	0.02
5	2.5	1.96	1.77
5	2.5	1.35	0.76
5	2.5	2.04	1.13
5	2.5	1.55	0.90
5	5	3.38	1.39
5	5	2.85	1.69
5	5	2.89	2.72

Hatch	Dose	Serum Conc ($\mu g\ g^{-1}$)	Liver Conc ($\mu g\ g^{-1}$)
5	5	4.34	2.27
5	10	5.74	4.27
5	10	8.54	5.53
5	10	9.76	6.93
5	10	8.16	7.75
5	15	7.63	6.01
5	15	10.7	7.34
5	15	8.65	7.58
5	15	15.5	11.75
5	20	12.6	6.63
5	20	17.3	11.37
5	20	28.5	11.67
5	20	13.5	6.19
6	0	0.023	0.03
6	0	0.016	0.33
6	0	0.026	0.38
6	0	0.034	0.03
6	2.5	1.83	1.13
6	2.5	1.52	1.24
6	2.5	1.08	0.69
6	2.5	2.67	1.17
6	5	3.79	1.96
6	5	4.60	2.35
6	5	3.83	2.94
6	5	5.58	2.59
6	10	8.95	5.25
6	10	13.2	5.94
6	10	10.1	6.47
6	10	9.06	6.71
6	15	13.2	10.64
6	15	13.8	8.93
6	15	15.6	10.16
6	15	14.2	5.78
6	20	18.8	10.54
6	20	13.5	15.78
6	20	23.5	7.21
6	20	11.3	12.28
7	0	0.027	0.05
7	0	0.034	0.03
7	0	0.020	0.03
7	0	0.019	0.04

Hatch	Dose	Serum Conc ($\mu g\ g^{-1}$)	Liver Conc ($\mu g\ g^{-1}$)
7	2.5	1.87	0.71
7	2.5	3.67	1.76
7	2.5	1.72	0.81
7	2.5	2.07	0.99
7	5	6.37	3.37
7	5	5.11	2.57
7	5	7.53	2.51
7	5	4.01	1.56
7	10	8.79	6.76
7	10	8.39	5.78
7	10	9.82	2.86
7	10	10.5	5.09
7	15	17.0	9.00
7	15	20.3	7.97
7	15	17.2	5.75
7	15	11.3	10.37
7	20	20.2	9.10
7	20	28.4	13.02
7	20	8.15	5.17
7	20	23.4	5.97
8	0	0.032	0.03
8	0	0.020	0.05
8	0	0.030	0.03
8	0	0.024	0.03
8	2.5	2.14	1.59
8	2.5	1.94	1.30
8	2.5	2.23	1.25
8	2.5	2.16	1.43
8	5	5.14	2.17
8	5	6.44	2.76
8	5	6.19	1.98
8	5	3.49	2.23
8	10	7.96	4.67
8	10	11.4	8.99
8	10	12.3	5.10
8	10	10.6	4.16
8	15	20.6	10.78
8	15	14.1	8.18
8	15	20.0	13.46
8	15	21.4	5.72
8	20	19.0	14.23

Hatch	Dose	Serum Conc ($\mu g\ g^{-1}$)	Liver Conc ($\mu g\ g^{-1}$)
8	20	14.2	11.23
8	20	17.8	9.49
8	20	24.9	10.23
9	0	0.016	0.015
9	0	0.022	0.033
9	0	0.029	0.014
9	0	0.015	0.013
9	2.5	2.30	1.61
9	2.5	1.44	1.16
9	2.5	1.93	1.27
9	2.5	3.41	2.32
9	5	4.05	2.60
9	5	3.38	1.98
9	5	3.07	2.01
9	5	5.93	3.49
9	10	8.82	4.32
9	10	10.6	5.89
9	10	6.62	4.53
9	10	9.53	5.16
9	15	17.3	7.22
9	15	11.6	7.27
9	15	11.2	7.41
9	15	11.4	7.13
9	20	13.6	6.89
9	20	13.0	8.39
9	20	22.2	11.51
9	20	18.7	10.97
10	0	0.031	0.022
10	0	0.020	0.015
10	0	0.028	0.019
10	0	0.027	0.027
10	2.5	3.07	0.972
10	2.5	1.92	0.885
10	2.5	1.25	0.752
10	2.5	4.41	1.40
10	5	4.60	2.46
10	5	4.75	2.89
10	5	7.23	2.36
10	5	5.86	2.02
10	10	8.14	4.42
10	10	9.39	4.46

Hatch	Dose	Serum Conc ($\mu g\ g^{-1}$)	Liver Conc ($\mu g\ g^{-1}$)
10	10	11.2	5.68
10	10	14.6	5.56
10	15	27.1	13.7
10	15	15.8	8.27
10	15	15.1	6.05
10	15	16.3	9.96
10	20	15.9	7.84
10	20	14.6	6.89
10	20	16.4	6.59
10	20	17.9	8.79

8.1.14 Chronic PFOS Egg White and Yolk Concentrations

ID	Date Collected	Dose	Yolk Conc ($\mu g\ g^{-1}$)	White Conc ($\mu g\ g^{-1}$)	ID	Date Collected	Dose	Yolk Conc ($\mu g\ g^{-1}$)	White Conc ($\mu g\ g^{-1}$)
158	5/1/18	0	0.057	0.001	2964	3/17/18	5	15.1	
166	5/1/18	0	0.023		2964	3/25/18	5	22.9	
172	2/23/18	0	0.076	0.001	2964	4/19/18	5	19.4	
172	3/3/18	0	0.032		2970	5/1/18	5	22.4	0.016
172	3/7/18	0	0.028		2972	2/23/18	5		0.021
172	4/15/18	0	0.030		2972	2/26/18	5	73.7	
172	4/24/18	0	0.047		2974	2/24/18	5	127	0.044
172	4/26/18	0	0.056	0.015	2976	5/1/18	5	27.7	0.041
174	2/25/18	0	0.045	0.001	2982	5/1/18	5	28.9	0.016
176	2/22/18	0	0.068	0.001	2982	3/5/19	5	73.7	
178	2/20/18	0	0.089	0.002	2986	2/26/18	5	66.4	0.015
180	5/1/18	0	0.044	0.001	2988	3/1/18	5	29.6	0.017
1584	3/2/18	10	250	2.74	2988	4/24/18	5	31.2	0.042
1594	2/26/18	10	225	0.144	2992	4/25/18	5	30.2	0.019
1594	5/1/18	10	45.0	0.024	4382	2/20/18	2.5	51.2	0.033
1598	3/4/19	10	274	0.137	4382	2/22/18	2.5	39.0	0.007
1600	5/1/18	10	52.8	0.020	4382	2/23/18	2.5	32.7	0.020
1604	3/4/18	10	69.2	6.36	4382	2/24/18	2.5	23.4	0.010
1604	3/9/18	10	54.2		4386	3/3/18	2.5	18.0	
1604	3/11/18	10	39.6		4390	2/25/18	2.5	33.1	
1604	3/16/18	10	39.3		4396	2/27/18	2.5		0.366
1604	3/25/18	10	63.4		4402	2/25/18	2.5		0.518
1604	4/8/18	10	55.4		4392	2/27/18	2.5	17.9	0.007
1604	4/12/18	10	77.7		4392	4/30/18	2.5	13.5	0.016
1606	4/25/18	10	59.7	0.033	4392	5/1/18	2.5	15.2	0.174
1610	5/1/18	10	54.8	0.082	4392	2/25/19	2.5	55.4	
2964	3/6/18	5	38.2		4394	2/22/18	2.5	50.4	0.010

ID	Date Collected	Dose	Yolk Conc (µg g^{-1})	White Conc (µg g^{-1})	ID	Date Collected	Dose	Yolk Conc (µg g^{-1})	White Conc (µg g^{-1})
4394	2/23/18	2.5	34.7	0.027	5696	3/5/18	20	70.4	0.090
4394	2/26/18	2.5	20.9	0.334	5696	4/29/18	20	89.0	
4394	3/8/18	2.5	8.52		5696	4/30/18	20	80.8	1.20
4394	3/22/18	2.5	12.9		5702	5/1/18	20	101	0.051
4394	4/1/18	2.5	10.1		6942	5/1/18	20	61.3	0.282
4394	4/12/18	2.5	16.7		6944	2/24/18	15	269	0.084
4396	4/27/18	2.5	19.1	0.128	6944	3/2/18	15	55.9	0.046
4402	2/24/18	2.5		0.008	6950	3/1/18	15	134	
4402	2/26/18	2.5	32.9		6950	3/6/19	15	80.7	
4404	5/1/18	2.5	17.5	0.077	6952	2/25/18	15	100	0.039
4412	5/1/18	2.5	3.97		6954	5/1/18	15	76.5	0.067
5674	5/1/18	2.5	109	0.082	6958	3/12/18	15	66.0	
5694	2/28/18	20	250	0.255	6958	3/17/18	15	90.6	
5694	5/1/18	20	124	0.082	6958	3/28/18	15	49.6	
5696	2/23/18	20	422	0.186	6958	4/8/18	15	78.1	
5696	3/2/18	20	110		6958	4/9/18	15	94.6	
5696	3/7/18	20	90.3		6958	5/1/18	15	74.3	0.041
5696	4/4/18	20	93.6		6964	2/28/18	15	112	0.041
5696	4/10/18	20	75.2						

8.1.15 Chronic 3M AFFF Adult Serum Concentrations

Control				
Quail	Gender	Serum PFHxS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})	Serum PFOA Conc (µg g^{-1})
202	Female	0.064	0.531	0.127
204	Female	0.037	0.326	0.111
206	Female	0.046	0.329	0.108
207	Male	0.039	0.259	0.107
208	Female	0.036	0.172	0.119
210	Female	0.032	0.092	0.127
211	Male	0.036	0.088	0.128
217	Male	0.033	0.100	0.131
227	Male	0.035	0.101	0.113
231	Male	0.032	0.096	0.076

2.5 µg g^{-1} PFOS from AFFF Dose				
Quail	Gender	Serum PFHxS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})	Serum PFOA Conc (µg g^{-1})
4502	Female	3.87	13.07	0.313
4503	Male	5.55	65.10	0.198
4506	Female	1.74	5.88	0.175
4507	Male	5.52	64.85	0.258
4508	Female	2.50	4.35	0.201
4509	Male	5.46	50.69	0.182
4511	Male	4.15	54.90	0.070
4512	Female	1.99	4.78	0.180
4513	Male	6.32	67.13	0.156
4514	Female	1.94	3.32	0.171

5 µg g^{-1} PFOS from AFFF Dose				
Quail	Gender	Serum PFHxS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})	Serum PFOA Conc (µg g^{-1})
3002	Female	4.95	14.41	0.415
3005	Male	9.56	142	0.236
3006	Female	3.85	9.87	0.398
3008	Female	3.52	8.95	0.376
3009	Male	15.9	161	0.417
3010	Female	5.12	7.42	0.403
3013	Male	13.9	135	0.288
3016	Female	3.36	6.73	0.253
3019	Male	17.9	135	0.389
3025	Male	8.61	124	0.195

10 µg g^{-1} PFOS from AFFF Dose				
Quail	Gender	Serum PFHxS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})	Serum PFOA Conc (µg g^{-1})
1613	Male	24.5	188	0.389
1614	Female	11.5	28.84	0.963
1615	Male	20.1	189	0.463
1616	Female	7.15	15.24	0.557
1617	Male	14.8	211	0.269
1618	Female	8.64	27.48	0.562
1619	Male	9.25	204	0.175
1620	Female	6.91	19.48	0.445
1621	Male	11.2	138	0.231
1624	Female	6.72	13.93	0.357

15 µg g^{-1} PFOS from AFFF Dose				
Quail	Gender	Serum PFHxS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})	Serum PFOA Conc (µg g^{-1})
6967	Male	21.0	209	0.367
6968	Female	13.4	67.8	0.479
6969	Male	54.7	209	1.15
6971	Male	17.2	232	0.373
6972	Female	10.7	30.7	0.558
6973	Male	38.5	304	0.897
6975	Male	20.1	210	0.369
6976	Female	14.0	40.1	0.791
6978	Female	15.3	40.0	1.02
6982	Female	15.0	40.5	0.995

20 µg g^{-1} PFOS from AFFF Dose				
Quail	Gender	Serum PFHxS Conc (µg g^{-1})	Serum PFOS Conc (µg g^{-1})	Serum PFOA Conc (µg g^{-1})
5901	Male	48.4	273	1.11
5902	Female	19.3	68.1	1.30
5903	Male	42.6	253	1.05
5905	Male	25.5	229	0.454
5907	Male	31.9	296	0.658
5908	Female	11.9	48.0	0.766
5909	Male	60.9	316	1.37
5912	Female	20.2	65.5	1.05
5914	Female	16.1	48.8	0.817
5916	Female	12.1	26.5	0.535

8.1.16 Chronic 3M AFFF Adult Liver Concentrations

Control				
Quail	Gender	Liver PFHxS Conc (µg g^{-1})	Liver PFOS Conc (µg g^{-1})	Liver PFOA Conc (µg g^{-1})
202	Female	0.012	0.157	0.033
204	Female	0.029	0.351	0.034
206	Female	0.040	0.359	0.045
207	Male	0.022	0.395	0.053
208	Female	0.015	0.202	0.042
210	Female	0.016	0.150	0.038
211	Male	--	0.364	0.050
217	Male	0.034	0.455	0.045
227	Male	0.012	0.188	0.044
231	Male	--	0.175	0.051
202	Female	0.012	0.157	0.033

-- Chromatography issues, unable to quantify

2.5 µg g^{-1} PFOS from AFFF Dose				
Quail	Gender	Liver PFHxS Conc (µg g^{-1})	Liver PFOS Conc (µg g^{-1})	Liver PFOA Conc (µg g^{-1})
4502	Female	1.70	14.9	0.150
4503	Male	0.984	24.9	0.059
4506	Female	0.898	8.839	0.107
4507	Male	--	30.0	0.059
4508	Female	1.12	6.541	0.115
4509	Male	0.842	17.7	0.035
4511	Male	0.698	24.4	0.044
4512	Female	0.920	6.785	0.105
4513	Male	0.944	26.6	0.039
4514	Female	0.544	4.146	0.067

-- Chromatography issues, unable to quantify

5 µg g^{-1} PFOS from AFFF Dose				
Quail	Gender	Liver PFHxS Conc (µg g^{-1})	Liver PFOS Conc (µg g^{-1})	Liver PFOA Conc (µg g^{-1})
3002	Female	2.27	22.5	0.200
3005	Male	1.66	58.1	0.064
3006	Female	1.50	15.5	0.236
3008	Female	0.839	9.686	0.101
3009	Male	3.41	59.0	0.125
3010	Female	--	11.4	0.202
3013	Male	2.08	39.8	0.062
3016	Female	1.10	5.758	0.086
3019	Male	3.03	60.1	0.087
3025	Male	1.14	43.3	0.036

-- Chromatography issues, unable to quantify

10 µg g^{-1} PFOS from AFFF Dose				
Quail	Gender	Liver PFHxS Conc (µg g^{-1})	Liver PFOS Conc (µg g^{-1})	Liver PFOA Conc (µg g^{-1})
1613	Male	3.98	96.5	0.142
1614	Female	3.48	26.5	0.361
1615	Male	--	180	0.200
1616	Female	1.88	17.8	0.208
1617	Male	2.12	80.6	0.062
1618	Female	3.18	26.7	0.208
1619	Male	2.03	132	0.057
1620	Female	2.42	28.2	0.247
1621	Male	1.87	75.6	0.070
1624	Female	2.92	22.9	0.375

-- Chromatography issues, unable to quantify

15 µg g^{-1} PFOS from AFFF Dose				
Quail	Gender	Liver PFHxS Conc (µg g^{-1})	Liver PFOS Conc (µg g^{-1})	Liver PFOA Conc (µg g^{-1})
6967	Male	8.90	147	0.279
6968	Female	6.73	212	0.193
6969	Male	4.99	65.1	0.275
6971	Male	24.6	173	0.674
6972	Female	3.44	32.8	0.402
6973	Male	2.46	107	0.280
6975	Male	4.00	26.4	0.303
6976	Female	4.92	143	0.153
6978	Female	4.34	35.5	0.411
6982	Female	5.82	37.2	0.639

20 µg g^{-1} PFOS from AFFF Dose				
Quail	Gender	Liver PFHxS Conc (µg g^{-1})	Liver PFOS Conc (µg g^{-1})	Liver PFOA Conc (µg g^{-1})
5901	Male	20.6	213	0.539
5902	Female	4.67	43.3	0.538
5903	Male	17.6	236	0.669
5905	Male	0.035	--	--
5907	Male	12.9	274	0.390
5908	Female	3.65	32.1	0.316
5909	Male	14.4	199	0.592
5912	Female	6.70	55.0	0.538
5914	Female	6.03	47.0	0.565
5916	Female	4.22	34.1	0.405

-- Chromatography issues, unable to quantify

8.1.17 Chronic 3M AFFF Chick Serum and Liver Concentrations

Hatch	Dose	Serum PFOS Conc (µg g^{-1})	Serum PFHxS Conc (µg g^{-1})	Liver PFOS Conc (µg g^{-1})	Liver PFHxS Conc (µg g^{-1})
1	0	0.244	0.015	0.035	0.002
1	0	0.123	0.010	0.029	0.001
1	0	0.128	0.008	0.027	0.002
1	0	0.119	0.008	0.025	0.001
1	2.5	3.91	0.754	3.66	0.126
1	2.5	1.87	0.567	1.33	0.103
1	2.5	2.84	0.270	2.21	0.055
1	2.5	2.00	0.322	1.54	0.048
1	5	6.08	1.33	3.60	0.248
1	5	4.43	2.09	2.52	0.293
1	5	13.3	1.32	8.07	0.201
1	5	5.37	1.38	3.99	0.299
1	10	12.4	4.30	5.74	0.483
1	10	19.4	1.31	12.6	0.199
2	0	0.104	0.007	0.035	0.002
2	0	0.115	0.008	0.030	0.001
2	0	0.114	0.009	0.025	0.002
2	0	0.103	0.009	0.034	0.001
2	2.5	2.24	0.345	1.05	0.045
2	2.5	1.45	0.418	1.24	0.091
2	2.5	2.09	0.758	1.25	0.081
2	2.5	3.55	0.664	1.98	0.113
2	5	3.78	0.928	2.38	0.185
2	5	6.45	1.69	5.42	0.366
2	5	6.67	1.36	3.89	0.230
2	5	5.55	1.71	5.55	0.299
2	10	10.6	2.10	9.28	0.518
2	10	10.0	1.68	7.92	0.314
2	10	14.8	3.88	12.9	0.846
2	10	7.53	1.81	4.86	0.330
2	15	16.8	4.44	12.9	1.08
2	15	19.1	4.58	12.9	1.10
2	15	9.04	2.72	4.26	0.510
2	15	13.1	2.33	7.94	0.451
2	20	28.2	1.56	15.1	0.342
2	20	15.1	1.71	8.33	0.349
2	20	20.9	3.22	12.7	0.655
2	20	21.5	2.28	11.1	0.466

Hatch	Dose	Serum PFOS Conc (µg g^{-1})	Serum PFHxS Conc (µg g^{-1})	Liver PFOS Conc (µg g^{-1})	Liver PFHxS Conc (µg g^{-1})
3	0	0.105	0.007	0.026	0.004
3	0	0.105	0.007	0.028	0.004
3	0	0.104	0.008	0.025	0.004
3	0	0.115	0.008	0.023	0.004
3	2.5	1.85	0.323	1.53	0.084
3	2.5	1.64	0.217	0.980	0.043
3	2.5	2.42	0.332	1.35	0.065
3	2.5	3.61	0.484	3.30	0.118
3	5	4.94	1.30	3.81	0.243
3	5	5.09	0.911	3.62	0.147
3	5	3.40	0.595	2.40	0.101
3	5	5.31	1.11	4.44	0.180
3	10	9.03	1.59	6.19	0.264
3	10	9.59	1.32	6.38	0.228
3	10	9.64	1.52	5.30	0.286
3	10	8.20	1.21	4.52	0.214
3	15	18.0	1.98	10.9	0.351
3	15	14.4	2.87	7.74	0.524
3	15	19.5	2.57	10.0	0.386
3	15	16.1	2.86	9.82	0.563
3	20	23.5	1.67	12.9	0.294
3	20	15.0	1.73	12.6	0.385
3	20	28.9	2.60	13.9	0.394
3	20	22.2	2.34	9.62	0.359
4	0	0.035	0.007	0.307	0.012
4	0	0.030	0.005	0.024	0.003
4	0	0.029	0.005	0.034	0.003
4	0	0.030	0.005	0.022	0.002
4	2.5	4.22	0.722	3.04	0.143
4	2.5	2.07	0.403	1.00	0.076
4	2.5	4.09	0.597	2.30	0.106
4	2.5	4.55	0.550	1.93	0.074
4	5	7.20	1.07	3.69	0.186
4	5	5.85	0.975	2.84	0.174
4	5	7.26	0.926	2.83	0.195
4	5	4.37	0.528	2.59	0.101
4	10	7.01	1.11	3.02	0.196
4	10	9.13	1.62	4.86	0.316
4	10	9.13	1.21	3.66	0.190
4	10	9.32	1.40	4.44	0.318
4	15	13.0	1.29	7.84	0.291
4	15	18.2	2.17	8.29	0.396

Hatch	Dose	Serum PFOS Conc (µg g^{-1})	Serum PFHxS Conc (µg g^{-1})	Liver PFOS Conc (µg g^{-1})	Liver PFHxS Conc (µg g^{-1})
4	15	18.0	1.48	9.65	0.271
4	15	28.3	2.22	9.35	0.348
4	20	30.9	2.53	11.2	0.422
4	20	28.4	2.82	11.4	0.443
4	20	19.7	1.77	8.98	0.440
4	20	31.2	2.52	13.9	0.605
5	0	0.030	0.007	0.039	0.004
5	0	0.033	0.005	0.021	0.003
5	0	0.033	0.011	0.029	0.004
5	0	0.034	0.005	0.041	0.005
5	2.5	2.87	0.347	1.44	0.059
5	2.5	4.40	0.749	1.96	0.106
5	2.5	2.69	0.386	1.50	0.063
5	2.5	3.05	0.363	4.97	0.208
5	5	5.65	0.872	5.55	0.344
5	5	6.81	1.10	5.66	0.310
5	5	8.19	1.36	5.86	0.235
5	5	6.27	0.970	4.63	0.283
5	10	15.6	1.80	8.17	0.539
5	10	16.5	2.50	7.38	0.456
5	10	15.4	2.03	8.95	0.621
5	10	11.5	0.596	5.00	0.136
5	15	13.9	1.27	5.56	0.320
5	15	9.9	1.60	10.1	0.573
5	15	15.8	1.48	7.18	0.315
5	15	16.0	2.10	7.52	0.504
5	20	17.7	2.14	10.2	0.622
5	20	18.2	1.83	11.1	0.391
5	20	17.9	2.77	15.2	1.01
5	20	24.0	2.58	16.7	0.540
6	0	--	--	0.155	0.008
6	0	--	--	0.095	0.009
6	0	--	--	0.088	0.008
6	0	--	--	0.070	0.005
6	2.5	3.62	0.500	2.53	0.105
6	2.5	3.45	0.533	2.00	0.107
6	2.5	3.45	0.479	2.26	0.049
6	2.5	2.15	0.315	1.47	0.078
6	5	7.73	1.47	5.56	0.326
6	5	6.55	0.956	4.44	0.220
6	5	6.07	1.05	5.34	0.306
6	5	5.72	1.07	7.25	0.392

--Analytical issues, unable to quantify

Hatch	Dose	Serum PFOS Conc (µg g^{-1})	Serum PFHxS Conc (µg g^{-1})	Liver PFOS Conc (µg g^{-1})	Liver PFHxS Conc (µg g^{-1})
6	10	7.11	1.10	6.16	0.308
6	10	7.47	1.14	--	--
6	10	7.86	1.27	--	--
6	10	7.96	1.26	--	--
6	15	13.0	1.94	7.966	0.590
6	15	17.2	2.77	8.932	0.713
6	15	26.9	2.20	13.846	0.527
6	15	20.0	3.60	8.32	0.573
6	20	15.5	0.753	7.93	0.221
6	20	26.6	3.23	11.152	0.737
6	20	21.5	3.88	14.174	1.15
6	20	13.9	2.30	--	--
7	0	0.033	0.005	0.063	0.006
7	0	0.032	0.005	0.081	0.005
7	0	0.031	0.004	0.066	0.004
7	0	0.031	0.004	0.069	0.004
7	2.5	3.04	0.462	1.61	0.081
7	2.5	2.67	0.310	1.57	0.093
7	2.5	4.45	0.450	1.62	0.671
7	2.5	1.60	0.384	2.57	0.193
7	5	3.51	0.676	2.18	1.53
7	5	4.25	0.758	2.51	1.56
7	5	3.41	0.617	0.825	1.47
7	5	6.22	1.23	1.99	1.56
7	10	5.63	0.590	5.39	2.73
7	10	9.9	1.66	7.46	3.01
7	10	6.75	1.48	1.99	1.52
7	10	11.6	2.04	2.12	1.91
7	15	14.0	2.31	10.2	4.09
7	15	13.2	3.68	6.48	2.95
7	15	17.9	2.56	7.86	3.29
7	15	15.0	2.57	5.97	2.89
7	20	11.1	1.86	6.40	3.07
7	20	15.4	2.15	8.23	3.48
7	20	13.8	2.13	7.02	2.77
7	20	13.1	3.04	9.90	4.26
8	0	0.033	0.004	--	1.07
8	0	0.030	0.004	--	0.585
8	0	0.036	0.004	--	0.771
8	0	0.032	0.004	--	0.879
8	2.5	2.24	0.325	--	1.60
8	2.5	2.61	0.475	0.660	1.07

--Analytical issues, unable to quantify

Hatch	Dose	Serum PFOS Conc (µg g^{-1})	Serum PFHxS Conc (µg g^{-1})	Liver PFOS Conc (µg g^{-1})	Liver PFHxS Conc (µg g^{-1})
8	2.5	2.68	0.379	1.29	1.45
8	2.5	2.38	0.375	--	0.755
8	5	6.20	0.860	4.10	0.230
8	5	5.25	0.466	2.47	0.128
8	5	4.30	0.604	5.39	0.180
8	5	8.13	1.13	9.99	0.417
8	10	12.7	1.62	8.94	0.286
8	10	9.8	1.23	6.56	0.226
8	10	8.87	1.06	6.74	0.302
8	10	5.50	0.766	10.9	0.352
8	15	17.9	2.34	14.7	0.492
8	15	10.6	2.09	11.4	0.441
8	15	16.0	2.29	7.94	0.437
8	15	15.9	2.46	11.6	0.516
8	20	13.9	2.50	19.9	0.574
8	20	12.3	1.70	10.3	0.564
8	20	21.9	2.24	8.57	0.379
8	20	13.5	2.88	9.56	0.498
9	0	0.029	0.005	0.060	0.017
9	0	0.037	0.004	0.068	0.007
9	0	0.026	0.005	0.086	0.016
9	0	0.026	0.004	0.089	0.016
9	2.5	1.38	0.178	2.74	0.138
9	2.5	1.54	0.410	1.24	--
9	2.5	2.85	0.357	1.18	0.125
9	2.5	4.03	0.539	2.19	0.113
9	5	6.40	0.774	3.02	0.206
9	5	6.61	1.26	5.35	0.283
9	5	4.85	0.901	4.67	0.247
9	5	6.21	1.04	3.12	0.164
9	10	8.72	1.62	4.51	0.147
9	10	9.04	1.78	4.52	0.267
9	10	4.16	0.733	6.71	0.480
9	10	7.48	0.724	4.75	0.332
9	15	15.0	2.54	12.5	0.632
9	15	16.1	2.46	10.1	0.571
9	15	15.8	1.61	11.8	0.639
9	15	13.7	1.27	11.0	0.522
9	20	28.9	1.48	7.75	0.512
9	20	19.3	1.55	23.0	0.916
9	20	14.6	1.14	16.3	0.881

--Analytical issues, unable to quantify

Hatch	Dose	Serum PFOS Conc (µg g^{-1})	Serum PFHxS Conc (µg g^{-1})	Liver PFOS Conc (µg g^{-1})	Liver PFHxS Conc (µg g^{-1})
10	0	0.031	0.002	0.059	0.012
10	0	0.032	0.003	0.041	0.014
10	0	0.032	0.003	0.046	0.010
10	0	0.030	0.003	0.066	0.016
10	2.5	1.43	0.129	1.47	0.046
10	2.5	1.95	0.306	1.81	0.112
10	2.5	2.80	0.548	1.10	0.060
10	2.5	3.44	0.901	2.63	0.181
10	5	4.48	1.09	2.76	0.179
10	5	3.30	0.520	2.39	0.149
10	5	4.75	1.20	2.99	0.188
10	5	6.99	1.12	3.98	0.264
10	10	9.37	1.42	16.5	0.940
10	10	8.75	1.44	10.1	0.371
10	10	19.0	2.91	6.64	0.363
10	10	18.0	1.94	5.22	0.205
10	15	17.6	3.73	12.7	0.724
10	15	17.7	3.05	9.35	0.642
10	15	24.0	5.36	9.68	0.732
10	15	22.4	3.54	10.2	0.584
10	20	25.5	1.53	12.8	0.220
10	20	23.2	2.36	18.5	0.829
10	20	16.7	0.803	14.9	0.668
10	20	19.0	3.80	20.6	0.483

8.1.18 Chronic 3M AFFF Egg White and Yolk Concentrations

Dose	Hen ID	Necropsy Date	Egg Yolk PFOS Conc ($\mu g\ g^{-1}$)	Egg White PFOS Conc ($\mu g\ g^{-1}$)	Egg Yolk PFHxS Conc ($\mu g\ g^{-1}$)	Egg White PFHxS Conc ($\mu g\ g^{-1}$)
0	202	10/30/18	0.109	0.000	0.017	0.000
0	210	8/26/18	0.375	0.001	0.043	0.000
0	210	10/30/18	0.258	0.001	8.65	0.000
0	224	8/28/18	230	0.065	10.2	0.015
0	228	8/28/18	0.056	0.000	0.016	0.000
0	230	8/21/18	0.119	0.001	0.015	0.000
0	232	10/30/18	0.376	0.000	0.03	0.000
2	4504	8/31/18	16.9	0.006	1.81	0.003
2	4505	10/30/18	21.5	0.004	1.74	0.002
2	4512	8/23/18	57.8	0.009	3.27	0.003
2	4514	10/30/18	20.7	0.006	1.66	0.004
2	4522	10/30/18	25.1	0.006	1.53	0.003
2	4524	8/27/18	100	0.019	3.83	0.005
2	4528	8/28/18	82.8	0.014	4.21	0.005
5	3004	10/30/18	32.8	0.013	3.14	0.006
5	3008	8/27/18	44.5	0.136	6.77	0.037
5	3012	8/28/18	42.3	0.012	7.15	0.010
5	3016	10/30/18	38.5	0.006	3.22	0.004
5	3020	8/26/18	102	0.023	6.07	0.009
5	3024	10/30/18	42.7	0.013	1.81	0.006
5	3028	8/27/18	103	0.014	10.3	0.007
10	1620	8/29/18	74.8	0.017	7.11	0.010
10	1622	10/30/18	64.3	0.034	5.53	0.019
10	1630	8/22/18	326	0.066	22.9	0.022
10	1630	8/24/18	178	0.042	21.4	0.027
10	1632	10/30/18	89.8	0.016	7.69	0.009
10	1634	8/31/18	53.7	0.009	6.01	0.006
10	1642	10/30/18	61.6	0.016	5.79	0.006
15	6976	10/30/18	118	0.079	17.8	0.031
15	6982	10/30/18	140	0.040	18.7	0.030
15	6984	9/1/18	99.3	0.018	7.88	0.013
15	6984	9/10/18	141	0.019	11.1	0.007
15	6988	8/31/18	205	0.025	15.8	0.012
15	6988	9/1/18	167	0.015	13.9	0.011
15	6988	10/30/18	103	0.042	23.8	0.034
20	5906	10/30/18	179	0.087	12.4	0.031
20	5918	10/30/18	190	0.062	11.9	0.018
20	5928	8/27/18	279	0.032	29.2	0.017
20	5928	8/31/18	140	0.037	14.8	0.018
20	5928	9/1/18	105	0.036	13.0	0.010
20	5928	10/30/18	207	0.156	10.1	0.013

8.1.19 Spike recoveries of PFOS, AFFF PFOS and AFFF PFHxS in liver, egg yolk and serum samples

	Concentration (mg kg^{-1})			
Compound	Nominal	Average	Recovery (%)	SD
Liver				
PFOS	0	0	---	---
	18	16	86	12
	36	33	90	12
	65	59	90	8
	95	77	80	3
	84	79	93	5
AFFF PFOS	0	0	---	---
	25	29	115	3
	50	51	103	5
	100	92	92	2
	150	147	98	4
	200	181	90	4
AFFF PFHxS	0	0	---	---
	2.0	1.9	95	8
	4.0	3.6	90	6
	8.1	7.8	97	3
	12	12	99	8
	16	15	91	10
Egg Yolk				
PFOS	0	0.07	---	---
	19	13	66	8
	32	19	59	7
	54	32	58	8
	76	60	68	13
	98	63	69	11
AFFF PFOS	0	0.1	---	---
	36	36	99	--- [a]
	57	66	115	---
	86	79	92	---
	114	99	87	---
	143	128	89	---
	171	148	86	---
	200	144	72	---
AFFF PFHxS	0	0.0	---	---
	2.9	3.0	102	---
	4.6	5.1	112	---
	6.9	5.9	86	---
	9.2	7.6	82	---
	12	11	92	---
	14	12	86	---
	16	12	75	---

Serum				
PFOS	0	0.9	---	---
	27	23	85	24
	54	50	93	13
	108	108	100	12
	162	160	99	13
	216	230	106	10
AFFF PFOS	0	0.3	---	---
	25	25	102	9
	50	47	95	16
	100	89	89	4
	200	246	123	9
	300	315	105	14
AFFF PFHxS	0	0.02	---	---
	2	1.7	84	12
	4	3.0	73	3
	8	6.0	73	4
	16	18	110	17
	24	22	90	17

[a]A single sample was analyzed, thus there is no standard deviation.

8.2 List of Scientific/Technical Publications

Bursian, S. J., Link, J.E., McCarty, M., Harr, K., Simcik, M.F. Dietary Exposure of Japanese Quail (*Coturnix japonica*) to Perfluorooctane Sulfonate (PFOS) and a Legacy Aqueous Film Forming Foam (AFFF) Containing PFOS: Effects on Reproduction and Chick Survivability and Growth. *In preparation*

Bursian, S. J., Link, J.E., McCarty, M., Simcik, M.F. The Subacute Toxicity of PFOS and/or PFOA and Legacy Aqueous Film Forming Foams to Japanese Quail (*Coturnix japonica*) chicks. *Environmental Toxicology and Chemistry*. **2020** doi:10.1002/etc.4684