INTERIM REPORT

Advancing the Understanding of the Ecological Risk of Per- and Polyfluoroalkyl Substances (PFAS)

SERDP Project ER-2627

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14. ABSTRACT
The objectives of this project were twofold: (a) conduct ecotoxicity studies that yield defensible and usable Toxicity Reference Values (TRVs) for wildlife potentially exposed to common per- and polyfluoroalkyl substances (PFASs) including perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and up to 4 "other" PFASs and (b) develop and validate a low-cost, relative-toxicity protocol for evaluating other PFAS that may drive risks at DOD sites. We are conducting PFAS toxicity studies on reptiles (*Anolis sagrei*), birds (*Colinus virginianus*), fish (*Pimephales promelas*) and chironomids (*Chironomus dilutus*). This report provides a summary of key findings to date and outlines remaining tasks. Task 1 of the project focused on identifying, PFAS that are of highest concern based on toxicity and analysis of soil and surface water PFAS data for a large number of Air Force Bases (AFBs). Toxicity data for fish and chironomids has revealed that PFOS was the most toxic PFAS under our test conditions. In fact, for all taxa tested including chironomids, fathead minnows, Anolis lizards, and bobwhite quail, PFOS has emerged as the most toxic of the tested PFAS which were those on the UCMR3 list and included perfluorooctanoic acid (PFOA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluoroheptanoic acit (PFHpA), and perfluoronanonic acid (PFNA).

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SUMMARY

The objectives of this project were twofold: (a) conduct ecotoxicity studies that yield defensible and usable Toxicity Reference Values (TRVs) for wildlife potentially exposed to common per- and polyfluoroalkyl substances (PFASs) including perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and up to 4 "other" PFASs and (b) develop and validate a low-cost, relative-toxicity protocol for evaluating other PFAS that may drive risks at DOD sites. We are conducting PFAS toxicity studies on reptiles (*Anolis sagrei*), birds (*Colinus virginianus*), fish (*Pimephales promelas*) and chironomids (*Chironomus dilutus*). This report provides a summary of key findings to date and outlines remaining tasks. Task 1 of the project focused on identifying, PFAS that are of highest concern based on toxicity and analysis of soil and surface water PFAS data for a large number of Air Force Bases (AFBs). Toxicity data for fish and chironomids has revealed that PFOS was the most toxic PFAS under our test conditions. In fact, for all taxa tested including chironomids, fathead minnows, Anolis lizards, and bobwhite quail, PFOS has emerged as the most toxic of the tested PFAS which were those on the UCMR3 list and included perfluorooctanoic acid (PFOA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluoroheptanoic acit (PFHpA), and perfluorонanonic acid (PFNA). While PFOS has emerged as the most toxic of tested PFAS, we also reported that perfluorohexane sulfonate (PFHxS) was frequently measured at relatively high concentrations in both surface water and soils at PFAS-contaminated sites on AFBs. PFOS and PFHxS commonly co-occur although there are few toxicity data related to this specific bi-chemical mixture. A series of toxicity studies in which PFOS + PFHxS toxicity was investigated points to an interaction or, more specifically, an apparent potentiation of toxicity when these chemicals co-occur. Several studies remain to be conducted with Anolis lizards and freshwater fish.

TASK 1: Identify and prioritize target PFAS for ecological risk assessment.
The key objectives of Task 1 are to (1.a) compile relevant field measurements of PFASs; (1.b) to generate toxicity estimates for PFAS that have yet to be studied (but were identified as important in 1.a); and (1.c) to rank the relative PFAS chemicals with regard to the likelihood to be important to consider in ecological risk assessments.

TASK 2: Determining the Toxicity of PFASs to Avian and Reptilian Species.
This task will utilize controlled laboratory toxicity testing protocols to support the development of Toxicity Reference Values for PFOS, PFOA and up to a total of five other PFASs (or mixtures) based on results from Task 1. **Note that since initiating this research, we have determined that PFHxS is a higher priority for research than PFOA for ecological receptors.*

TASK 3: Develop tools, TRV recommendations and conceptual models of PFASs to facilitate the use of products developed in Task 1 and 2 in ecological risk assessment.
This Task serves as a synthesis of research efforts undertaken in Tasks 1 and 2 of this project as well as other developments in PFAS ecotoxicology and ecological risk assessment.

Objective 1.a: Review analytical results from environmental field sites to assess the occurrence and concentrations of key PFASs in the context of environmental relevance.

A key concern regarding the toxicity and risk of PFAS to ecological receptors is that there are many different PFAS and they commonly occur in the environment as mixtures. In an effort to determine whether there are patterns of PFAS mixtures that could be used to prioritize mixture toxicity testing and risk assessment, we conducted an extensive analysis of surface water and soil data from actives U.S. Air Force Bases (AFBs) where Aqueous Film Forming Foams (AFFFs) were used. A data science approach was used to better understand and communicate the frequency of occurrence and magnitude of up to 18 PFAS measured in surface water and soils. This analysis also helped to solidify our conceptual model which has also been corroborated by recently published research. Essentially, use of AFFF has frequently occurred in the terrestrial environment which has then led to contamination of soils. Percolation of these soils and eventual movement to groundwater results, ultimately, in a release of AFFF to surface waters.

<u>Results Synopsis:</u> PFOS and PFHxS are generally the most dominant PFAS in surface water (East et al. 2021) and soil samples collected from U.S. Air Force installations (Figure 1). In general, PFOS is the most dominant PFAS in both sediment and soils with an even higher relative concentration of PFOS in soils. Analysis suggests that 4-chemical mixtures likely capture a significant proportion of PFAS mixtures in surface waters. Results of these analyses can provide insight into subsequent toxicity testing for PFAS mixtures relevant to DoD installations, particularly where AFFF has been used.

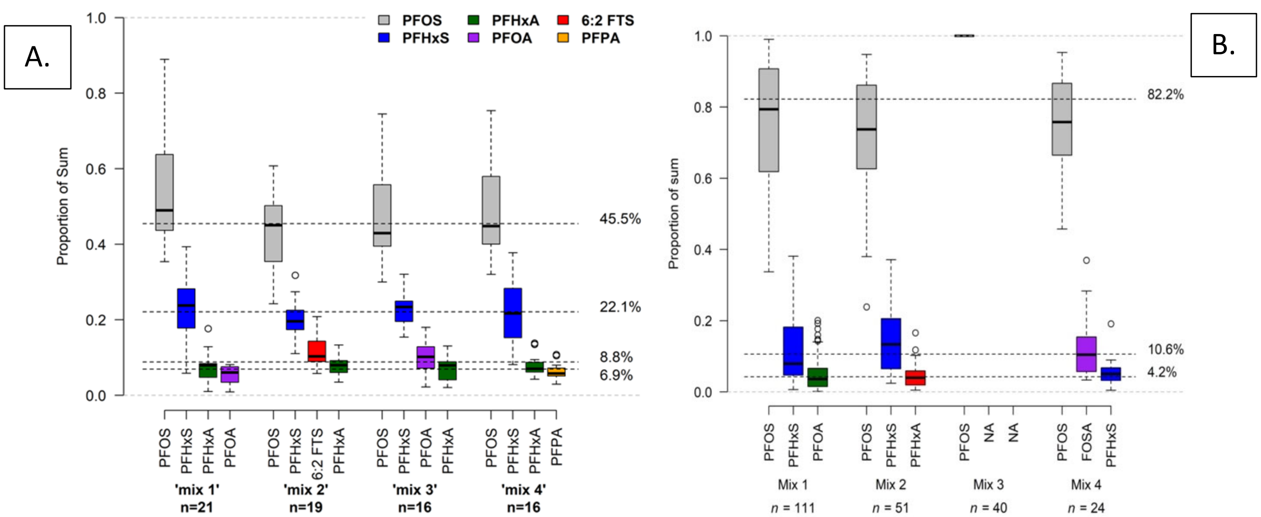


Figure 1. Relative composition of the 4-PFAS mixtures in surface water (A) and soil (B) at AFBs; the 4-chemical mixture accounts for approximately 80% of the total sum site-specific PFAS. In essence, this represents the simplest, most representative PFAS mixture profile for the surface water and soil data set. Note, Mix 3 in the soil boxplot (B) is dominated by PFOS.

Remaining Tasks: A manuscript has been published reporting the results of the analysis for PFAS-contaminated surface water (East et al. 2021). A companion paper is in draft form focused on PFAS in soil from the same contaminated sites as in East et al. (2021).

Objective 1.b: Freshwater invertebrate (*Chironomus dilutus*) toxicity testing to facilitate PFAS prioritization.

Originally, we proposed that toxicity studies using freshwater invertebrates and fish which, are generally cheaper and easier to perform, could be used to help prioritize PFAS for toxicity tests involving taxa that are generally more challenging to work with. While this is true, it also became apparent (see Objective 1 above) that surface water contamination with PFAS is very common and likely to be the dominant exposure media for wildlife. As such, toxicity data for freshwater invertebrates and fish provides value in prioritizing PFAS for testing and can also be used for toxicity threshold development for applications in ecological risk assessment.

For freshwater invertebrates, single chemical spiked-water bioassays were conducted with freshwater non-biting midge (*Chironomus dilutus*) exposed to PFOS, PFOA, PFHxS, PFNA, PFHpA, and PFBS. *Chironomus sp.* was selected since they are one of the most sensitive species to aquatic exposures of PFOS (Giesy et al. 2004; Qi et al. 2011; Salice et al. 2018). Short term tests were intended to inform prioritization of infrequently and understudied PFAS such as PFNA, PFHpA, and PFHxS. Ten-day range finding (acute) and 20-day (short-term chronic) bioassays with midge followed ASTM E1706-05 protocols. For each chemical, five different exposure concentrations were used for each bioassay with concentrations capturing environmental relevance. Measured endpoints included survival in 10-day tests, and survival, growth, and biomass in 20-day tests. Additionally, as mentioned above, PFOS and PFHxS are the most frequently detected PFAS in water samples collected at DoD installations (East et al. 2021). Therefore, a final binary mixture bioassay with PFOS+PFHxS followed the 10-day and 20-day single chemical tests. *C. dilutus* were exposed to environmentally relevant concentrations of PFOS in combination with PFHxS at higher concentrations shown to be toxic in single-chemical tests and at a lower environmentally relevant concentration.

Results Synopsis: Results of single chemical tests agreed with previous studies in which PFOS was the most toxic to midge (MacDonald et al. 2004; Marziali et al. 2019) but the results also showed that PFHxS was more toxic than PFOA. The more toxic response to PFHxS than to PFOA, combined with PFOS response, supports a conclusion that perfluorosulfonic acids (PFSAs, including PFHxS and PFOS) may be more toxic to freshwater non-biting midges than perfluorocarboxilic acids (PFCAs, including PFOA, PFNA, and PFHpA).

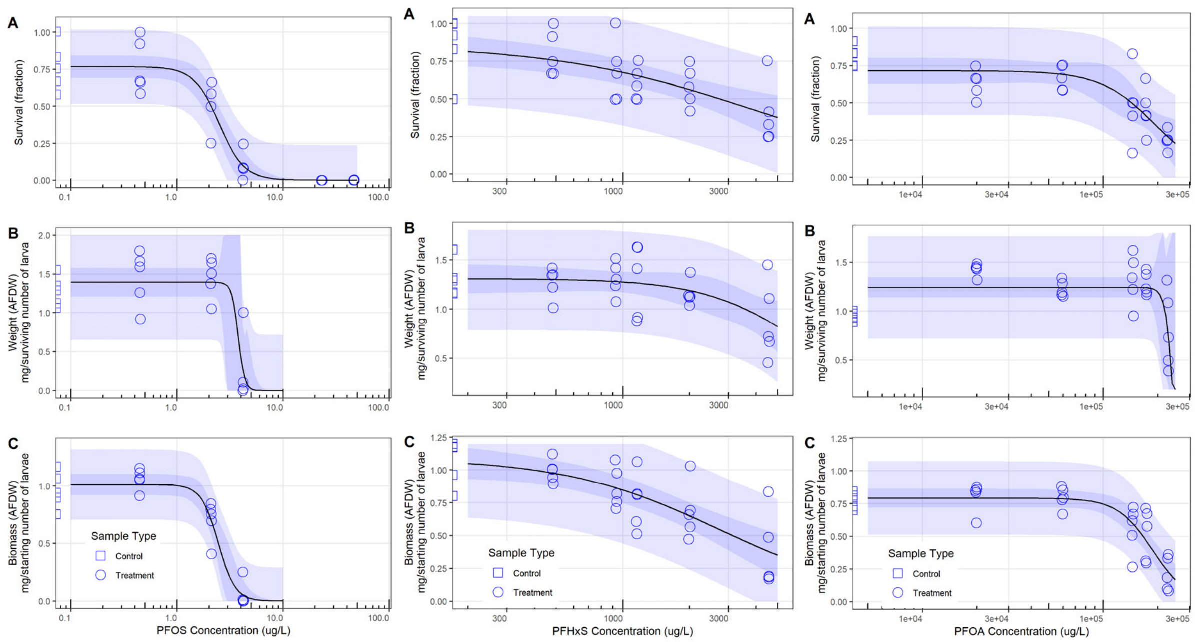


Figure 2. Exposure-response relationships for survival, body weight, and biomass of *C. dilutus* exposed to PFOS, PFHxS and PFOA. Similar relationships could not be developed for PFNA, PFHpA, and PFBS due to a lack of toxicity.

PFNA, PFHpA, and PFBS showed limited or no toxicity to *C. dilutus* at environmentally relevant concentrations reported by East et al. (2021). Conversely, responses for PFOS, PFHxS, and PFOA all yielded robust concentration response relationships. Results of this study suggest toxicity of PFNA, PFHpA, and PFBS, to *C. dilutus* and possibly to other midge larvae in natural freshwater ecosystems is highly unlikely even at high-end environmentally relevant concentrations. No observed effect concentrations (NOECs) for survival and growth of PFNA, PFHpA, and PFBS were all in the mg/L range and higher than 99th percentile concentrations observed at DOD sites (e.g., compare to East et al. 2021).

Another important finding was that non-biting midge could be more sensitive to PFOS than previously demonstrated. The present study with *C. dilutus* produced a PFOS EC50 of 3.77 µg/L for growth which is in line with results of Marzialli et al. (2019) who noted reduced individual growth in many generations of a 10-generation study with *C. riparius* at 3.5 µg/L PFOS. The present study also resulted in an LC50 of 2.49 µg/L from 20-day tests whereas the 20-d LC50 for *C. tentans* reported by MacDonald et al. (2004) was 92.2 µg/L.

Survival and growth were both lower in the PFOS +PFHxS mixture than in single chemical exposures. However, there were no statistically significant differences. Survival and biomass reductions were suggestive of an additive or synergistic interaction, though multivariate analysis showed no statistical significance. Results showed evidence of increased response to exposures of PFOS+PFHxS within the range of environmentally relevant PFOS.

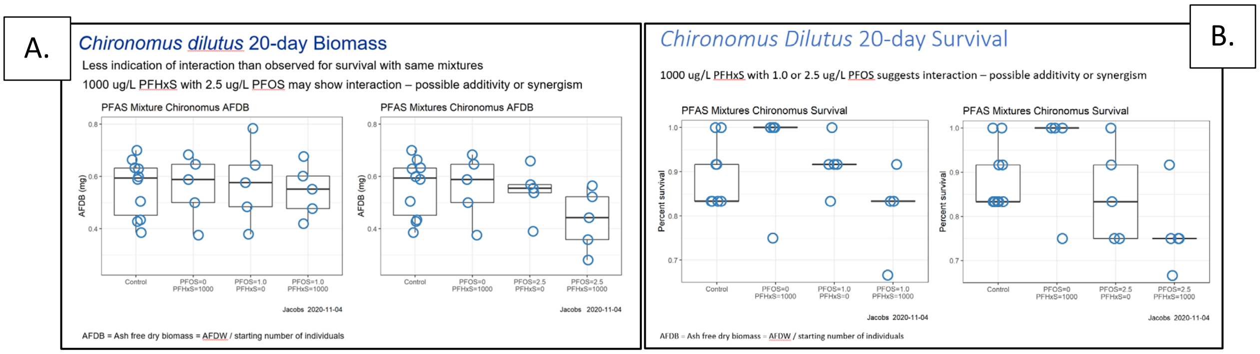


Figure 3. Biomass (A) and survival (B) of *C. dilutes* exposed to mixtures of PFOS and PHFxS.

The binary mixture study with PFOS+PFHxS suggests further mixtures studies with the same chemicals and with more complex mixtures is warranted to fully understand what may be occurring in natural environments. The mixture study was preliminary with a limited factorial design. Future work could be informed by the results of this completed work.

Objective 1.c: Freshwater Fish (*Pimephales promelas*) Toxicity Testing establish toxicity estimates and to facilitate PFAS prioritization:
PFOS-PFHxS Mixture

There were no significant differences in endpoints of survival and growth to adult fish. However, these endpoints were significantly decreased fish from the second-generation. The high PFAS mixture (300 µg PFOS/L: 200 µg PFHxS/L) had a significant impact on survival compared to the control and other PFAS exposures (Chi square = 47.7, df = 5, $p < 0.0001$; Figure A). Additionally, biomass is significantly reduced in the high PFAS mixture concentration compared to control and the low PFOS (150 µg/L) exposure ($F_{5,18} = 4.6$, $p < 0.01$; Figure B).

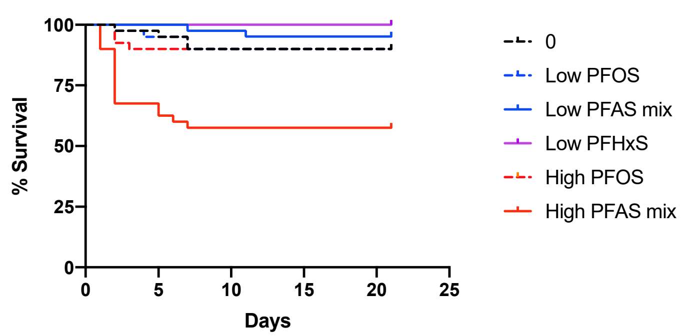
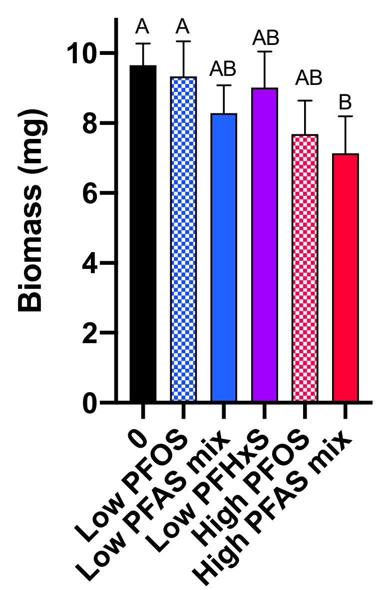


Figure A. Survival analysis of juvenile fathead minnows exposed to PFOS, PFHxS and two mixtures. Exposure treatments are defined as Low PFOS (150 µg/L), Low PFAS mix (150 µg PFOS/L: 100 µg PFHxS/L), Low PFHxS (200 µg/L), High PFOS (300µg/L) and High PFAS mix (300µg PFOS/L: 200 µg PFHxS/L)



Selected tissues were analyzed for PFAS accumulation in adult fish following the 42-day exposure period. Tissues with enough replication for statistical analysis were brain and ovary; there is exposure dependent increases in the sum of PFAS concentrations in the brain and ovary compared to controls ($p < 0.001$; Figure C). Interestingly, mean PFOS tissue concentrations are greater than mean PFOS concentrations of the corresponding mixture. This may be due to competitive binding during chemical accumulation in fish exposed to mixture treatments of PFOS and PFHxS.

Figure B. Biomass of juvenile fathead minnows exposed to PFOS, PFHxS and two mixtures. Letters indicate significant differences from exposure treatments ($p<0.05$). Exposure treatments are defined as Low PFOS (150 µg/L), Low PFAS mix (150 µg PFOS/L: 100 µg PFHxS/L), Low PFHxS (200 µg/L), High PFOS (300µg/L) and

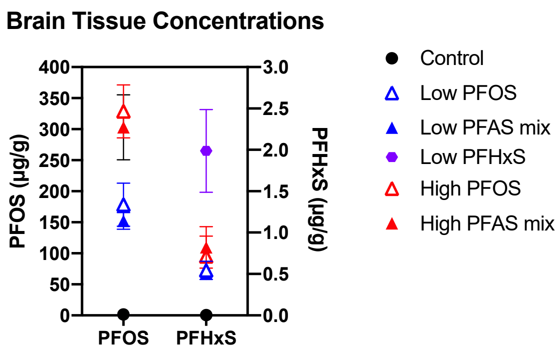
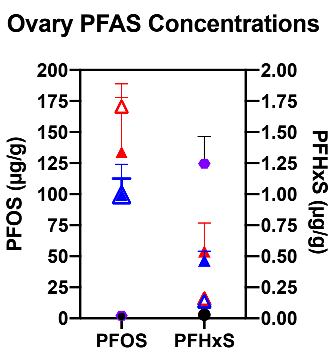


Figure C. Tissue concentrations of PFAS in adult fathead minnows exposed to PFOS, PFHxS and two mixtures. Letters indicate significant differences from exposure treatments ($p<0.05$). Exposure treatments are defined as Low PFOS (150 µg/L), Low PFAS mix (150 µg PFOS/L: 100 µg PFHxS/L), Low PFHxS (200 µg/L), High PFOS



Results Synopsis: The toxicity testing with the fathead minnow has gone well with the completion now of 4 toxicity tests including PFOS, PFHxS, PFNA, and a mixture of PFOS:PFHxS. PFOS was toxic at the highest tested concentrations (500 ppb) while PFHxS alone was not. Interestingly, fish exposed to the mixture of PFOS:PFHxS showed toxicity at 200 ppb PFOS when combined with 132 ppb PFHxS suggesting a potential synergistic effect.

Objective 2.a. Avian Toxicity Testing:

Despite evidence suggesting that avian species are likely to be vulnerable to exposure and effects of PFAS (Larson et al., 2018; Suski et al., 2020), there are few data on the toxicity of PFAS precluding robust ecological risk assessments for this taxa. The objective of this task was to develop toxicity data for Northern bobwhite quail, *Colinus virginianus*, exposed to PFAS in drinking water. No existing data suggest that acute toxicity to avian (or other species) is common or likely as a result of PFAS exposure but the general lack of acute toxicity data for PFAS other than PFOS provided the impetus to explore acute toxicity. Quail were also exposed to PFAS in drinking water for 90 days to include the reproductive period. These data have appeared in several manuscripts and may inform future development of Toxicity Reference Values for avian species.

Results Synopsis: For acute toxicity testing, we used the “up and down method” for estimating acute LD50s for test PFAS in adult Northern bobwhite quail. The up and down method provides similar results to traditional LD50 testing but uses much fewer animals (OECD 2008). Stock dosing solutions of test PFAS (PFBS, PFHxS, PFOS, PFHpA, PFOA, PFNA) were generated in milli-Q water. Adult quail (both male and female) were dosed by pseudo gavage, placed in cages based on test chemical, and monitored for 24 h. Briefly, the test occurred in two phases starting with a limit test and followed by a main test if a test chemical did not pass the limit test. Three (3) PFASs (PFHxS, PFOS, and PFNA) passed the limit test, and were not evaluated further using the main test; one of five birds died in the PFHxS treatment, and no deaths occurred in the PFOS or PFNA treatments. Three (3) PFASs (PFBS, PFHpA, and PFOA) failed the limit test and were further evaluated using the main test; all five birds died within 1.5 h in the PFBS treatment, all five birds died within 24 h in the PFHpA treatment, and four of five birds died in the PFOA treatment. Results of the main test for PFBS, PFHpA, and PFOA indicated acute LD50s between 1750 mg/kg - 2000 mg/kg. This result was somewhat surprising given how rapidly PFBS and PFHpA produced mortality in the limit test. However, the limited acute toxicity data available in the literature suggests that PFAS are not very acutely toxic (PFOS oral LC50 in quail 220 mg/kg for 5 days; PFOS oral LC50 in duck 628 mg/kg for 5 days). Our observations are consistent with these previous reports.

For chronic toxicity studies, we exposed pairs of adult birds to three (3) concentrations of PFAS, either individually or as simple mixtures, via drinking water for 90 d. The exposure concentrations were chosen to bracket PFAS concentrations and mixture ratios present in surface water samples (East et al. 2021). PFAS dose (average daily intake, $mg{\bullet}kg^{-1}\ bw{\bullet}d^{-1}$) was determined at the conclusion of each study based on water consumption. Two trials with individual PFAS or simple mixtures were conducted each year beginning in January. Each trial included monitoring several biological endpoints generally classified as relevant to avian reproduction, growth, and survival. Water exposures provide advantages in quantification and uniform delivery; a water source is also required for wild birds during significant growth and reproduction. However, exposure through water does complicate comparisons to other studies utilizing food exposures (e.g.,) as there may be differences in rates of absorption and excretion of the target contaminants that merit consideration.

Effects of PFOA, PFHpA, PFBS, and PFHxS to bobwhite quail over 90-days were general minimal, non-monotonic, and sometimes equivocal with regard to ecological relevance. For example, hatchling weight was increased in some PFOA exposure groups suggesting that PFOA may act as a mild growth stimulator in juveniles via altered metabolism. Similarly, there was evidence of increases hatchling weight from exposure to PFBS at the lower concentrations and decreased hatchling weight at the highest PFBS exposure level. For high PFHxS exposure levels, hatchlings weighed more than control hatchlings at the lower exposure levels (0.1 and 1.0 ng/ml) and less than controls at the high PFHxS exposure level (10 ng/ml). PFHxS also decreased overall egg production

As a result of efforts under Task 1, it became clear that PFOS and PFOS + PFHxS toxicity data would be highly relevant for AFFF use sites. Hence, quail were exposed to PFOS and PFOS + PFHxS mixtures (2:1 PFOS:PFHxS) guided by results of a data science analysis of PFAS occurrence in surface water (East et al. 2021). For the PFOS and 2:1 PFOS:PFHxS exposures, nominal water concentrations were 0.1 ng/mL, 1 ng/mL, and 20 ng/mL. We used water consumption and measured PFAS concentrations to determine the average daily intakes (ADI) over the 90-d study (**Table 1**).

Table 1. Mean water consumption per bird per day and average daily intake (ADI) from a 90-d PFOS or PFOS:PFHxS exposure study.

Measured Exposure Concentration	Water consumption ± SE (mL/bird/day)	ADI (mg• kg^{-1} bw• d^{-1})
Control	31.8 ± 0.9	NA
0.216 ng/mL PFOS	31.1 ± 1.4	$2.97x10^{-5}$
0.596 ng/mL PFOS	33.0 ± 1.5	$8.50x10^{-5}$
18.7 ng/mL PFOS	29.7 ± 0.4	$2.45x10^{-3}$
0.375 ng/mL PFOS:PFHxS (1.2:1)	30.8 ± 0.6	$5.04x10^{-5}$
0.958 ng/mL PFOS:PFHxS (1.8:1)	32.6 ± 0.4	$1.31x10^{-4}$
22.9 ng/mL PFOS:PFHxS (1.2:1)	31.8 ± 0.9	$3.10x10^{-3}$

Detailed results of this study are reported in 2 manuscripts (Dennis et al. 2020; 2021) published in *Environmental Toxicology and Chemistry*, so only a very brief summary is provided here. There were no effects on quail survival of any treatments. However, female weight gain was adversely affected at an ADI of $3.10x10^{-3}$ mg• kg^{-1} bw• d^{-1} for the mixture exposure. For reproduction endpoints, successful development to the pipping stage resulting in complete hatching once pipped was adversely affected at an ADI of $2.45x10^{-3}$ mg•kg^{-1} bw•d^{-1} for the PFOS exposure. These values are comparatively much lower than the current dietary avian toxicity reference value (TRV) of 0.021 mg PFOS•kg^{-1} bw•d^{-1} where birds were exposed via the feed (Newsted et al.). Relationships between test chemical (PFOS) and test substance (PFOS:PFHxS) showed that PFOS and PFHxS have possible interacting effects in avian receptors and likely differing mechanisms of toxicity depending on chemical co-occurrence and dose.

Species- and tissue-specific PFAS LOAEL Chronic Toxicity Value (CTV) were determined through residue analyses of adult quail liver tissue, juvenile quail liver tissue, and whole egg contents. These values (**Table 2**) represent burdens at which population-level adverse health effects could be expected to occur in wild avian populations at this historically vulnerable life stage and are lower than current class wide avian TRVs.

Table 2. Mean PFOS, PFHxS, and sum PFAS concentration in quail tissues producing the lowest observable adverse effect levels (LOAELs) from chronic exposure to either 18.7 ng/mL PFOS or a 1.2:1 PFOS:PFHxS mixture.

Tissue Type	PFAS	Single Chemical Exposure	Mixture Exposure Concentration
Adult liver (ng/g ww)	PFOS	348	342
	PFHxS	ND	263
	ΣPFAS	348	561
Female liver (ng/g ww)	PFOS	159	268
	PFHxS	ND	149
	ΣPFAS	159	367
Male liver (ng/g ww)	PFOS	536	417
	PFHxS	ND	339

	ΣPFAS	536	756
Offspring liver (ng/g ww)	PFOS	32	27
	PFHxS	ND	11
	ΣPFAS	32	35
Whole egg (ng/g ww)	PFOS	92	47
	PFHxS	ND	36
	ΣPFAS	92	82

ND = not detected

The last two studies on bobwhite quail involved exposure to PFHxA and a simple mixture exposure (4:1 PFOS:PFHxA) again guided by results of a meta-analysis of PFAS occurrence in surface water (East et al. 2021). For the PFHxA exposures, we tested nominal water concentrations of 0.1 ng/mL, and 5 ng/mL. For the 4:1 PFOS:PFHxA exposures, nominal water concentrations were 0.1 ng/mL, 1 ng/mL, and 20 ng/mL. We used water consumption to determine the average daily intakes (ADI) over the 90-d study. This study was just completed in Fall 2020 and analyses are still ongoing so these results should be considered preliminary. There were no effects of any exposure on survival, body weight, or number of eggs laid of adult birds. However, there was a negative association was found (Pearson's Chi-squared analysis) between treatment and hatching success ($p < 0.001$), specifically lower hatching success for the 20 ng/mL 4:1 PFOS:PFHxA mixture exposures compared to controls. Statistical investigation of unhatched eggs to determine the stage of arrest revealed that the average day of arrested embryonic development (i.e., arrested development day) was unequal among exposure groups ($p = 0.007$). Embryos arrested development earlier on average in the 20 ng/mL 4:1 PFOS:PFHxA exposure group compared to controls.

Collectively, the data generated from these studies on Northern bobwhite quail point to the relatively high toxicity of PFOS and mixtures of PFOS and PFHxS. This is also the first study we are aware of to report avian toxicity data for PFAS other and PFOS and PFOA. Notably, there were some observed and potentially ecologically relevant effects of PFHxA. These data will help inform ecological risk assessments for avian species inhabiting PFAS contaminated habitats.

Objective 2.b. Reptilian Toxicity Testing:

While there are considerable toxicity data for PFOS and PFOA compared to other PFASs, there are still considerable data gaps. A critical data gap lies in the lack of data for what are historically understudied taxa such as reptiles (Hopkins 2002). To date, only PFAS tissue concentrations have been reported for reptiles which provides substantial evidence that exposure to PFAS in this taxa occurs but provides little to no insight into toxicity (Keller et al. 2012; Bangma et al. 2016; Christie et al. 2016; Tipton et al. 2017). This lack of data precludes ecological risk assessments on PFAS contaminated sites where reptiles may be abundant or where reptiles listed as threatened and endangered may reside. We developed a reptilian toxicity test model using anolis lizard based on studies published previously by the primary investigator on the effects of chemical contaminants on the Western fence lizard, *Sceloporus occidentalis* (Suski et al. 2008; Salice et al. 2009). Further, we were successful in establishing a breeding colony for brown Anoles that have allowed us to conduct several studies using eggs and have also allowed us to conduct two reproductive studies. Our testing protocol involves 60-90-day toxicity studies in which lizards are dosed via pseudo-gavage three times per week for the duration of the study. Anoles are very amenable to laboratory study and we have had very few (<5) mortalities in control animals in ten pilot or definitive studies.

Results Synopsis: A reptile toxicity testing model based on the brown anole (*Anolis sagrei*) has been developed and successfully implemented for up to 90 days. We have evaluated the toxicity of several PFAS including PFOS, PFHxS, PFNA, and PFOA. Data thus far indicate that PFOS is the most toxic of the tested PFAS causing adverse effects on size and/or growth of subadult male and adult female lizards. In a study in which mated female brown anoles were exposed to PFOS, there appeared to be somewhat of a stimulation in egg production at the highest PFOS dose but with a concomitant reduction in size of exposed females. Moreover, offloading of PFOS to eggs by exposed females resulted in PFOS concentrations in eggs in excess 5.0 x 10^4 ng PFOS/g egg. Several studies confirm that PFOS causes adverse effects at doses of 2.0 mg/kg-bw/day and above. Because of the prominence of PFXxS in soil and surface water at contaminated AFBs, we also conducted a series of studies on PFHxS and in no case was toxicity observed. Additionally, given the co-occurrence of PFOS and PFHxS, we recently complated a study in which A longer duration, refined toxicity test is ongoing for PFOS. We have also developed a breeding colony for eventual use in developing a reproductive toxicity test with the brown anole. A mixture of PFOS:PFHxS will be tested in spring 2019.

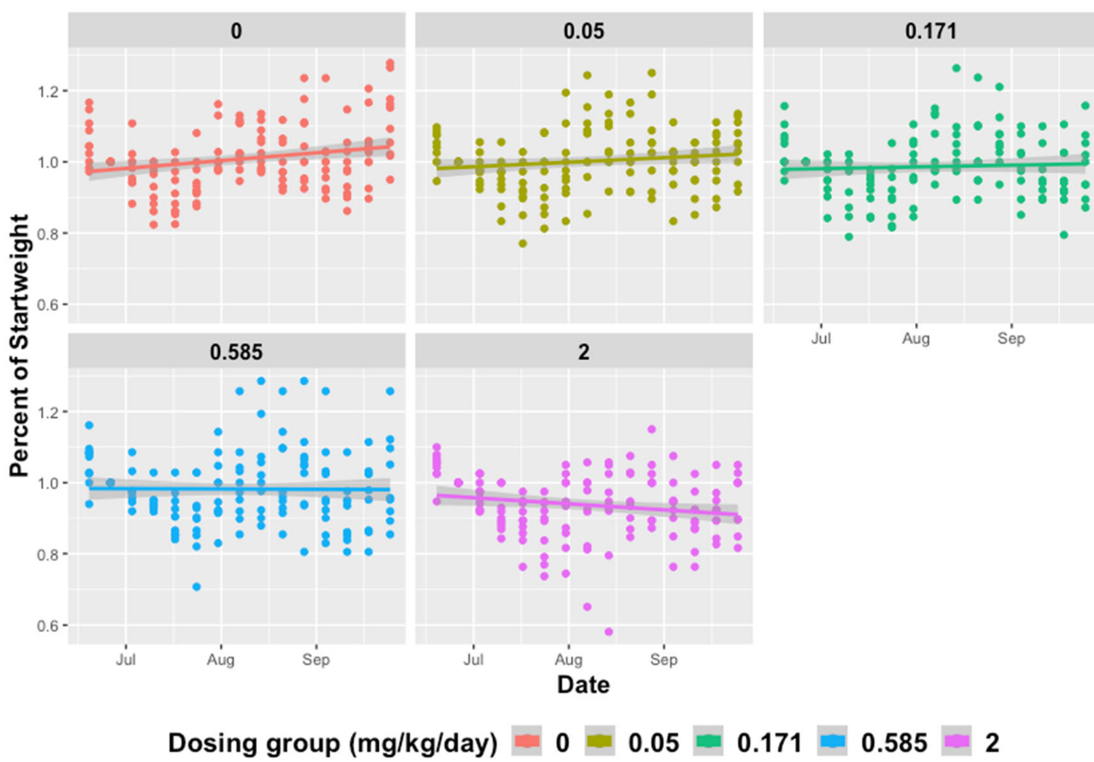


Figure 3. Percent of start weight over 90-Day period PFOS exposure period for female brown anoles after 2-week in lab acclimation period. Each treatment group was based on a mg/kg/day PFOS dosing regimen. 2-weeks of acclimation period are included in this figure prior to exposure to show that no individuals began exposure less than 90% of the weight they entered

the lab as. Percent of start weight for exposure was calculated using the individual's mass at the start of exposure (day 0).

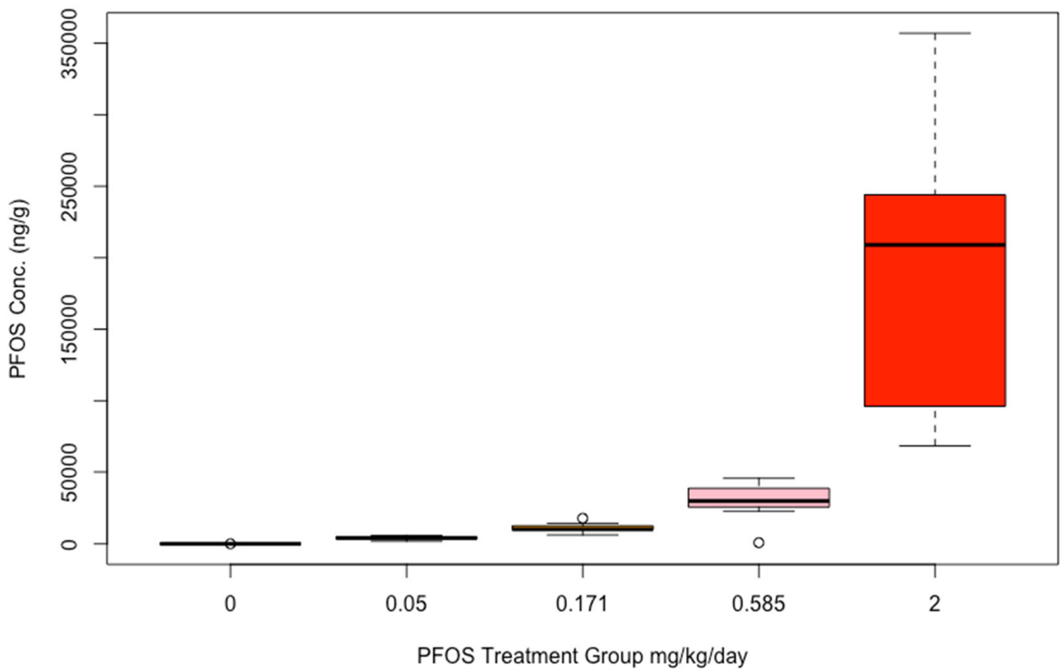


Figure 3.3. PFOS concentrations detected in viable eggs in each treatment group. There is a clear dose dependent relationship between the amount of PFOS exposure to female brown anoles and amount offloaded into their eggs. ANOVA p << 0.001.

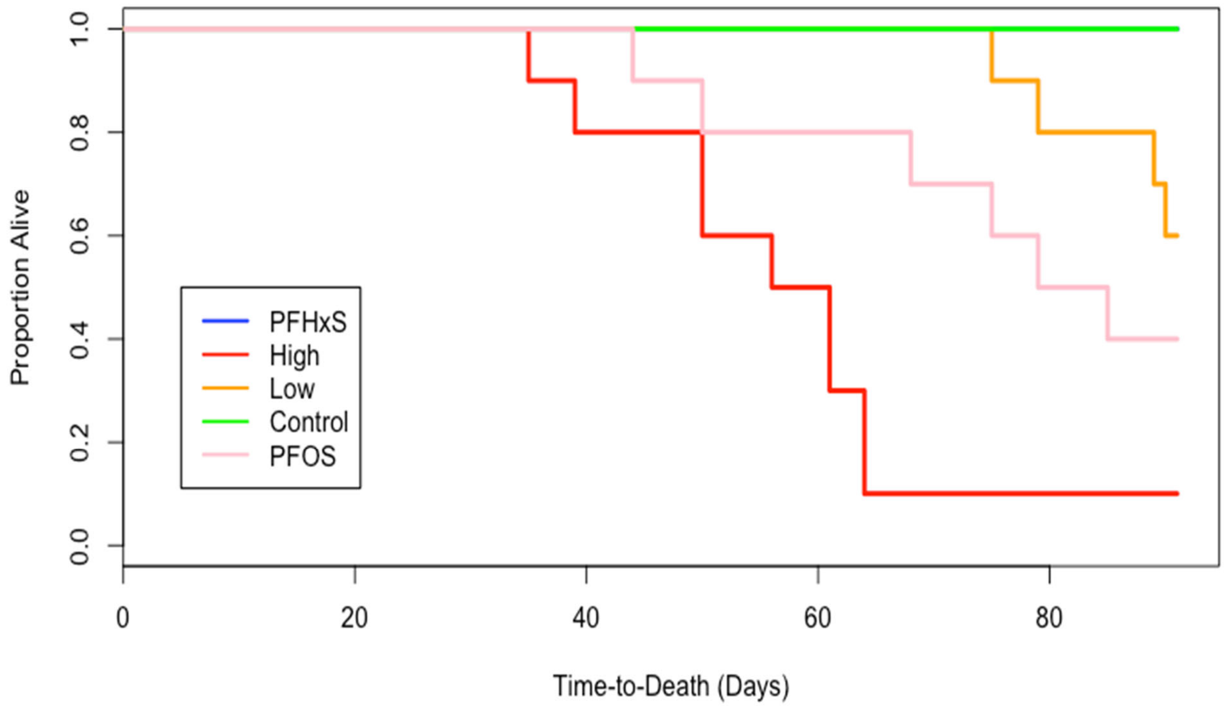


Figure 3.3. Time to death for each treatment group throughout a 90-Day PFAS exposure. PFHxS and Control were identical in their lack of mortality, thus the blue line (PFHxS) is occluded in the graph. Log-rank test revealed a Chi-sq value= 43.2 and a p = <0.001.

Objective 3: Develop tools, TRV recommendations and conceptual models of PFASs to facilitate the use of products developed in Task 1 and 2 in ecological risk assessment.

The ultimate goal of the proposed research is to generate data and tools that can facilitate ecological risk assessment of PFASs on DOD installations and elsewhere. Therefore, an important product of the proposed effort will be the conversion of data and insights into revised conceptual models, robust TRVs, and a guidance document that provides the information and framework needed to (a) apply current tools/data and (b) a process for identifying which new data may be needed to effectively estimate risk to wildlife receptors. Decision trees and algorithms (e.g., the Prioritization Algorithm of Task 1) will be built based on current state of knowledge and provided so that they can used for ecological risk assessment. The Transition Plan (section 1.3.3.3.f) will play an important role here as it outlines how information will be disseminated to DOD personnel and interested stakeholders.

Subtask 3.a: Modify and finalize PFAS conceptual model:

The working conceptual model for PFAS exposure to ecological receptors is shown in Figure 3.a.1. A rendition similar to this figure was published in 2018 (Salice et al. 2018).

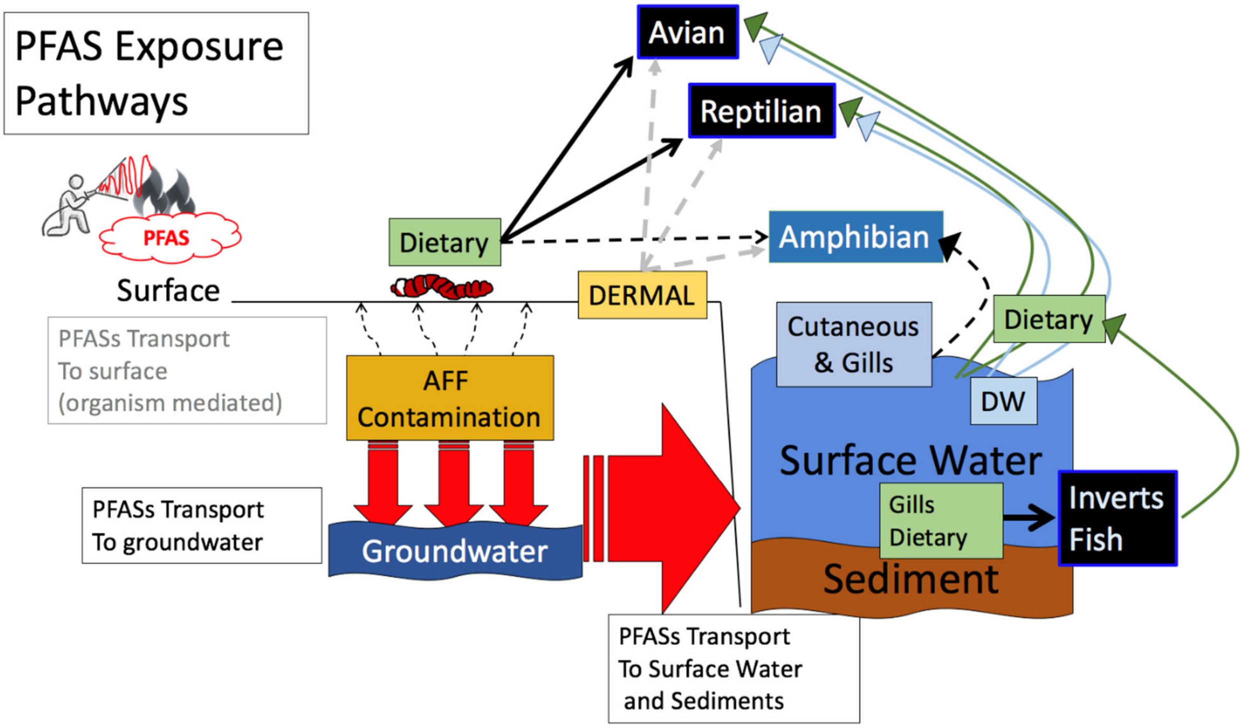


Figure 3.a.1. Diagram Showing the Conceptual Model for PFAS Release to the Environment and Subsequent Exposure to Key Ecological Receptors.

Subtask 3.b: Develop and Recommend Toxicity Reference Values

As of February, 2019 toxicity studies are still ongoing. That said, based on results from Task 1, our prevailing hypothesis is that PFOS is the most likely dominant PFAS and no data has yet surfaced to suggest that it is also NOT the most toxic. Toxicity studies with avian receptors and chironomids have not shown any toxicity (or equivocal effects) for PFAS other than PFOS. The second most likely PFAS in DoD soild and surface water is PFHxS. PFHxS alone does not appear to be toxic at high-end environmentally relevant exposure concentrations for fish, birds, chironomids and reptiles. However, a study in fish exposed to PFOS+PFHxS indicated potential synergistic effects of the two chemicals at environmentally relevant ratio. The combined effects of PFOS + PFHxS warrants additional study since this appears to be the most likely environmental exposure.