AN ECOLOGICAL RISK ASSESSMENT OF THE POTENTIAL EFFECTS OF PFOS ON INVERTIVOROUS BIRDS NESTING NEAR TWO POINT SOURCES IN TEXAS

A Thesis

by

LARK HESTON

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Chair of Committee,	Miguel Mora-Zacarias
Committee Members,	Masami Fujiwara
	Thomas McDonald
Head of Department,	Cliff Lamb

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic organic compounds that have a large number of congeners and are used as surfactants in a wide range of consumer products, including firefighting foams, pesticides, and cleaning supplies. The two main point sources of environmental contamination of PFAS are releases from manufacturing facilities and from the use of firefighting foams at fire training facilities, airports, military bases, and fire suppression sites. Two potential point sources of PFAS in College Station, TX occur along White Creek: the Brayton Fire Training Field (BFTF) and the Easterwood Airport. The goal of this ecological risk assessment is to determine the magnitude and effects that PFAS coming from these two sources may pose on the reproductive success of Carolina Wrens, an indicator species for invertivorous birds. Field-collected concentrations of PFAS around the facilities and literature-based equations and values were used to assist in the estimation of exposure of PFAS to Carolina Wrens. Specifically, a total daily intake value of PFOS (0.006 $mg_{PFOS}/kg_{body}/day$) was calculated and then compared to a lab-derived no-observed-adverse-effect-level (0.77 $mg_{PFOS}/kg_{body}/day$) from the literature to yield a risk quotient (0.008). The risk quotient in this ecological risk assessment was well below 1, indicating that PFOS does not represent a risk for negative effects on the reproduction of Carolina Wrens nesting around the Easterwood Airport and Brayton Fire Training Field.

DEDICATION

To my mom for her endless love and support

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Contributors

This work was supervised by a thesis committee consisting of my advisor, Dr. Miguel Mora-Zacarias of the Department of Wildlife and Fisheries Sciences, Dr. Masami Fujiwara of the Department of Ecology and Conservation Biology, and Dr. Thomas McDonald of the School of Public Health.

The collection of soil and water samples was assisted by undergraduate assistant Andrew Austin. The analyses of the samples were led by Dr. Yina Liu and Michael Shields of the Geochemical and Environmental Research Group.

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NOMENCLATURE

AFFF	Aqueous film-forming foam
AUF	Area use factor
DWI	Daily water intake
EPC	Exposure point concentration
IR	Ingestion rate
NOAEL	No-observed-adverse-effect-level
P	Proportion of invertebrates potentially exposed to PFAS
PFAS	Perfluoroalkyl substances
PFOS	Perfluorooctanesulfonate
RQ	Risk quotient
TDI	Total daily intake

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INTRODUCTION

Perfluoroalkyl acids

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic organic compounds that have a large number of congeners and are used as surfactants in a wide range of consumer products (ITRC 2018). These products include textiles, cookware, personal care products, firefighting foams, pesticides, and many other different types of manufactured items (Wang et al. 2011; ITRC 2018). There are two groups of per- and polyfluoroalkyl substances: perfluoroalkyl acids (PFAA) and polyfluoroalkyl substances (PFS) (Gomis 2017; ITRC 2018). The general structure of PFAA is a chain of carbons of varying length, each bonded to a hydrogen-replaced fluorine, plus an associated side group attached at the end of the chain (Gomis 2017). The bond between carbon and fluorine in these substances is strong, allowing PFAA, which have this bond at each carbon, to be resistant to degradation (Gomis 2017).

Polyfluoroalkyl substances include a subgroup called fluorotelomer alcohols (FTOH) (Wang et al. 2011; Gomis 2017). Unlike PFAA, these compounds are able to degrade in the environment by microbes, in organisms by metabolism, or in the atmosphere (Wang et al. 2011; Krafft and Reiss 2015). Fluorotelomer alcohols such as fluorotelomer sulfonic acids (FTSA) are precursors to some congeners of PFAA, such as PFOA, which increases environmental concentrations of PFAA when degraded (Wang et al. 2011).

The first global assessment of accumulation of PFAS in wildlife reported detectable amounts of these chemicals in the tissues of fish, birds, reptiles, amphibians, and marine mammals sampled throughout the world (Giesy and Kannan 2001). Every sample tested contained detectable amounts of PFAS, and perfluorooctanesulfonate (PFOS) was discovered to be the most prominent congener, which aligns with other studies of the global distribution of PFAS in the environment and organisms (Giesy and Kannan 2001; Houde et al. 2011). Samples from organisms living in urbanized locations were found to have 2-10 times higher concentrations of PFOS than organisms of the same or similar species living in remote locations (Giesy and Kannan 2001). Although PFOS production ceased in 2003, the congener remains widely distributed (Houde et al. 2011). This may be due to the continued production of congeners of PFAS that are precursors to PFOS (Houde et al. 2011).

The two main point sources of environmental contamination of PFAS are releases from manufacturing facilities and from the use of firefighting foams (Cousins et al. 2016; ITRC 2018). The use of firefighting foams occurs at fire training facilities, airports, military bases, and fire suppression sites (ITRC 2018). These foams have the ability to contaminate many environmental media, such as sediments and surface- and groundwater, with chemicals such as PFAS (ITRC 2018). Perfluoroalkyl substances may be released atmospherically or through water runoff (NRWA n.d.). Due to the low degradability of these chemicals, PFAS released from fire training facilities have been measured in detectable concentrations even 20 years after the training had ceased (Mcguire et al. 2014; Flipovic et al. 2015).

Site descriptions

White Creek, a tributary to the Brazos River, runs through College Station, Texas and passes two potential point sources of PFAS: the Brayton Fire Training Field (BFTF) and the Easterwood Airport (Figure 1). Both of these facilities are owned by Texas A&M University. These facilities are located in the Post Oak Savannah ecoregion of Texas (TPWD n.d.). The soils surrounding them are classified as Sandow series, which is comprised of frequently flooded but moderately well drained loam, and are on a zero to one percent slope (Soil Survey Staff n.d.). The average temperatures recorded at the Easterwood Airport, which is located adjacent to the

BFTF, range from 6 to 35 °C throughout the year (Weather Spark n.d.). During the wet season, the average rainfall is 10.5 cm, and during the dry season it is 4 cm (Weather Spark n.d.).

Brayton Fire Training Field

The Brayton Fire Training Field in College Station, TX is used to train thousands of people each year in fire suppression techniques (TEEX n.d.). The Brayton Fire Training Field is nearly 300 acres, is the largest fire training facility in the world, and has been training firefighters since 1929 (TEEX n.d.). The land surrounding the BFTF was used as a landfill until 1983 and a landfarm from 1993-2011 when a parking lot was constructed on top of that land (Holloway 2003). As a landfill, the treatment of retention pond sediment containing PCBs and petroleum hydrocarbons was promoted by the addition of microorganisms and nutrients (Craig Holloway, Texas A&M Engineering Extension Service, personal correspondence). As a landfarm, the land supported the treatment of petroleum products originating from the fire training activities (Craig Holloway, Texas A&M Engineering Extension Service, personal correspondence). Since then, there has been a closed-loop waste water treatment system put into place (Holloway 2003). There have also been recent individual research studies using various firefighting foams that are occasionally conducted at the fire training field, such as Suardin et al. (2009), Qi et al. (2010) and Yun et al. (2011). The constant use of foams leads to the potential for chemicals to disperse into the surrounding air, water, and soil.

Aqueous film-forming foams (AFFFs) have never been used at the Brayton Fire Training Field, and three brands of non-PFAS Class-A foams are currently being used: Williams Thunderstorm training foam, Verde Environmental Micro-Blaze Out training foam, and Solberg training foam (Howard Meek, Texas A&M Engineering Extension Service, personal correspondence). As these are foams used for training purposes, the goal is not to extinguish the fire as thoroughly as possible, but rather extinguish the fire just enough to be able to reignite the training structure. However, foams may be labeled by the manufacturer as having a small percentage of proprietary ingredients, which may actually include PFAS. As the exact makeup of foams is known only by the manufacturer, there may be unintentional use of PFAS by fire training facilities. As detailed in Table 1, Williams Thunderstorm training foam contains 12-23% of labeled non-PFAS ingredients, with the remaining percentage of the foam being proprietary ingredients (*TSTFP* 2019). However, the data sheet for this foam states that there are no fluorinated chemicals present in the foam (T-STORM® TSTF Training Foam Concentrate 2019). Although Solberg training foam and Verde Environmental Micro-Blaze Out training foam are also described as not containing PFAS ingredients, the ingredient lists on the safety data sheets do not amount to 100% labeled ingredients (*MBO* 2019; *Solberg Foam TF5X* 2007) (Table 1). Specifically, the Solberg foam lists five ingredients that all make up less than 20% of the foam, with the remaining ingredients being proprietary (*Solberg Foam TF5X* 2007) (Table 1).

The Brayton Fire Training Field uses a catchment system that fully surrounds the impervious surface of the fire training area to prevent runoff (Holloway 2003). The facility also contains two retention ponds to further minimize environmental contamination. These retention ponds, along with an Equalization Basin and additional tanks, work as a closed-loop wastewater treatment system that is closely monitored by TEEX. The ponds supply the water for fire training operations, and the used water gets treated chemically and microbially before it is returned to the ponds (Holloway 2003). The pond that contains the treated water is fitted with an 8-inch outfall pipe and totalizer, which the water can flow through if there is an excess of volume in the pond during, for example, heavy precipitation events (Holloway 2003).

Easterwood Airport

The Easterwood Airport sits on about 900 acres of land and is directly adjacent to the College Station Fire Department's Fire Station #4 (Madison Environmental Group, Inc. 2019). Two fire trucks that use aqueous film forming foams (AFFFs) are also on site at all times (Madison Environmental Group, Inc. 2017). The airport was constructed in 1992 and has expanded twice since its original construction (Madison Environmental Group, Inc. 2019). It currently operates every day, although the fire station is likely not utilized as often. There are five outfall pipes around the facility, and these outfall pipes direct all stormwater from paved ground and drainage ditches into White Creek and its tributaries (Madison Environmental Group, Inc. 2019). Additionally, there is one retention pond that is used as a halfway point of one of the stormwater routes to White Creek, allowing solids to settle at the bottom of the pond before the stormwater reaches the creek (Madison Environmental Group, Inc. 2017). If any stormwater manages to travel from Easterwood Airport to the parking lots of the Brayton Fire Training Field, this stormwater is directed to White Creek and is not added to the wastewater treatment system (Holloway 2003). During airport operations, such as cleaning aircrafts or equipment with surfactants, the water can be diverted to the sanitary sewage system via a valve on the drain inlet (Madison Environmental Group, Inc. 2019). Substances that are known to be potential contaminants through the stormwater system include fuels, solvents, surfactants, metallic products, pesticides, and fertilizers (Madison Environmental Group, Inc. 2019). Surfactants and pesticides can include PFAS, but this is not specified in the airport's Stormwater Pollution Prevention Program (Madison Environmental Group, Inc. 2019).

Figure 1. Map of the Brayton Fire Training Field and Easterwood Airport. The yellow crosses represent the upstream and downstream locations of the samples. The white arrow that is located between the crosses indicates the direction of the flow of White Creek.

Objectives

The goal of this ecological risk assessment is to determine the presence and magnitude of effects that PFAS may pose on the reproductive success of the Carolina Wren, which is an indicator species for invertivorous birds. This will be accomplished by examining the total daily intake of PFAS that these birds may experience by living and breeding near two points sources, the Easterwood Airport and Brayton Fire Training Field.

PROBLEM FORMULATION

Stressors

Exposure: bioaccumulation and biomagnification

The potential for bioaccumulation and biomagnification of these compounds increases with increasing carbon chain length and depends on the organism's life history (Houde et al. 2011; Krafft and Reiss 2015; ATSDR 2018). Life history plays a role because the degree of bioaccumulation and biomagnification may vary based on an organism's developmental stage, age, life cycle, feeding ecology, breeding ecology, etc. (Houde et al. 2011). Additionally, bioaccumulation can occur from mother to offspring during development (Gebbink and Letcher 2012). Bioaccumulation of PFAS from an environmental media to an organism can be calculated using a bioaccumulation factor (BAF). Environmental media that organisms may bioaccumulate PFAS from include soil, water, and sediment, as well as from their diet. For example, birds may uptake PFAS directly from the water via ingestion, and invertebrates may uptake PFAS directly from sediment during their larval stage.

Congeners of PFAS with a higher number of carbons are generally more bioaccumulative than congeners with a shorter chain length (Buck et al. 2011). Congeners of PFAS that are more bioaccumulative have a higher potential for biomagnification. Biomagnification is measured as the concentration of contaminants in the predator divided by the concentration in its prey and may include values representing the trophic level of each organism (Houde et al. 2011). This value is presented as the biomagnification factor (BMF).

Effects on avian species

Laboratory and field studies have provided insight on the potential effects that different congeners of PFAS may have on various organisms. Most studies on the effects of these chemicals are done in an experimental laboratory setting and administer concentrations of PFAS that are usually much higher than what is found in field studies (CRC CARE 2018). This allows us to understand the upper limit of organisms' tolerances to the chemicals but does not provide insight on how organisms might be adversely affected in a natural setting. Field studies use measures of tolerance that are produced by laboratory studies, such as the no-observed-adverse-effect-level (NOAEL), lowest-observed-effect-level (LOAEL), and lethal concentration of 50% of the test subjects (LC50), to determine if the concentrations found in wildlife may reach the upper limit of the study organisms' tolerance. Extrapolation from laboratory study species to wild receptors should be done with caution, as there may be interspecies differences in the effects that PFAS pose on various receptors with different morphology, physiology, and life history (Norden et al. 2016).

Avian species are commonly used in laboratory studies to determine the effects of chemicals on organisms, although only Mallards (*Anas platyrhynchos*), Northern Bobwhites (*Colinus virginianus*), Japanese Quail (*Coturnix japonica*), and chickens (*Gallus spp.*) have been used as species for experimental PFAS studies (Ankley et al. 2020). In these studies, PFAS have been determined to cause lethality, reduced body weight, reduced weight gain, and adverse reproductive effects (Newstead et al. 2007; Bursian and Link 2018; Dennis et al. 2020). Reproductive effects include decreased pipping success, decreased embryo mass, decreased embryo survival, and decreased imprinting ability (Pinkas et al. 2010; Cassone et al. 2012; Norden et al. 2016; Bursian et al. 2020). These effects are avian-specific and do not encompass the full range of effects that may be found for other organisms, such as invertebrates, fish, and amphibians (Ankley et al. 2020).

Field studies have also been conducted on avian species, but there is usually a difference in species or genus from the experimental studies. Due to this factor and other major discrepancies between laboratory and field studies, the effects observed in laboratory studies may not accurately translate to the organisms being studied in the wild (Ankley et al. 2020). Birds of various trophic levels, such as insectivores, piscivores, and birds of prey, are popular study organisms for avian field studies. Various field studies have concluded that PFAS may cause reduced hatching success, eggshell thickness, and overall breeding success in Tree Swallows (*Tachycineta bicolor*) and Great Tits (*Parus major*), which are insectivores (Custer et al. 2012; Groffen et al. 2019). Increased fledgling success has also been found in these species, and this might be a result of less sibling competition due to increased embryo mortality (Custer et al. 2014; Groffen et al. 2019). Reduced hatching success has also been found in Black-legged Kittiwakes (*Rissa tridactyla*), a piscivore (Tartu et al. 2014).

It is important to note that not all laboratory and field studies have found adverse effects of PFAS in wildlife, which might be due to differences in concentrations of PFAS or methodology (Bustnes et al. 2008). One study on Northern Cardinals (*Cardinalis cardinalis*) found concentrations at higher levels than those determined to possibly cause adverse effects in immune function in chickens but lower levels than those determined to cause adverse effects in Northern Bobwhites and Mallards. Despite the high concentrations of PFAS, the effects of the PFAS on the Northern Cardinals were not investigated (Russell et al. 2019). In a laboratory study, there was no effect of PFAS found on the egg production of Northern Bobwhite Quail (Dennis et al. 2020). In a field study that used Tree Swallows as receptors, Custer et al. (2019) found no adverse reproductive effects in one population that experienced higher levels of PFOS than laboratory-derived toxicity reference values (TRVs), but they did find reduced hatching success in populations that had lower PFOS concentrations. This discrepancy was thought to be caused by the potential presence of additional contaminants in the populations that experienced reduced hatching success (Ankley et al. 2020).

Receptors

Invertivorous avian wildlife, particularly passerines such as Tree Swallows and Great Tits, are often used as indicator species to measure accumulation of PFAS in tissues and examine the reproductive success of potentially affected individuals (Custer et al. 2019; Groffen et al. 2019). Invertivorous species are exposed to PFAS mainly through eating invertebrates that have an aquatic or soil-based larval stage; however, they may also drink surface water that potentially could have concentrations of PFAS (Winkler et al. 2011). Also, many species are exposed to contaminated sediments when building their nests and probing for invertebrates on the ground. Birds may also uptake contaminants through dust bathing and preening. Invertivorous birds that nest near the Brayton Fire Training Field and Easterwood Airport may accumulate PFAS through any of these routes.

Assessment endpoints

In adherence to the Guidelines for Ecological Risk Assessment (EPA 1998), assessment endpoints were chosen based on their ecological relevance, susceptibility to the stressor, and value. In 2020, wildlife surveys were conducted at the nearby Ecology and Natural Resources Teaching Area in late winter to early spring to produce a list of receptors for the assessment endpoints.

The ecological receptor chosen from this list to represent the year-round resident avian invertivores, specifically species whose breeding range includes the area around the facilities, was the Carolina Wren (*Thryothorus ludovicianus*). The assessment endpoint chosen for this ecological risk assessment was the reproductive success of the wrens. This species was chosen for the following reasons: the surrounding ecosystem is composed of quality Carolina Wren habitat, as well as quality habitat for the prey of the Carolina Wren; this species is susceptible and sensitive to the contaminants due to its foraging behavior, diet composition, trophic level, and small size; there is a recreational value of the Carolina Wren to hikers, birders, and photographers due to its morphological and vocal aesthetics; and there is an adequate amount of available data on this species' life history and toxicology in the literature.

Carolina Wrens do not migrate and are monogamous throughout their entire breeding life (Taylor et al. 1983). They may breed up to three times within one breeding season, laying four or five eggs in each brood (Gill and Haggerty 2012). In Texas, the breeding season has been reported to be from mid-March to mid-July (Bent 1948). Various studies have reported this species' territory size to range from 0.7 to 4.1 hectares on average (Haggerty 2009). The foraging behavior of Carolina Wrens mainly consists of turning over leaves and other forest litter to search for terrestrial invertebrates, as well as feeding from tree limbs and shrubs (Strain and Mumme 1988). Ninety-four percent of the Carolina Wren's diet consists of animal matter, of which 67% are invertebrates that have the potential to be contaminated by PFAS given their life history strategies, such as having an aquatic or benthic larval stage (Beal et al. 1941). Their diet consists mainly of insects and spiders, but these invertebrates specifically include dipterans, hemipterans, coleopterans, orthopterans, hymenopterans, arachnids, millipedes, snails, and sowbugs (Beal et al. 1941).

Conceptual model

The conceptual model of this ecological risk assessment depicts the routes of contamination of PFAS to the environment, as well as the routes of exposure from the environment to the Carolina Wren as a surrogate for invertivorous avian species (Figure 2).

The fire training foams that are used at the Brayton Fire Training Field are a part of the wastewater treatment system, where they eventually end up in the retention ponds for future use.

At the Easterwood Airport, surfactants or firefighting foams may enter a retention pond or be directed straight into White Creek. From the water in the retention ponds at both facilities, PFAS can accumulate in the sediment or in aquatic invertebrates. These invertebrates are then ingested by Carolina Wrens. The retention pond water may also be ingested directly by the birds. During a major outfall event, such as heavy rain, some water from the BFTF retention pond is directed into White Creek to maintain the water level of the pond. The PFAS that are released into the creek from both facilities can accumulate in aquatic invertebrates or the sediment, which may later be ingested by the birds. Any remaining PFAS that are not taken up by aquatic organisms are either deposited into the sediment or washed downstream towards the Brazos River.

Due to wind, atmospheric deposition of PFAS during fire training activities may result in soil accumulation of PFAS in the immediate area surrounding BFTF. From the soil, PFAS can accumulate in soil invertebrates, which are then ingested by the birds. The soil that contains PFAS can also be unintentionally directly ingested by the birds during ingestion of invertebrates. The exposure of Carolina Wrens to PFAS likely occurs on a daily basis due to the consistent use of the training foams and surfactants at these facilities.

Risk hypothesis

The hypothesis of this risk assessment is that the use of AFFFs and surfactants at the Easterwood Airport and Class-A fire training foams at the Brayton Fire Training Field results in PFAS in the environment, which leads to the exposure of these organisms. However, the potentially low or absent amount of PFAS in the training foams and infrequent use of surfactants at the airport likely results in very low environmental concentrations of PFAS. I hypothesize that due to the potentially low concentrations of PFAS, despite the daily exposure of the birds to the chemicals, there will be no adverse effects on the reproductive success of the wrens.

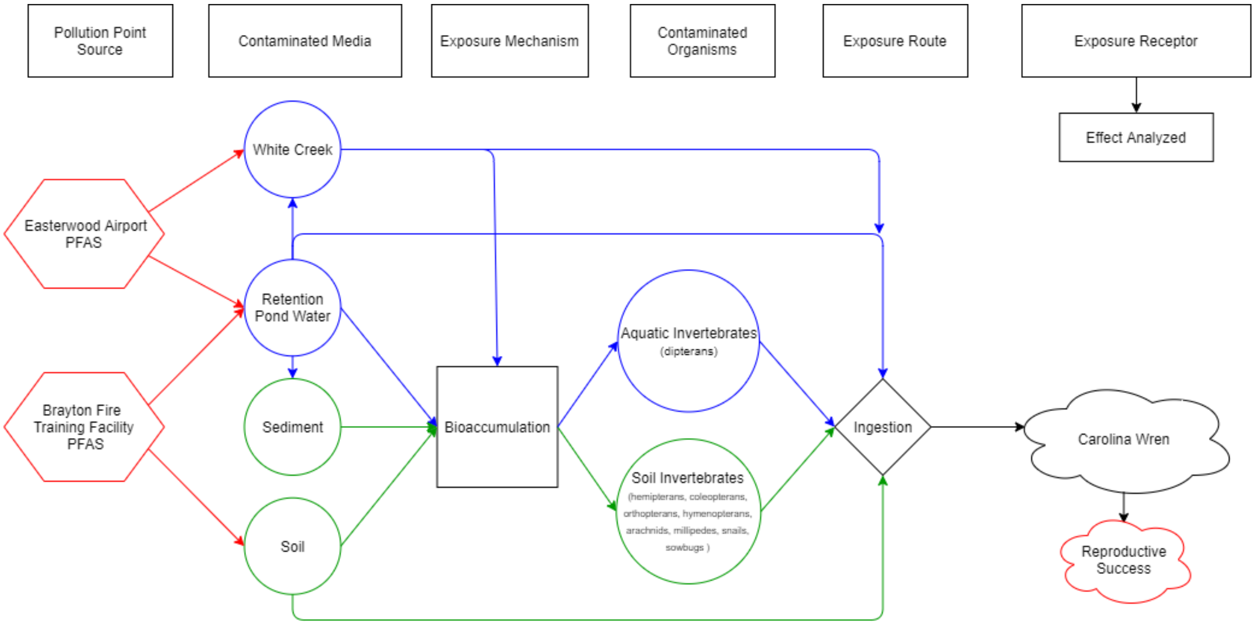

Figure 2. Conceptual model of pathways PFAS can take from the Brayton Fire Training Field and Easterwood Airport to the ecological receptor.

Analysis plan

This ecological risk assessment will use field-collected concentrations and literature-based equations and values to assist in the estimation of exposure of PFAS to Carolina Wrens. These estimated exposure values will then be compared to lab-derived values of effects from the literature. This comparison will yield a risk quotient that will determine whether or not the concentrations present in the environment are high enough to cause adverse effects in the assessment endpoints.

Characterization of exposure plan

To estimate the exposure of Carolina Wrens to PFAS around the two point sources, the airport and fire training facility, I collected samples of water and soil along White Creek and analyzed them to determine the concentration of PFAS in the environment. In November 2020, I collected three samples of water and three samples of soil downstream from the facilities’ outfall pipes, as well as three samples of water and three samples of soil upstream from the facilities.

The three samples of each media in each location were taken from within the same sampling plot and act as replicates in the analysis. Water samples were collected in 250 ml Thermo Scientific HDPE bottles, and soil samples were collected in gallon Ziploc bags. The upstream water samples were collected from 30° 35' 19" N, 96° 21' 08" W, and the upstream soil samples were collected from 30° 35' 19" N, 96° 21' 09" W. The downstream water and soil samples were collected from 30° 34' 57" N, 96° 21' 15" W.

Sample preparation and analysis

The soil and water samples were analyzed at Texas A&M University's Geochemical and Environmental Research Group laboratory. The congeners of PFAS that were analyzed and their respective abbreviations are listed in Table 2. The samples were analyzed using a different protocol for each media. To analyze the water samples, the Silcock et al. (2014) protocol was followed, involving the use of Oasis WAX cartridges (6cc vac, 500mg sorbent per cartridge, 60μm particle size).

The methods for the analysis of soil samples were adapted from the "Protocol for Determination of PFAS Compounds in Soils/Sediments", a protocol following Strivens et al. (2021). The analyzed contents included three replicate samples of upstream soil, three replicate samples of downstream soil, a matrix spike, and a blank. To prepare the six soil samples for extraction, each sample was dried and weighed out to approximately 1.00 g (any variation from 1.00 g was corrected post-analysis). Each sample, the matrix spike, and the blank were spiked with 100 μL of extraction standards consisting of isotopically labelled PFAS compounds. The matrix spike was also spiked with 100 μL of PFAS spike containing all PFAS analytes measured in this study. All eight tubes then received 3 mL of 0.1% NH_4OH in methanol, were vortexed for 30 seconds, sonicated for 30 minutes at 30°C, and centrifuged for 10 minutes at 3500 rpm. Three milliliters of the supernatant from each tube were then transferred to new 15 mL tubes. The steps from 3 mL of 0.1% NH_4OH in methanol through the transferring of the supernatant were repeated twice more before extraction. For extract cleanup and matrix removal, Supelco ENVI-CARB cartridges and an SPE vacuum manifold were used with each tube. Each of the eight cartridges were conditioned with 6 mL of 0.1% NH_4OH in methanol before the samples, matrix spike, and blank were passed through each of the respective cartridges. The cartridges were then washed with another 2 mL of the 0.1% NH_4OH in methanol. The sample extracts were concentrated to 1 mL with a gentle stream of nitrogen gas. All samples were analyzed via liquid chromatography tandem mass spectrometry (LC-MS/MS) on an Agilent 1290 liquid chromatograph coupled to an Agilent 6470 Triple Quadripole Mass Spectrometer. 100 µL of the concentrated samples, matrix spike, and blank, plus 10 µL of injection standards (IS) were added to individual 250 µL polypropylene vials. A linear calibration was conducted prior to running each sequence.

The resultant concentrations from this analysis were averaged among replicates of each media in each location to yield the final results for the environmental concentrations. Using the averaged results from the analysis of the field samples downstream from the facilities, along with equations and values from the literature, I estimated the total daily intake (TDI) of PFAS to Carolina Wrens.

Characterization of effects plan

The literature was examined for effects that PFAS have on avian endpoints, particularly Mallard (*Anas platyrhynchos*), Northern Bobwhite Quail (*Colinus virginiana*), Japanese quail (*Coturnix japonica*), and chicken (*Gallus spp.*). A no-observed-adverse-effect level (NOAEL) value was chosen based on its relevance to reproductive effects.

Risk characterization plan

To estimate the effects that PFAS near the Easterwood Airport and Brayton Fire Training Field have on the Carolina Wrens, the calculated TDI of PFOS and the lab-derived NOAEL from the literature were used to calculate a risk quotient (RQ) by dividing the TDI by the NOAEL. The value of this RQ indicates whether or not there are adverse effects on the reproductive success of these birds near the two point sources.

ANALYSIS

Characterization of exposure

Environmental PFAS concentrations

Thirty congeners of PFAS were analyzed from the soil and water samples taken near the Brayton Fire Training Field and Easterwood Airport. The results of the sample analyses are summarized in Table 3. Downstream of both facilities' outfall pipes, 21 congeners were detected in the soil samples and 12 were detected in the water samples. Upstream of the facilities, two congeners were found in the soil and seven were found in the water. Only one congener, PFBA, was found in all four sample sites; it was 47 times higher in the downstream soil samples ($\bar{x}$ = 0.0188 mg/kg) than in the upstream samples ($\bar{x}$ = 0.0004 mg/kg), and it was four times higher in the downstream water samples ($\bar{x}$ = 0.0000093 mg/L) than in the upstream samples ($\bar{x}$ = 0.0000023 mg/L). The congener with the highest average concentration in the soil samples was PFTrDA, which was detected downstream of the facilities at 0.1984 ± 0.0672 mg/kg. The congener with the highest average concentration in the water samples was PFHxS, which was detected downstream of the facilities at 0.0000460 ± 0.0000070 mg/L. In the downstream soil samples, the average concentration of PFOS was 0.1691 ± 0.0446 mg/kg, and in the downstream water samples, the average concentration was 0.0000404 ± 0.0000057 mg/L.

Calculation of exposure

I estimated the total daily intake of PFAS by Carolina Wrens by using values of PFOS because they have been found to be the most abundant congener of PFAS at fire training facilities and airports, they are known to be bioaccumulative, and there is a large amount of available data on this congener in the literature (Larson et al. 2018). The following equations and associated values are summarized in Table 4.

I used the equation below which combines the TDI of PFOS through various exposure routes: diet, surface water, and soil (Geosyntec Consultants, Inc. 2019),

Total Daily Intake ($mg_{PFOS}/kg_{body}/day$) = $TDI_{diet} + TDI_{water} + TDI_{soil}$ Eq. 1

where TDI_{diet} is the total daily intake of PFOS through the diet of Carolina Wrens, TDI_{water} is the total daily intake of PFOS through ingestion of water, and TDI_{soil} is the total daily intake of PFOS through ingestion of soil.

TDI of PFOS in the Carolina Wren's diet:

TDI_{diet} ($mg_{PFOS}/kg_{body}/day$) = IR_{diet} ($kg_{prey}/kg_{body}/day$) * EPC_{diet} (mg_{PFOS}/kg_{prey}) * P * AUF Eq. 2

Where:

- IR_{diet} = 0.25 $kg_{prey}/kg_{body}/day$, which was calculated by dividing the avian-specific food ingestion rate of prey (0.005 kg_{prey}/day) by the Carolina Wren's average body weight (0.02 kg) (Nagy 1987; Pyle 1997)
- EPC_{diet} = 0.03 mg_{PFOS}/kg_{prey}, which is the exposure point concentration of PFOS in the prey and was calculated by multiplying the biota-sediment accumulation factor of PFOS in earthworms (*Eisenia fetida*) (0.17) by the average exposure point concentration of PFOS in soil downstream of the facilities (0.169 mg_{PFOS}/kg_{soil}) (Rich et al. 2015)
- P = 0.67, which is the proportion of the Carolina Wren's diet that is potentially exposed to PFOS via soil and water and was calculated as the sum of 19% hemipterans, 14% coleopterans, 13% orthopterans, 11% arachnids, 5% hymenopterans, 3% dipterans, 2% millipedes, and 0.16% sowbugs that make up the Carolina Wren diet (Beal et al. 1941)
- AUF is the area use factor that accounts for the proportion of the Carolina Wren's home range that is located within the contaminated site and is assumed to be 1 in each of the TDI equations

- TDI_{diet} = 0.005 mg_{PFOS}/kg_{body}/day (Table 4)

TDI of PFOS in the Carolina Wren's drinking water:

TDI_{water} (mg_{PFOS}/kg_{body}/day) = DWI (L_{water}/kg_{body}/day) * EPC_{water} (mg_{PFOS}/L_{water}) * AUF Eq. 3

Where:

- DWI = 0.215 L_{water}/kg_{body}/day, which is the daily water ingestion rate and was calculated by dividing the avian-specific daily water ingestion rate (0.0043 L_{water}/day) by the average body weight of Carolina Wren (0.02 kg) (Calder and Braun 1983; Pyle 1997)
- EPC_{water} = 0.00004 mg_{PFOS}/L_{water}, which was the average concentration of PFOS from the three samples of water downstream of the facilities
- TDI_{water} = 0.000009 mg_{PFOS}/kg_{body}/day (Table 4)

TDI of PFOS in the soil that Carolina Wren ingest while feeding:

TDI_{soil} (mg_{PFOS}/kg_{body}/day) = IR_{soil} (kg_{soil}/kg_{body}/day) * EPC_{soil} (mg_{PFOS}/kg_{soil}) * P * AUF Eq. 4

Where:

- IR_{soil} = 0.01 kg_{soil}/kg_{body}/day, which was calculated by multiplying the IR_{diet} (0.25 kg_{prey}/kg_{body}/day) by the proportion of soil content of terrestrial invertebrates (0.05) (Nagy 1987; Pyle 1997; JACOS 2010)
- EPC_{soil} = 0.169 mg_{PFOS}/kg_{soil}, which was the average concentration of PFOS from the three samples of soil downstream of the facilities
- P = 0.64, which is the proportion of the Carolina Wren's diet that is potentially exposed to PFOS and resides in the soil, and it was calculated as the sum of 19% hemipterans, 14% coleopterans, 13% orthopterans, 11% arachnids, 5% hymenopterans, 2% millipedes, and 0.16% sowbugs that make up the Carolina Wren diet (Beal et al. 1941)

- TDI_{soil} = 0.001 mg_{PFOS}/kg_{body}/day (Table 4)

The sum of TDI_{diet}, TDI_{water}, and TDI_{soil} (Eq. 1) resulted in a TDI rate of 0.006 mg_{PFOS}/kg_{body}/day (Table 4). Specifically, this is the "average" TDI rate, as the equations were inputted with average PFOS concentrations from soil and water samples.

In order to reduce uncertainties associated with statistical errors, the TDI was calculated twice more, using low and high values for the PFOS concentrations analyzed in soil and water samples. The calculation of the above equations was repeated, but the averages of the PFOS concentrations were replaced with the low and high concentrations, indicated by the lower and higher standard deviations. The low and high concentrations in the soil were 0.1245 mg_{PFOS}/kg_{soil} and 0.2137 mg_{PFOS}/kg_{soil}, respectively (Table 3). The low and high concentrations in the water were 0.0000347 mg_{PFOS}/L_{water} and 0.0000461 mg_{PFOS}/L_{water}, respectively (Table 3). The low TDI resulted in a rate of 0.004 mg_{PFOS}/kg_{body}/day, and the high TDI resulted in a rate of 0.007 mg_{PFOS}/kg_{body}/day.

Characterization of effects

Calculation of effects

Laboratory studies on the effects of PFAS on avian receptors have used only four species: Mallard (*Anas platyrhynchos*), Northern Bobwhite Quail (*Colinus virginiana*), Japanese quail (*Coturnix japonica*), and chicken (*Gallus spp.*) (Ankley et al. 2020). One experimental study also used wild individuals of the Great Cormorant (*Phalacrocorax carbo sinensis*) and the Herring Gull (*Larus argentatus*) in addition to the domesticated chicken (*Gallus gallus domesticus*) (Norden et al. 2016). Common endpoints of laboratory studies include effects of PFAS on gene expression, various hormones, body weight, reproductive success, and survival (Ankley et al.

2020). Reproductive success can be measured in various ways, including male fertility, female body weight, embryo development, embryo survival, pipping success, hatching success, offspring survival, offspring body weight, and imprinting ability. It is usually reported as a LOAEL or NOAEL, whereas survival is usually reported as an LD50 or LC50.

In the study that compared effects on domesticated chickens to wild cormorants and gulls, PFOS in the chickens resulted in an LD50 of 8.5 µg/g and PFOA resulted in an LD50 of 2.5 µg/g (Norden et al. 2016). Based on embryo survival, the NOAEL in chickens was 2.73 µg/g for PFOS and 0.48 µg/g for PFOA (Norden et al. 2016). The results of the cormorants and gulls indicated that these species were less sensitive to PFOS and PFOA than the chickens, as they could tolerate 1.6-2.6 times higher doses (Norden et al. 2016).

In another study, the ADD50 (average daily dose that resulted in 50% mortality) was found to be 38 mg/kg/day for PFOS and 68 mg/kg/day for PFOA in Japanese Quail (Bursian et al. 2020). When using reduced body weight as the endpoint, the LOAEL for PFOS was 62 mg/kg (11 mg/kg/day ADD) and 262 mg/kg (52 mg/kg/day ADD) for PFOA (Bursian et al. 2020). This study also looked at the effects of the combination of PFOS and PFOA, finding an ADD50 of 28 mg/kg/day PFOS + 28 mg/kg/day PFOA, and a LOAEL that resulted in reduced body weight of 43 mg/kg PFOS + 45 mg/kg PFOA (8.5 + 8.7 mg/kg/day ADD) (Bursian et al. 2020). Overall, this study concluded that the combination of PFOS and PFOA seemed to result in additive effects.

Bobwhite Quail and Mallards began experiencing mortality at 50 and 150 mg/kg doses (Newsted et al. 2007). However, LD50 values for these receptors were not calculated. The NOAEL for PFOS in male Bobwhite Quail, based on reduced testes size, was 10 mg/kg (0.77 mg/kg/day ADD) (Newsted et al. 2007). Although the NOAEL for PFOS in females was not determined, the LOAEL, based on offspring survival and increased female liver weight, was also

10 mg/kg (0.77 mg/kg/day ADD) (Newsted et al. 2007). In Mallards, the NOAEL, based on egg hatchability and offspring survival, was 10 mg/kg (1.49 mg/kg/day ADD), but the LOAEL was not determined (Newsted et al. 2007). There were no effects on adult survival, adult body weight, spermatogenesis, egg fertility, egg production, or embryo viability at a dose of 10 mg/kg of PFOS (Newsted et al. 2007). Newsted et al. (2007) suggested that PFOS concentrations at or below 6.2 mg/kg should not adversely affect the reproductive success or survival of Bobwhite Quail.

To estimate the effects of the exposure to PFAS on reproductive success of Carolina Wrens, I used a NOAEL of PFOS from the literature. The highest NOAEL value of the effect of PFOS on avian reproductive success found in the literature was 0.77 mg_{PFOS}/kg_{body}/day (Newsted et al. 2007). This NOAEL value represents the highest concentration of PFOS that does not produce adverse effects on male testes size, female liver weight, and offspring survival on 20% of bobwhites tested (Newsted et al. 2005). This value has been used in the literature as a toxicity reference value to model PFOS accumulation in aquatic avian food systems (Larson et al. 2018).

RISK CHARACTERIZATION

Risk estimate

The calculation of the "average" risk quotient to determine the magnitude of effect the "average" total daily intake (TDI) of PFOS has on Carolina Wrens is shown in Equation 5,

Risk Quotient (RQ) = TDI / NOAEL Eq. 5

Where:

- TDI = 0.006 $mg_{PFOS}/kg_{body}/day$
- NOAEL = 0.77 $mg_{PFOS}/kg_{body}/day$ (Newsted et al. 2007)
- RQ = 0.008

The low and high TDIs were also used to estimate low and high risk quotients. The low risk quotient, calculated with the low TDI (0.004 $mg_{PFOS}/kg_{body}/day$), was 0.005. The high risk quotient, calculated with the high TDI (0.007 $mg_{PFOS}/kg_{body}/day$), was 0.009.

Risk description

If the TDI is larger than the NOAEL, it would result in a risk quotient above 1, indicating potential adverse reproductive effects from daily exposure to PFAS. The risk quotients in this ecological risk assessment were all well below 1, indicating that PFOS does not represent a risk for negative effects on the reproduction of Carolina Wrens nesting around the Easterwood Airport and Brayton Fire Training Field. The use of low and high values provides a broader scope of the potential effects that may occur from exposure to various potential concentrations of PFOS.

CONCLUSIONS

Fire training facilities and airports are known point sources for environmental contamination of PFAS (ITRC 2018). The historic use of the Brayton Fire Training Field’s land as a landfill and landfarm adds another point source of PFAS to the soil and water of White Creek. Landfills have been reported as significant sources of PFAS in European Starling (*Sturnus vulgaris*), with higher concentrations in eggs from landfills than in eggs near industrial sites (Gewurtz et al. 2018). One landfill even resulted in higher concentrations of PFCAs and comparable concentrations of PFDS and PFOA in European Starling eggs than in Great Tit eggs near a fluorochemical plant (Gewurtz et al. 2018). The aforementioned landfill differs from the current study’s historically present landfill in that it likely contained various consumer items, whereas the landfill that used to be present on the site in the current study was used only for the treatment of sediment from the fire training field’s retention pond. The implication of this difference is that various landfills may have different magnitudes of effects in terms of PFAS contamination.

Fire training facilities may be operated as individual facilities or as part of a larger airport or military base. There have been a handful of studies that aim to understand the distribution of various PFAS congeners in the soil and water around these facilities (Moody et al. 2003; Custer et al. 2010; Karrman et al. 2011; Houtz et al. 2013; Gewurtz et al. 2014; McGuire et al. 2014). Unlike the facility in this ecological risk assessment, many of the facilities examined in the literature use aqueous film-forming foams (AFFFs), which contain PFAS, rather than the non-PFAS Class A foams that the Brayton Fire Training Field uses. In one study, the concentration of PFOS in the soil at the fire training area of an airport was 273 ng/g (0.273 mg/kg), and 10-20 m from the fire training area it increased to 1905 ng/g (1.905 mg/kg) (Karrman et al. 2011). The latter concentration is approximately 11 times higher than the concentration of PFOS sampled in the soil downstream of the Easterwood Airport and Brayton Fire Training Field. After the initial increase 10-20 m from the airport's training area, the concentration of PFOS decreased with increasing distance from the airport's fire training area (Karrman et al. 2011). Concentrations of PFOS in sport fish species, such as smallmouth bass (*Micropterus dolomieu*), were highest in the fish sampled closest to a fire training facility located at an airport that had historical use of AFFFs (Gewurtz et al. 2014). Eggs of Great Blue Herons (*Ardea herodias*) sampled in 1993 (but analyzed in 2007) had higher concentrations of PFOS nearby a 3M plant than in the upstream and even downstream sample sites (Custer et al. 2010). Although only one upstream and one downstream group of samples were taken in the current study, it may be presumed that soil and water (and surrounding wildlife) farther downstream in White Creek, until its deposition into the Brazos River, might be impacted less than those at the sampled downstream site.

At a fire training facility located on a U.S. Air Force Base that was operating with AFFFs from 1970 to 1990, the median concentration (during sampling more than 20 years later in 2011) of PFOS in the soil was 2400 μg/kg (2.4 mg/kg) (Houtz et al. 2013). This is approximately 14 times higher than the concentration of PFOS sampled in the soil downstream of this study's facilities. Unlike the fire training facility in the current study, the facility at the Air Force Base was unlined and underwent remedial treatment after operations ceased rather than having physical barriers to environmental contamination and consistent use of wastewater treatment, which might explain the differences between sites (Houtz et al. 2013). Remediation of surface soils following cessation of fire training activities has been found to alter the composition of PFAS congeners present in the soil (McGuire et al. 2014). Specifically, post-remediation concentrations of PFHxS surpass those of PFOS, whereas pre-remediation concentrations have the opposite trend (McGuire et al. 2014). This is suggested to be due to the transformation of PFAS precursor congeners that degrade into PFAS congeners such as PFHxS (but not PFOS) during remediation (McGuire et al. 2014). Remediation methods used prior to the study included "soil vapor extraction (SVE), groundwater pump and treat, and installation of a dual phase extraction trench using various wells" (McGuire et al. 2014). In downstream soil samples of the current study, concentrations of PFOS surpass concentrations of all other congeners except PFTrDA, a perfluoroalkyl carboxylic acid, which are common products of precursors (Table 3).

Some studies that determine the distribution of PFAS in water sources around fire training facilities sample groundwater rather than surface water. In a comparison to another study of the surface water concentrations of PFAS downstream from an accidental spill, Moody et al. (2003) reported that the concentrations of PFOS and PFOA were significantly higher in their groundwater samples near the Wurtsmith Air Force Base (WAFB) fire training facility than the reported surface water downstream of the spill. However, concentrations of PFOS and PFOA in surface water downstream of a different accidental spill was comparable, but still slightly lower, to the concentrations of these congeners at WAFB (Moody et al. 2003). Groundwater in the current study was not sampled, but the concentration of PFOS in the surface downstream of the airport and fire training field was 0.0000404 mg/L, which is 2723 times lower than the highest reported PFOS concentration in the groundwater sampled at WAFB, which was 110 μg/L (0.110 mg/L) (Moody et al. 2003).

The concentrations of PFAS in soil and water samples in the current study are lower than in the literature. This is likely due to the use of non-PFAS Class A foams at the Brayton Fire Training Field and the physical and chemical efforts put in place via the wastewater diversion and treatment system at the fire training field and Easterwood Airport. Specifically, the Brayton Fire Training Field uses three brands of foams that are labeled as PFAS-free Class A firefighting foams, and the wastewater treatment system in place at the facility prevents any discharge of water except during heavy rain events (Holloway 2003). Easterwood Airport also has protections in place to minimize discharge into White Creek, such as a valve-operated system that allows the water to be directed to the sewage system rather than to the outfall pipes during cleaning operations (Madison Environmental Group, Inc. 2019). It is possible that the presence of PFAS in the soil and water near these two point sources is due to the historic use of the land as a landfill and landfarm. Because of the minimal amount of PFAS present in the soil and water, the amount of bioaccumulation and biomagnification from these media to Carolina Wrens, or other passerine species with similar diet, likely does not pose a threat to their reproductive success, but other sublethal effects were not addressed. This assertion is supported by the small values of the range of risk quotients that were calculated using the estimated total daily intake (TDI) of PFOS and laboratory-derived no-observed-adverse-effect-level (NOAEL). Further studies of the composition of Class A foams used at the Brayton Fire Training Field, use of AFFFs at Easterwood Airport, potential additional point and nonpoint sources of PFAS to White Creek, and the characteristics of biomagnification of PFAS up avian-related food chains are all needed to resolve the main uncertainties of this ecological risk assessment.

UNCERTAINTIES AND ASSUMPTIONS

In Introduction:

- Uncertainty in number and frequency of outfall events per year at each facility
- Uncertainty in identity of proprietary ingredients in foams
- Uncertainty in PFAS composition of surfactants used at the Easterwood Airport
- Uncertainty in use of other firefighting foams used in individual research at the Brayton Fire Training Field
- Uncertainty in exact cause of environmental concentrations of PFAS due to history of facilities, unknown surrounding infrastructure, and uncertainty in exact use of surfactants at the airport

In Problem Formulation:

- Uncertainty in cumulative effects of mixtures of PFAS on receptors
- Uncertainty in population density of Carolina Wrens around the two facilities
- Uncertainty in proportion of PFAS in each pathway (e.g. proportion of contamination to soil versus retention pond)
- Uncertainty in exposure and effects of other chemicals from airport and fire training facility
- Uncertainty in timing of most recent outfall event prior to PFAS sampling

In Analysis:

- Food ingestion rate is average of three estimations of Marsh Wren, a closely related species but not the receptor species
- Exposure point concentrations are values from field site analysis, which might be uncharacteristic of the surrounding areas

- BSAF of PFOS to invertebrates is estimated from a study that used earthworms (which are not a part of Carolina Wren diet) because there are no studies in the literature that use prey of Carolina Wren (Rich et al. 2015)
- Proportion of diet is calculated from percentages of diet that are invertebrates that may be contaminated (excludes proportion of diet that is lepidopterans which would not be exposed to water or soil during at least one stage of their life cycle)
    - From only one study of 291 individuals Carolina Wrens' stomach contents sampled throughout entire year (Beal et al. 1941)
- Area use factor (AUF) is assumed to be 1, although it may be lower if receptor forages outside of contaminated site
- Estimations do not take into account the skin absorption from soil (Carolina Wrens take dust baths)
- Bioavailability and uptake of PFOS in prey, water, and soil is assumed to be 100%
- NOAEL was derived from a study that used Northern Bobwhite Quails because no laboratory studies use Carolina Wrens or closely related species
- NOAEL was based off of the male testes size, female liver weight, and offspring survival on 20% of bobwhites tested, which is not all-encompassing for ways to measure reproductive success

REFERENCES

[ATSDR] Agency for Toxic Substances and Disease Registry. 2018. ToxGuide for perfluoroalkyls. https://www.atsdr.cdc.gov/toxguides/toxguide-200.pdf

Ankley G, Cureton P, Hoke R, Houde M, Kumar A, Kurias J, Lanno R, McCarthy C, Newsted J, Salice C et al. 2020. Assessing the ecological risks of per- and polyfluoroalkyl substances: Current state-of-the science and a proposed path forward. *Environ Toxicol Chem* 40(3):564-605.

Beal F, McAtee W, Kalmbach E. 1941. Common birds of southeastern United States in relation to agriculture. Washington, D.C.: U.S. Dept. of Agriculture. p 246.

Bent AC. 1948. Life histories of North American nuthatches, wrens, thrashers, and their allies. *Bull US Natl Mus*. p 195.

Buck R, Franklin J, Berger U, Conder J, Cousins I, de Voogt P, Jensen A, Kannan K, Mabury S, van Leeuwen S. 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integr Environ Assess Manag* 7(4), 513–541.

Bursian SJ, Link JE. 2018. Dietary exposure of Japanese quail (*Coturnix japonica*) to perfluorooctane sulfonate: Effects on reproduction and chick survivability and growth. *Proc*, 39th Annual North American Meeting of the Society of Environmental Toxicity and Chemistry (SETAC), Sacramento (US), November 4-8, 2018.

Bursian S, Link J, McCarty M, Simcik MF. 2020. The subacute toxicity of PFOS and/or PFOA and legacy aqueous film forming foams to Japanese quail (*Coturnix japonica*) chicks. *Environ Toxicol Chem* 40(3):695-710.

Bustnes JO, Erikstad KE, Lorentsen SH, Herzke D. 2008. Perfluorinated and chlorinated pollutants as predictors of demographic parameters in an endangered seabird. *Environ Pollut* 156(2):417-424.

Calder WA, Braun, EJ. 1983. Scaling of osmotic regulation in mammals and birds. *Am J Physiol* 244(5):601-606.

Cassone CG, Vongphachan V, Chiu S, Williams KL, Letcher RJ, Pelletier E, Crump D, Kennedy SW. 2012. *In ovo* effects of perfluorohexane sulfonate and perfluorohexanoate on pipping success, development, mRNA expression, and thyroid hormone levels in chicken embryos. *Toxicol Sci* 127(1):216–224.

[CRC CARE] Cooperative Research Centre for Contamination Assessment and Remediation of the Environment. 2018. Practitioner guide to risk-based assessment, remediation and management of PFAS site contamination, CRC CARE Technical Report no. 43., Newcastle (AU).

Cousins IT, Vestergren R, Wang Z, Scheringer M, McLachlan MS. 2016. The precautionary principle and chemicals management: The example of perfluoroalkyl acids in groundwater. *Environ Int* 94:331-340.

Custer CM, Custer TW, Schoenfuss HL, Poganski BH, Solem L. 2012. Exposure and effects of perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA. *Reprod Toxicol* 33(4):556-562.

Custer CM, Custer TW, Dummer PM, Etterson MA, Thogmartin WE, Wu Q, Kannan K, Trowbridge A, McKann PC. 2014. Exposure and effects of perfluoroalkyl substances in tree swallows nesting in Minnesota and Wisconsin, USA. *Arch Environ Contam Toxicol* 66:120–138.

Custer CM, Custer TW, Delaney R, Dummer PM, Schultz S, Karouna-Renier N. 2019. Perfluoroalkyl contaminant exposure and effects in Tree Swallows nesting at Clarks Marsh, Oscoda, Michigan, USA. *Arch Environ Contam Toxicol* 77(1):1-13.

Custer TW, Tao L, Saxena A, Route B. 2010. Perfluorinated compounds and polybrominated diphenyl ethers in great blue heron eggs from Indiana Dunes National Lakeshore, Indiana. *J Great Lakes Res* 35:401-405.

Dennis NM, Karnjanapiboonwong A, Subbiah S, Rewerts JN, Field JA, McCarthy C, Salice CJ, Anderson TA. 2020. Chronic reproductive toxicity of perfluorooctane sulfonic acid and a simple mixture of perfluorooctane sulfonic acid and perfluorohexane sulfonic acid to northern bobwhite quail (*Colinus virginianus*). *Environ Toxicol Chem* 39:1101-1111.

Environmental Protection Agency (EPA). 1998. Guidelines for ecological risk assessment. *Fed* 63(93):26846-26924.

Filipovic M, Woldegiorgis A, Norström K, Bibi M, Lindberg M, Österås AH. 2015. Historical usage of aqueous film forming foam: A case study of the widespread distribution of perfluoroalkyl acids from a military airport to groundwater, lakes, soils and fish. *Chemosphere*, 129:39-45.

Gebbink WA, Letcher RJ. 2012. Comparative tissue and body compartment accumulation and maternal transfer to eggs of perfluoroalkyl sulfonates and carboxylates in Great Lakes herring gulls. *Environ Pollut* 162:40-7.

Geosyntec Consultants, Inc. (2019). APPENDIX G: Ecological screening level exposure assessment of Table 3+ PFAS. Geosyntec Project Number TR0795.

Gewurtz SB, Bhavsar SP, Petro S, Mahon CG, Zhao X, Morse D, Reiner EJ, Tittlemier SA, Braekevelt E, Drouillard K. 2014. High levels of perfluoroalkyl acids in sport fish species downstream of a firefighting training facility at Hamilton International Airport, Ontario. *Canada Environ Int*, 67:1-11.

Gewurtz SB, Martin P, Letcher R, Burgess N, Champoux L, Elliott J, Idrissi A. 2018. Perfluoroalkyl acids in European starling eggs indicate landfill and urban influences in Canadian terrestrial environments. *Environ Sci Technol* 52(10):5571–5580.

Giesy JP, Kannan K. 2001. Global distribution of perfluorooctane sulfonate in wildlife. *Environ Sci Technol*, 35(7):1339-1342.

Gill SA, Haggerty TM. 2012. A comparison of life-history and parental care in temperate and tropical wrens. *J Avian Bio* 43:461-471.

Gomis MI. 2017. From emission sources to human tissues: Modelling the exposure to per- and polyfluoroalkyl substances [PhD dissertation]. Stockholm (SE): Department of Environmental Science and Analytical Chemistry, Stockholm Univ.

Groffen T, Lasters R, Lopez-Antia A, Prinsen E, Bervoets L, Eens M. 2019. Limited reproductive impairment in a passerine bird species exposed along a perfluoroalkyl acid (PFAA) pollution gradient. *Sci Total Environ* 652:718-728.

Haggerty TM. 2009. Alabama breeding bird atlas 2000-2006. [accessed 2021 Mar 12]. http://www.una.edu/faculty/thaggerty/BBA%20Homepage.htm.

Holloway CS. 2003. Technical memorandum BFTF wastewater and stormwater management system assessment, Project No. 9-2919, URS Corporation, Austin, TX (US)

Houde M, De Silva AO, Muir DC, Letcher RJ. 2011. Monitoring of perfluorinated compounds in aquatic biota: An updated review. *Environ Sci Technol* 45(19):7962-7973.

Houtz EF, Higgins CP, Field JA, Sedlak DL. 2013. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. *Environ Sci Technol* 47 (15), 8187-8195.

[ITRC] Interstate Technology & Regulatory Council. 2018. Environmental fate and transport for per- and polyfluoroalkyl substances. [accessed on 2020 Feb 18]. https://pfas-1.itrcweb.org/wp-content/uploads/2018/03/pfas_fact_sheet_fate_and_transport__3_16_18.pdf

[JACOS] Japan Canada Oil Sands Limited. 2010. JACOS Hangingstone expansion project: Application for approval to the Energy Resources Conservation Board and Alberta Environment: Appendix 19A-8: Parameters for Key Indicator Resources, Edmonton (CA)

Karrman A, Elgh-Dalgren K, Lafossas C, Moskeland T. 2011. Environmental levels and distribution of structural isomers of perfluoroalkyl acids after aqueous fire-fighting foam (AFFF) contamination. *Environ Chem* 8:372-380.

Krafft MP, Riess JG. 2015. Per- and polyfluorinated substances (PFASs): Environmental challenges. *Curr Opin Colloid Interface Sci* 20(3):192-212.

Madison Environmental Group, Inc. 2017. Stormwater pollution prevention plan. Multi-Sector General Permit #TXR05CE76. Prepared for Easterwood Airport Management, College Station, TX (US)

Madison Environmental Group, Inc. 2019. Spill prevention, control, and countermeasure plan. Prepared for Astin Aviation, College Station, TX (US)

Micro-Blaze® Out (MBO) [Online]; Verde Environmental, Inc.: Houston, TX, January 22, 2021. [accessed 2021 Feb 07] https://www.micro-blaze.com/wp-content/uploads/MECrg.pdf

McGuire ME, Schaefer C, Richards T, Backe WJ, Field JA, Houtz E, Sedlak DL, Guelfo JL, Wunsch A, Higgins CP. 2014. Evidence of remediation-induced alteration of subsurface poly- and perfluoroalkyl substance distribution at a former firefighter training area. *Environ Sci Technol*, 48(12):6644-52.

Moody CA, Hebert GN, Strauss SH, Field JA. 2003. Occurrence and persistence of perfluorooctanesulfonate and other perfluorinated surfactants in groundwater at a fire-training area at Wurtsmith Air Force Base, Michigan, USA. *J Environ Monitor* 5(2), 341–345.

Nagy, K. 1987. Field metabolic rate and food requirement scaling in mammals and birds. *Ecol Monogr* 57:111-128.

[NWRA] National Rural Water Association. (n.d.). Is your state or system being affected by PFAS? [accessed 2020 Feb 18]. https://nrwa.org/initiatives/pfas/

Norden M, Berger U, Engwall M. 2016. Developmental toxicity of PFOS and PFOA in great cormorant (*Phalacrocorax carbo sinensis*), herring gull (*Larus argentatus*) and chicken (*Gallus gallus domesticus*). *Environ Sci Pollut Res Int* 23(11):10855-10862.

Pinkas A, Slotkin TA, Brick-Turin Y, Van der Zee EA, Yanai J. 2010. Neurobehavioral teratogenicity of perfluorinated alkyls in an avian model. *Neurotoxicol Teratol* 32(2), 182-186.

Pyle P. 1997. Identification guide to North American birds, Part I: Columbidae to Ploceidae. Slate Creek Press, Bolinas, CA (US)

Qi R, Ng D, Cormier BR, Mannan SM. 2010. Numerical simulations of LNG vapor dispersion in Brayton Fire Training Field tests with ANSYS CFX. *J Hazard Mater* 183(3):51-61.

Rich CD, Blaine AC, Hundal L, Higgins CP. 2015. Bioaccumulation of perfluoroalkyl acids by earthworms (*Eisenia fetida*) exposed to contaminated soils. *Environ Sci Technol* 49(2):881-888.

Russell MC, Newton SR, McClure KM, Levine RS, Phelps LP, Lindstrom AB, Strynar MJ. 2019. Per- and polyfluoroalkyl substances in two different populations of northern cardinals. *Chemosphere* 222:295-304.

Silcock P, Karrman A, van Bavel B. 2014. Advancing perfluorinated compound analysis using simultaneous matrix monitoring. Waters Corporation, Milford, MA (US)

Strivens JE, Kuo LJ, Liu Y, Noor KL. 2021. Spatial and temporal baseline of perfluorooctanesulfonic acid retained in sediment core samples from Puget Sound, Washington, USA. *Mar Pollut Bull* 167:112381.

Suardin JA, Wang Y, Willson M, Mannan MS. 2009. Field experiments on high expansion (HEX) foam application for controlling LNG pool fire. *J Hazard Mater* 165(3):612-622.

Soil Survey Staff. (n.d.). Web Soil Survey. Natural Resources Conservation Service, United States Department of Agriculture. [accessed 2020 Aug 17]. http://websoilsurvey.sc.egov.usda.gov/

Solberg Foam TF5X [Online]; Solberg Scandinavian AS: Sæbøvågen, Norway, August 21, 2007. [accessed 02/07/2021]. https://www.solbergfoam.com/getattachment/feefb270-5ad5-4b95-8e92-8960aeb7fb35/SDS_TF5X_Safety_Data_ENG.aspx

Strain JG, Mumme RL. 1988. Effects of food supplementation, song playback, and temperature on vocal territorial behavior of Carolina wrens. *The Auk* 105(1):11–16.

Tartu S, Gabrielsen G, Blevin P, Ellis H, Jo B, Herzke D, Chastel O. 2014. Endocrine and fitness correlates of long-chain perfluorinated carboxylates exposure in Arctic breeding black-legged kittiwakes. *Environ Sci Technol* 48:13504-13510.

Taylor WK, Crawford RL, Kershner M, Gravel S. 1983. House Wren migration compared with other wrens: An emphasis on Florida. *J Field Ornithol* 54:17-28.

Texas A&M Engineering Extension Service (TEEX). (n.d.). History. [accessed 2020 Feb 22]. https://teex.org/about-us/history/

[TPWD] Texas Parks and Wildlife Department. (n.d.). Texas Ecoregions. [accessed 2020 Feb 22]. https://tpwd.texas.gov/education/hunter-education/online-course/wildlife-conservation/texas-ecoregions#:~:text=Generally%2C%20Texas%20is%20divided%20into,%2C%20and%20the%20Trans%2DPecos.

[*TSTFP*] *T-Storm TSTF Training Foam*; SDS No. 770195 [Online]; WILLIAMS Fire and Hazard Control: Port Arthur, TX, May 28, 2019. [accessed 02/07/2021].

T-STORM® TSTF Training Foam Concentrate. 2019. Form No. WM-2019063-00. WILLIAMS Fire and Hazard Control: Port Arthur, TX (US)

Wang N, Liu J, Buck RC, Korzeniowski SH, Wolstenholme BW, Folsom PW, Sulecki LM. 2011. 6:2 fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. *Chemosphere*, 82:853–858.

Weather Spark. (n.d.). Average weather at Easterwood Field. [accessed 02/07/2021]. https://weatherspark.com/y/145982/Average-Weather-at-Easterwood-Field-Texas-United-States-Year-Round

Winkler DW, Hallinger KK, Ardia DR, Robertson RJ, Stutchbury BJ, Cohen RR. 2011. Tree swallow (*Tachycineta bicolor*), version 2.0. In The Birds of North America (A.F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY (US)

Yun G, Ng D, Mannan MS. 2011. Key findings of liquefied natural gas pool fire outdoor tests with expansion foam application. *Ind Eng Chem Res* 50(4):2359-2372.

APPENDIX A: TABLES

Table 1. Ingredients of the three fire training foams that are used at the Brayton Fire Training Field throughout the year.

Fire Training Foam Brand	Ingredients	CAS No.	Percentage of Ingredient	Reference
Williams Thunderstorm training foam	sodium alkene sulphonate	68439-57-6	7–13%	*TSTFP* (2019)
	2-(2-butoxyethoxy)ethanol	112-34-5	5–10%	
Solberg training foam	alcohols, C12-14, ethoxylated, sulfates, sodium salts (> 1 < 2.5 mol EO)	68891-38-3	< 20%	*Solberg Foam TF5X* (2007)
	2-(2-butoxyethoxy)ethanol	112-34-5	< 20%	
	tris(2-hydroxyethyl)ammonium dodecylsulfate	139-96-8	< 20%	
	poly(oxy-1,2-ethanediyl), alpha-sulfo-omega-hydroxy-, C6-10-alkyl ethers, sodium salts	73665-22-2	< 20%	
	1-propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivs., hydroxides, inner salts	61789-40-0	< 20%	
Verde Environmental Micro-Blaze Out training foam	water and proprietary viable spore forming cultures	7732-18-5	> 60%	*MBO* (2021)
	ammonium lauryl sulfate	68081-96-9	> 30%	
	proprietary blend of ethoxylated alcohols and other oganic materials	NA	1–3%	
	additives	NA	0.1–0.8%	
	polysaccharide xanthum gum	11138-66-2	< 0.1%	

Table 2. Congeners and respective abbreviations of PFAS.

PFAS Congener	Abbreviation
4:2 Fluorotelomer sulfonic acid	42FTS
6:2 Fluorotelomer phosphate diester	62diPAP
6:2 Fluorotelomer sulfonic acid	62FTS
Sodium bis(perfluorohexyl)phosphinate	66PFPi
8:2 Fluorotelomer phosphate diester	82diPAP
8:2 Fluorotelomer sulfonic acid	82FTS
Sodium bis(perfluorooctyl)phosphinate	88PFPi
Perfluoro-1-octanesulfonamide	FOSA-I
Sodium perfluoro-1-dodecanesulfonate	L-PFDoS
Sodium perfluoro-1-decanesulfonate	L-PFDS
Sodium perfluoro-1-nonanesulfonate	L-PFNS
Sodium perfluoro-1-pentanesulfonate	L-PFPeS
N-ethylperfluoro-1-octanesulfonamide	N-EtFOSA-M
N-methylperfluoro-1-octansulfonamide	N-MeFOSA-M
Perfluorobutanoic acid	PFBA
Perfluorobutanesulfonic acid	PFBS
Perfluorodecanoic acid	PFDA
Perfluorododecanoic acid	PFDoA
Perfluoroheptanoic acid	PFHpA
Perfluorohexanoic acid	PFHxA
Perfluorohexadecanoic acid	PFHxDA
Perfluorohexanesulfonic acid	PFHxS
Perfluorononanoic acid	PFNA
Perfluorooctanoic acid	PFOA
Perfluorooctadecanoic acid	PFODA
Perfluorooctanesulfonic acid	PFOS
Perfluoropentanoic acid	PFPeA
Perfluorotetradecanoic acid	PFTeDA
Perfluorotridecanoic acid	PFTrDA
Perfluoroundecanoic acid	PFUdA

Table 3. Mean ± SD concentrations of PFAS in soil (mg/kg) and water (mg/L) samples from White Creek upstream and downstream of the Easterwood Airport and Brayton Fire Training Field, College Station, Texas, 2020.

Congener	**Soil**		**Water**	
	Upstream	**Downstream**	**Upstream**	**Downstream**
42FTS	<LOQ	<LOQ	<LOQ	<LOQ
62diPAP	<LOQ	<LOQ	<LOQ	<LOQ
62FTS	<LOQ	0.0262 ± 0.0099	<LOQ	0.0000173 ± 0.0000043
66PFPi	<LOQ	<LOQ	<LOQ	<LOQ
82diPAP	<LOQ	<LOQ	<LOQ	<LOQ
82FTS	<LOQ	0.0421 ± 0.0116	<LOQ	<LOQ
88PFPi	<LOQ	<LOQ	<LOQ	<LOQ
FOSA-I	<LOQ	0.0077 ± 0.0077	<LOQ	<LOQ
L-PFDoS	<LOQ	0.0015 ± 0.0007	<LOQ	<LOQ
L-PFDS	<LOQ	0.0048 ± 0.0011	<LOQ	<LOQ
L-PFNS	<LOQ	0.0008 ± 0.0002	<LOQ	<LOQ
L-PFPeS	<LOQ	0.0040 ± 0.0013	<LOQ	0.0000074 ± 0.0000005
N-EtFOSA-M	<LOQ	<LOQ	<LOQ	<LOQ
N-MeFOSA-M	<LOQ	<LOQ	<LOQ	<LOQ
PFBA	0.0004 ± 0.0001	0.0188 ± 0.0026	0.0000023 ± 0.0000004	0.0000093 ± 0.0000029
PFBS	<LOQ	0.0030 ± 0.0006	0.0000059 ± 0.0000008	0.0000139 ± 0.0000011
PFDA	<LOQ	0.0040 ± 0.0015	<LOQ	<LOQ
PFDoA	<LOQ	0.0119 ± 0.0025	<LOQ	<LOQ
PFHpA	<LOQ	0.0049 ± 0.0012	0.0000015 ± 0.0000002	0.0000078 ± 0.0000007
PFHxA	<LOQ	0.0240 ± 0.0044	0.0000041 ± 0.0000003	0.0000321 ± 0.0000009
PFHxDA	<LOQ	<LOQ	<LOQ	<LOQ
PFHxS	<LOQ	0.0433 ± 0.0119	0.0000015 ± 0.0000003	0.0000460 ± 0.0000070
PFNA	<LOQ	0.0245 ± 0.0071	<LOQ	0.0000434 ± 0.0000040
PFOA	<LOQ	0.0089 ± 0.0027	0.0000019 ± 0.0000001	0.0000092 ± 0.0000013
PFODA	<LOQ	<LOQ	<LOQ	<LOQ
PFOS	0.0007 ± 0.0001	0.1691 ± 0.0446	<LOQ	0.0000404 ± 0.0000057
PFPeA	<LOQ	0.0344 ± 0.0047	0.0000144 ± 0.0000017	0.0000342 ± 0.0000052
PFTeDA	<LOQ	0.0010 ± 0.0003	<LOQ	<LOQ
PFTrDA	<LOQ	0.1984 ± 0.0672	<LOQ	<LOQ
PFUdA	<LOQ	0.1226 ± 0.0271	<LOQ	0.0000014 ± 0.0000014

Table 4. Estimated average total daily intake of PFOS from water, soil, and diet of Carolina Wrens foraging around the Easterwood Airport and Brayton Fire Training Field, College Station, Texas.

Input	**Calculation**	**References**
IR_{diet} (FIR / body weight)	$0.005\ kg_{prey}/day\ /\ 0.02\ kg_{body} = 0.25\ kg_{prey}/kg_{body}/day$	Nagy (1987); Pyle (1997)
EPC_{diet} (BSAF * soil PFOS conc)	$0.17 * 0.169\ mg_{PFOS}/kg_{soil} = 0.03\ mg_{PFOS}/kg_{prey}$	Rich et al. (2015); analysis of PFOS in downstream soil
P (sum of potentially exposed prey as proportion of diet)	0.19 + 0.14 + 0.13 + 0.11 + 0.05 + 0.03 + 0.02 + 0.0016 = 0.67	Beal et al. (1941)
AUF	1	
TDI_{diet} (IR_{diet} * EPC_{diet} * P * AUF)	$0.25 * 0.03 * 0.67 * 1 = 0.005\ mg_{PFOS}/kg_{body}/day$	
DWI (DWI / body weight)	$0.0043\ L_{water}/day\ /\ 0.02\ kg_{body} = 0.215\ L_{water}/kg_{body}/day$	Calder and Braun (1983); Pyle (1997)
EPC_{water}	$0.00004\ mg_{PFOS}/L_{water}$	analysis of PFOS in downstream water
AUF	1	
TDI_{water} (DWI * EPC_{water} * AUF)	$0.215 * 0.0004 * 1 = 0.000009\ mg_{PFOS}/kg_{body}/day$	
IR_{soil} (IR_{diet} * prop. soil in prey)	$0.25\ kg_{prey}/kg_{body}/day * 0.05 = 0.01\ kg_{soil}/kg_{body}/day$	Nagy (1987); Pyle (1997); JACOS (2010)
EPC_{soil}	$0.169\ mg_{PFOS}/kg_{soil}$	analysis of PFOS in downstream soil
P (sum of potentially exposed prey as proportion of diet)	0.19 + 0.14 + 0.13 + 0.11 + 0.05 + 0.02 + 0.0016 = 0.64	Beal et al. (1941)
AUF	1	
TDI_{soil} (IR_{soil} * EPC_{soil} * P * AUF)	$0.01 * 0.169 * 0.64 * 1 = 0.001\ mg_{PFOS}/kg_{body}/day$	
TDI (TDI_{diet} + TDI_{water} + TDI_{soil})	$0.005 + 0.000009 + 0.001 = 0.006\ mg_{PFOS}/kg_{body}/day$	