FINAL REPORT

A Framework for Assessing Bioaccumulation and Exposure Risks of Per- and Polyfluoroalkyl Substances in Threatened and Endangered Species on Aqueous Film Forming Foam (AFFF)-Impacted Sites

SERDP Project ER18-1502

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14. ABSTRACT
This project generally follows conventional methods employed for ecological risk assessment, including exposure characterization, effects characterization and risk estimation. In particular, the approach utilizes a combination of field-based measurements and bioaccumulation modeling to evaluate exposure in T&E species. Toxicity reference values (TRVs) are derived using the available toxicity data, along with species-sensitivity distributions (SSDs) and resulting 5th percentile of the hazardous concentration levels (HC5s) or application of uncertainty factors to the lowest observed toxicity values.

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Executive Summary

For several decades, the U.S. Department of Defense (DoD) widely used aqueous film forming foams (AFFF) formulations for training and operations involving fire suppression. These AFFF formulations contained relative high quantities of perfluorooctane sulfonate (PFOS), as well a range of other of per- and poly-fluoroalkyl substances (PFASs). The objective of the present study is to develop a framework for conducting scientifically sound risk assessments for PFAS in Threatened and Endangered (T&E) species at these sites. The study involved (i) a comprehensive literature review of physical-chemical properties, bioaccumulation metrics and environmental concentrations, (ii) development of a risk assessment framework for assessing PFAS bioaccumulation and exposure risks in T&E species at AFFF-impacted DoD sites and (iii) application of the proposed framework to several DoD for which existing PFAS monitoring data are available. The study aims to help guide future research efforts and risk assessment initiatives related to exposure of legacy PFASs in T&E species at AFFF-impacted DoD sites.

The proposed approach generally follows conventional methods employed for ecological risk assessment, including exposure characterization, effects characterization and risk estimation. In particular, the proposed approach utilizes a combination of field-based measurements and bioaccumulation modeling to evaluate exposure in T&E species. Toxicity reference values (TRVs) are derived using the available toxicity data, along with species-sensitivity distributions (SSDs) and resulting 5th percentile of the hazardous concentration levels (HC5s) or application of uncertainty factors to the lowest observed toxicity values.

In the proposed framework, a chemical activity-based risk assessment approach is used. The chemical activity of a contaminant (a, unitless) in a given medium is the ratio of the concentration (C, mol/m^3) of the contaminant in the medium and the chemical's apparent solubility (S, mol/m^3) in the medium (i.e., $a = C/S$). The approach involves three key steps, including (i) determining chemical activities of PFAS in external environmental media (e.g., water, soil, prey items), (ii) determining internal chemical activities of PFAS in T&E species and (iii) comparing those estimated activities to activities related to biological effects observed in vivo and/or in vitro. The merits of this approach are that monitoring data from several or diverse environmental media and sampling devices can be included in a risk assessment and monitoring data of multiple PFAS contaminants can be interpreted in terms of toxicity. This approach increases the weight of evidence in risk evaluations and facilitates coordination of research efforts by different research groups by expressing available information (i.e., from monitoring, modelling and toxicity assays) in terms of a common metric.

The chemical activity-based risk assessment approach also can incorporate ToxCast AC50 and other in vitro assay data in a risk assessment, which is particularly useful for T&E species which can often not be used in in-vivo toxicity studies. This approach is consistent with the goal of minimizing animal studies in toxicity testing, highlighted in the National Research Council's vision and strategy for exposure and toxicity testing in the 21st Century. While the chemical activity-based approach can increase the information that is used in a risk assessment, the risk assessment remains primarily focused and reliant on ecologically relevant metrics (e.g., growth, development, reproduction) in wildlife.

Evaluation of the available ToxCast data for individual PFASs indicates that commonly detected perfluoroalkyl acids (e.g., PFOS, PFHxS, PFOA, PFNA, PFDA) exhibit specific mode of toxic action, in the chemical activity range of between 10-6 to 10-3, generally below levels related to narcosis (a = 0.01). Chemical activities of PFAA associated with effects in vitro (i.e., ToxCast AC50 values) are generally similar to those associated with toxic effects in vivo. ToxCast data for PFAA precursors (N-Et-FOSA and PFOSA) suggests these neutral hydrophobic compounds tend to exhibit baseline toxicity behavior, with effect levels occurring in the range known to be associated with nonpolar narcosis (a = 0.01). As PFAAs exhibit toxic effects in the same chemical activity range, a simple additivity approach may be adopted to incorporate mixture effects. However, as PFOS is typically the predominant PFAA (> 95%), contribution of other PFAAs to the toxicity of PFAA is often negligible. Risk assessments based solely on PFOS may adequately represent the overall PFAS risk at a given site, especially if PFAAs are the main PFAS class of concern.

A preliminary mechanistic PFAS food web bioaccumulation model was developed to predict internal exposure levels (concentrations and activities) and external exposure (daily intake, µg/kg BW/d) of individual PFASs in various aquatic and terrestrial organisms that include T&E species and their prey items. The model was parameterized and applied to simulate PFAS bioaccumulation in T&E species at several DoD sites that have existing PFAS monitoring data. The model was shown to predict internal PFAS exposure levels in biota at DoD sites reasonably well, with model predicted values generally within a factor of three of the observed field data. The developed PFAS food web bioaccumulation model indicates this mechanistic modeling approach may be useful for future risk assessments of T&E species potentially exposed to PFAS at AFFF-impacted DoD sites. However, further development and testing of this modeling approach is still needed. In particular, information is needed on the partitioning properties of PFAS in biological media. This information is not only crucial to the development of a chemical activity-based risk assessment approach but also for other approaches.

T&E species with habitat ranges overlapping AFFF-impacted DoD sites included the bog turtle (*Clemmys muhlenbergii*), northern long-eared bat (*Myotis septentrionalis*), red-cockaded woodpecker (*Picoides borealis*) and eastern massasauga rattlesnake (*Sistrurus catenatus*). For sites with relatively high PFAS concentrations, risk quotients (RQs) related to PFOS exposure in T&E species often exceeded the level of concern (LOC) of 0.1. Omnivorous and carnivorous birds, mammals and reptiles are shown to exhibit a relatively high degree of PFAS bioaccumulation and hence exposure risk, compared to aquatic organisms at a given site. Model predictions indicate that at some sites with elevated PFAS concentrations in sediments, concentrations in benthic invertebrates can attain levels similar to those expected to induce acute effects in aquatic organisms. Biomagnification of PFAS in aquatic insectivorous bird species (feeding on benthos) cause very high exposure levels and associated risks.

PFAS concentrations in soils were found to be very important for exposure risks in numerous T&E species within terrestrial food webs, including terrestrial reptiles (eastern massasauga rattlesnake, Kirtland' warblers). PFAS exposure risks to upper trophic terrestrial wildlife were in many cases high. Risk quotients often exceed the level of concern (LOC) of 0.1. Sites exhibiting high PFAS concentrations in soils, such as those at several active USAF sites, are expected to cause high levels of risks to terrestrial organisms. In some cases, the estimated dose in terrestrial wildlife exceeds the PFOS LD50 of 150 mg/kg BW/d. Our initial findings show that risks of PFAS to T&E species of terrestrial food-webs are of particular concern.

It is important to note that risk estimates for T&E species in the present study are based on scenarios that assume exposure occurs via concentrations at the studied DoD sites. The extent of interaction of T&E species and their prey with AFFF-impacted soils and surface waters is a major knowledge gap in the present assessment of PFAS exposure risks of these species at DoD sites. Other knowledge gaps include the frequency and duration of various T&E species at AFFF-impacted DoD sites. In particular, studies to determine PFAS concentrations in prey and relative prey consumption rates would be useful. Other important research needs include investigations to better understand the transfer of PFAS from to insect-consuming animals and upper trophic terrestrial wildlife.

Lastly, this study demonstrates the potential and merit of a chemical activity-based approach for assessing bioaccumulation and exposure risks of PFASs to T&E species of concern. A limitation of this approach is that the apparent solubility values used to estimate chemical activities are based on numerous assumptions regarding physicochemical properties, phase partitioning, protein-binding and toxicokinetics. Currently, there is a need for further laboratory-based measurements of PFAS solubilities (S, mol/m^3) in different environmental and biological media, as well as media-water distribution coefficients for different transporter proteins and distribution coefficients for different transporter proteins ($D_{TP,W}$), structural proteins ($D_{SP,W}$), phospholipids ($D_{PL,W}$), neutral lipids ($D_{NL,W}$), carbohydrates (D_{CW}) and organic carbon (D_{OC}). Accurate estimates of solubility and distribution coefficient values will undoubtedly strengthen the reliability of the activity-based risk assessment approach. This will also aid PFAS bioaccumulation modeling efforts, as the various distribution coefficients are key parameters within the proposed mechanistic food web bioaccumulation model.

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AC50	concentration at 50% maximum activity for in vitro assays
AFB	Air Force Base
AFFF	aqueous film forming foam
ADMET	absorption-distribution-metabolism-excretion-toxicity
ATSDR	Agency for Toxic Substances and Disease Registry
BAF	bioaccumulation factor
BCF	bioconcentration factor
BMF	biomagnification factor
BW	body weight
CTLBB	critical target lipid body burden
D_{BSA-W}	serum albumin-water distribution coefficient
DI	daily intake
D_{MP-W}	muscle protein-water distribution coefficient
D_{MW}	membrane-water distribution coefficient
D_{OW}	octanol-water distribution coefficient
D_{PW}	protein-water distribution coefficient
$D_{SP,W}$	structural protein-water distribution coefficients
$D_{TP,W}$	transporter protein-water distribution coefficients
DoD	U.S. Department of Defense
EC50	concentration causing sublethal effects in 50% of the test organisms
EEC	estimated environmental concentration
EPA	United States Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
f_{OC}	organic carbon fraction
FTOH	fluorotelomer alcohol
FTSA	fluorotelomer sulfonate
PFOSA	N-alkyl perfluorooctanesulfonamide
FOSAA	per- fluorooctanesulfonamidoacetates
FOSE	per- fluorooctanesulfonamidoethanols
GIS	geographic information system
H	Henry's Law constant
HC5	hazardous concentration corresponding to 5% of affected species
HAL	health advisory level
IOC	ionizable organic compound
IRIS	U.S. EPA Integrated Risk Information System
ITRC	Interstate Technology Regulatory Council
K_{OA}	octanol-air partition coefficients
K_{OC}	organic carbon-water partition coefficient
K_{OW}	octanol-water partition coefficients

LC50	concentration causing 50% mortality in the test organisms
LD50	dose causing 50% mortality in the test organisms
LOEL	lowest observed effect level
LOC	level of concern
NAPL	non-aqueous phase liquid
N-Et-FOSE	N-ethyl perfluorooctanesulfonamidoethanol
N-Et-FOSA	N-ethyl per- fluorooctanesulfonamide
N-Me-FOSA	N-methyl per- fluorooctanesulfonamide
N-Me-FOSE	N-methyl per- fluorooctanesulfonamidoethanol
NOEL	no-observable effect level
OCP	organochlorine pesticide
PCB	polychlorinated biphenyl
PCDD	polychlorinated-p-dibenzo dioxin
PCDF	polychlorinated dibenzo furan
PFAA	perfluoroalkyl acid
PFAS	per- and polyfluoroalkyl substance
PFCA	perfluoroalkyl carboxylate
PFSA	perfluoroalkyl sulfonate
PFBA	perfluorobutanoic acid
PFBS	perfluorobutanesulfonic acid
PFCA	perfluoroalkyl carboxylate
PFHpA	perfluoroheptanoic acid
PFHxS	perfluorohexanesulfonic acid
PFNA	perfluorononanoic acid
PFNS	perfluorononane sulfonate
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
pK_a	acid dissociation constants
RfD	reference dose
RI	remedial investigation
RQ	risk quotient
SERDP	Strategic Environmental Research and Development Program
SPME	solid-phase microextraction
SSD	species-sensitivity distribution
T&E	threatened and endangered
TEQ	toxic equivalent
TL	trophic level
TLM	target lipid model
TMF	trophic magnification factor
TRV	toxicity reference value
TU	toxic units
UCMR	Unregulated Contaminant Monitoring Rule
UF	uncertainty factor

USAF	United States Air Force
WHO	World Health Organization
95% CI	95% confidence interval

1. Introduction

1.1. Background and rationale

Per- and poly-fluoroalkyl substances (PFASs) are widely used in commercial products such as liquid repellents for paper, packaging, textiles, leather goods, carpets, industrial surfactants and aqueous film forming foams (AFFF), (Kissa, 2001). These compounds have emerged as an important class of organic contaminant, due to evidence of environmental persistence, bioaccumulation potential and toxicity (Key et al., 1997; Moody and Field, 2000; Panaretakis et al., 2001; Yang et al., 2001; Xie et al., 2003; Kennedy et al., 2004; Nakayama et al., 2005; Prevedouros et al., 2006; Lau et al., 2007; Tilton et al., 2008; Fromme et al., 2009).

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), two extensively produced perfluoroalkyl acids (PFAAs), have received the most attention. PFOS and PFOA are currently the only PFASs for which EPA has defined a lifetime health advisory for drinking water. Based on laboratory and epidemiological studies, the drinking water advisory level for human exposure has been set at 70 ng/L for the sum of PFOA and PFOS, or either compound individually.

For several decades, the U.S. Department of Defense (DoD) widely used AFFF formulations for training and operations related to fire suppression. These AFFF formulations contained relative high quantities of PFOS, as well a range of other of PFASs, including poly-fluoroalkyl compounds that can precursors to PFAAs via various abiotic and/or biotic degradation pathways. Currently, 139 DoD installations exhibit PFOS/PFOA levels above the US EPA lifetime health advisories in drinking and groundwater (i.e., > 70 ng/L). Consequently, DoD is responsible for management and remediation of legacy PFAS contamination at numerous AFFF-impact sites.

There is currently a critical need to better assess the potential for legacy PFASs at AFFF-impacted DoD sites to bioaccumulate and cause adverse biological effects in threatened and endangered (T&E) species. In particular, evaluation of exposure pathways to T&E species of concern was highlighted as a critical priority area in the recent The Strategic Environmental Research and Development Program (SERDP) and the Environmental Security Technology Certification Program (ESTCP) Workshop on Research and Demonstration Needs for Management of AFFF-Impacted Sites in Washington, D.C (https://www.serdp-estcp.org/Featured-Initiatives/Per-and-Polyfluoroalkyl-Substances-PFASs/2017-Workshop-Report-on-Per-and-Polyfluoroalkyl-Substances).

The present SERDP project is in response to the recent Fiscal Year 2018 limited-scope Statement of Need (SON), “Defining Knowledge Gaps in the Understanding of PFASs in the Subsurface”, with focus on the DoDs interest in developing the basis for an approach for assessing PFAS risks to T&E species at AFFF-impacted sites.

Ecological risk assessment is a well-established field, frequently applied by government agencies, private industry, as well as academics. While there are different techniques and tools utilized for ecological risk assessment, the two fundamental components of any ecological risk assessment include exposure characterization and effects characterization, which comprise the critically important risk analysis phase of the risk assessment (U.S. Environmental Protection

Agency, 1998). The resulting exposure and effects profiles, which typically include a measure of uncertainty in the exposure and effects estimates, can then be utilized for risk estimation.

Exposure assessment may involve field measurements and/or model simulations to determine estimated environmental concentrations (EECs), daily intake (DI) values and/or internal tissue residue concentrations in different organisms of concern. Bioaccumulation metrics and mechanistic bioaccumulation models are also important for contaminant exposure assessment. Effects characterization generally involves a comprehensive assessment of the available toxicity data in order to derive reference values (TRVs). The TRVs aim to provide a representation of the threshold effect concentration, dose or internal residue concentration, above which adverse ecological effects may occur.

There are various techniques and tools that can be utilized during risk analysis (exposure and effects characterization) and risk estimation phases of an ecological risk assessment. Ecological risk assessments of PFAS exposure in T&E species at AFFF-impacted sites should be robust and scientifically defensible. These assessments should be conducted utilizing best practices, with sound understanding of key chemical and biological factors and include sources of uncertainty associated with PFAS exposure and effects.

1.2. Fundamental Research questions

- What types of data can be utilized for assessing risk to T&E species potentially exposed to PFASs at AFFF-impacted sites?
- What T&E species may be potentially exposed to PFASs related to AFFF-impacted DoD sites?
- What surrogate species should be used when extrapolating PFAS exposure and effects data to assess T&E species?
- How does life-history and migration play a role in PFAS exposure in T&E species?
- What are the key exposure pathways and bioaccumulation mechanisms governing PFAS concentrations in different T&E species?
- What approaches can be used to effectively quantify exposure in T&E species?
- What TRVs should be used to adequately protect T&E species?
- What approaches can be utilized to evaluate exposure risks of PFAS mixtures and PFAA precursors?
- How can models be used to complement environmental monitoring and toxicity data?
- How do we characterize risks related to direct and indirect PFAS effects?
- What level of risk is acceptable for T&E species?

- What are the major sources of uncertainty related to risk assessment of T&E species potentially exposed to PFASs at AFFF-impacted sites?

- What are the key research needs to help mitigate these uncertainties?

1.3. Study objectives

The objective of the present SERDP project is to develop a framework for conducting robust, scientifically defensible risk assessments of PFASs exposure to T&E species at AFFF-impacted DoD sites. Further, the study aims to highlight key issues related to several techniques and tools that may aid T&E species risk assessments at these sites.

1.4. Study Approach

1.4.1. Literature review and data compilation

This component of the project involved a comprehensive literature review, compilation and assessment of pertinent data, techniques and tools regarding PFASs and AFFF-impacted DoD sites. This included compilation of a range of information related to PFAS exposure and effects, with particular focus on AFFF related contamination at DoD sites. Specifically, literature surveys focused on compilation of (i) environmentally relevant PFAS physicochemical properties and toxicokinetic parameters, (ii) available PFAS monitoring data for AFFF-impacted DoD sites, (iii) information related to T&E species near AFFF-impacted DoD sites, (iv) laboratory and field-based bioaccumulation metrics, (v) environmental fate and bioaccumulation models applicable to PFASs and (vi) PFAS toxicity data for various aquatic and terrestrial organisms. The investigation focused on key PFASs that are commonly detected at AFFF-impacted DoD sites, including several PFAAs and PFAA precursors. Spreadsheet databases of the compiled data were generated and provided as Electronic Supplementary Material (ESM-1 to ESM-5).

For the present study, we did not apply a formalized set of criteria to evaluate the quality of the compiled bioaccumulation, toxicity and environmental concentration data. However, these data were generally obtained from peer-reviewed articles published in reputable scientific journals. The source articles containing these data were reviewed to assess experimental design, field sampling protocols, analytical methods, instrumental analyses and quality assurance/quality control (QA/QC) measures. The compiled data presented in this report were deemed suitable for use for the purpose of this study. It is important to note that future investigations of PFAS exposure risks at specific DoD sites may utilize a different approach for assessing data quality. For example, laboratory-derived toxicity data such as lowest-observable effect level (LOEL) may be deemed unsuitable for use, due to a lack of QA/QC and/or other information. Data quality screening criteria and protocols will ultimately be investigation specific.

1.4.2. Development of a framework for risk assessment of PFASs in T&E species

Following the literature review and data compilation, we developed a framework for risk assessment of PFAS exposure in T&E species. Specifically, this component involved the formulation of possible approaches for characterizing PFAS exposure and effects, as well as risk estimation techniques.

1.4.3. Application of the proposed framework to select DoD sites

The last component of the study involved demonstration of the framework at select AFFF-impacted DoD sites. Demonstration of the framework was accomplished by implementing the proposed techniques for exposure characterization, ecological effects characterization and risk estimation to assess the risk of PFAS exposure to T&E species at the selected DoD sites. The studied DoD sites were selected based on the availability of PFAS monitoring data. Based on our review of the available monitoring data, five DoD sites had sufficient data, including Former Wurtsmith Air Force Base (AFB), Barksdale AFB, Former Pease Air Force Base AFB, Joint Base McGuire-Dix-Lakehurst. These DoD sites are located in counties with several federally listed T&E species, including plants, fish, reptiles, birds and mammals.

1.4.4. Report organization

This Final Report for SERDP project ER18-1502 summarizes the completed project tasks including a comprehensive literature review and data compilation, development of a PFAS risk assessment framework and application of the proposed framework. **Section 2** of the report provides an overview of the current state of knowledge regarding AFFF-impacted DoD sites, as well as important physicochemical properties, environmental concentrations, bioaccumulation behavior, exposure and effects of PFASs. **Section 3** provides a general overview of a proposed data-driven approach for assessing the potential risks of PFAS exposure in T&E species at AFFF-impacted DoD sites, including details related to exposure characterization, effects characterization and risk estimation. **Section 4** summarizes the results the potential risk to T&E species potentially exposed to PFOS at the studied DoD sites. Lastly, **Section 5** includes a summary of key findings, uncertainties and knowledge gaps, as well as some recommendations regarding future research priority areas.

2. Current State of Knowledge

2.1. PFAS classification and physicochemical properties

Buck et al., (2011) recently presented a comprehensive assessment of the classification and terminology for perfluoroalkyl and polyfluoroalkyl substances (PFASs), which are a specific sub-class of the broader class of "Fluorinated" substances. In particular, perfluoroalkyl substances are defined as aliphatic substances for which all of the H atoms attached to C atoms in the nonfluorinated substance from which they are notionally derived have been replaced by F atoms, except those H atoms whose substitution would modify the nature of any functional groups present. Polyfluoroalkyl substances are defined as aliphatic substances for which all H atoms attached to at least one (but not all) C atoms have been replaced by F atoms, in such a manner that they contain the perfluoroalkyl moiety C_nF_{2n+1}–. Molecular structures of three important PFAS classes are shown in Figure 2-1, including polyfluorinated fluorotelomer based substances, perfluoroalkane sulfonamido substances and perfluoroalkyl acids (PFAAs).

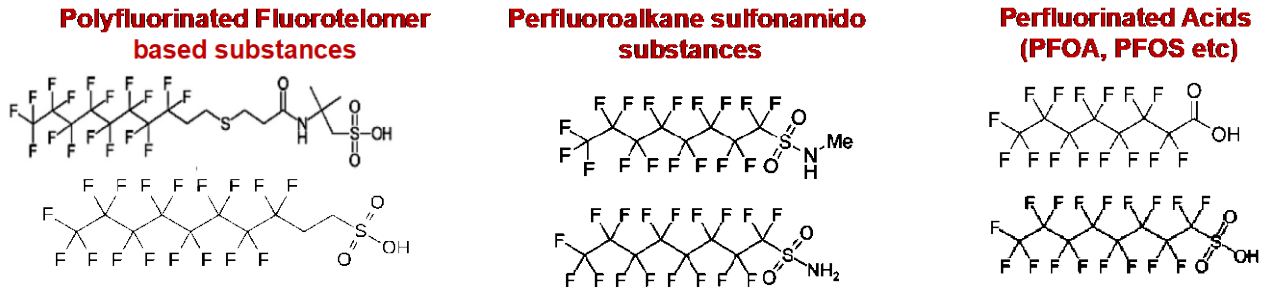

Figure 2-1. Molecular structures of key classes of poly and perfluoroalkyl substances.

The compiled physicochemical properties of PFASs investigated in the present study are shown in Appendix I. Chemical property information was compiled from various sources, including property estimation software (e.g., EPI Suite, COSMOtherm, ALOGPS), databases (e.g., EPA Chemical Dashboard, PubChem) and research papers. The compiled properties include molecular structure, perfluoroalkyl chain length, acid dissociation constants (pK_a), aqueous solubility (S_W), octanol-water partition coefficient of neutral species ($K_{OW,N}$), octanol-air partition coefficient of neutral species ($K_{OA,N}$), organic carbon-water partition coefficient (K_{OC}), Henry's Law Constant of neutral species (H_N), octanol-water distribution coefficients (D_{OW}), membrane-water distribution coefficients (D_{MW}) and protein-water distribution coefficients (D_{PW}).

Figure 2-2 illustrates the potential chemical constituents and degradation pathways for AFFF produced by electrochemical fluorination (3M) and telomerization (Ansul). PFASs are generally synthesized by either electrochemical fluorination or telomerization processes. AFFF formulations originally sold by 3M contained PFASs synthesized by electrochemical fluorination and therefore contained fully fluorinated perfluoroalkyl sulfonic acids (PFSAs), including perfluorooctane sulfonate (PFOS), as well as various perfluoroalkane sulfonamido compounds. AFFF formulations produced by other manufacturers such as Ansul, National Foam and Buckeye were produced via telomerization and hence contain various PFCAs and polyfluorinated fluorotelomer based compounds.

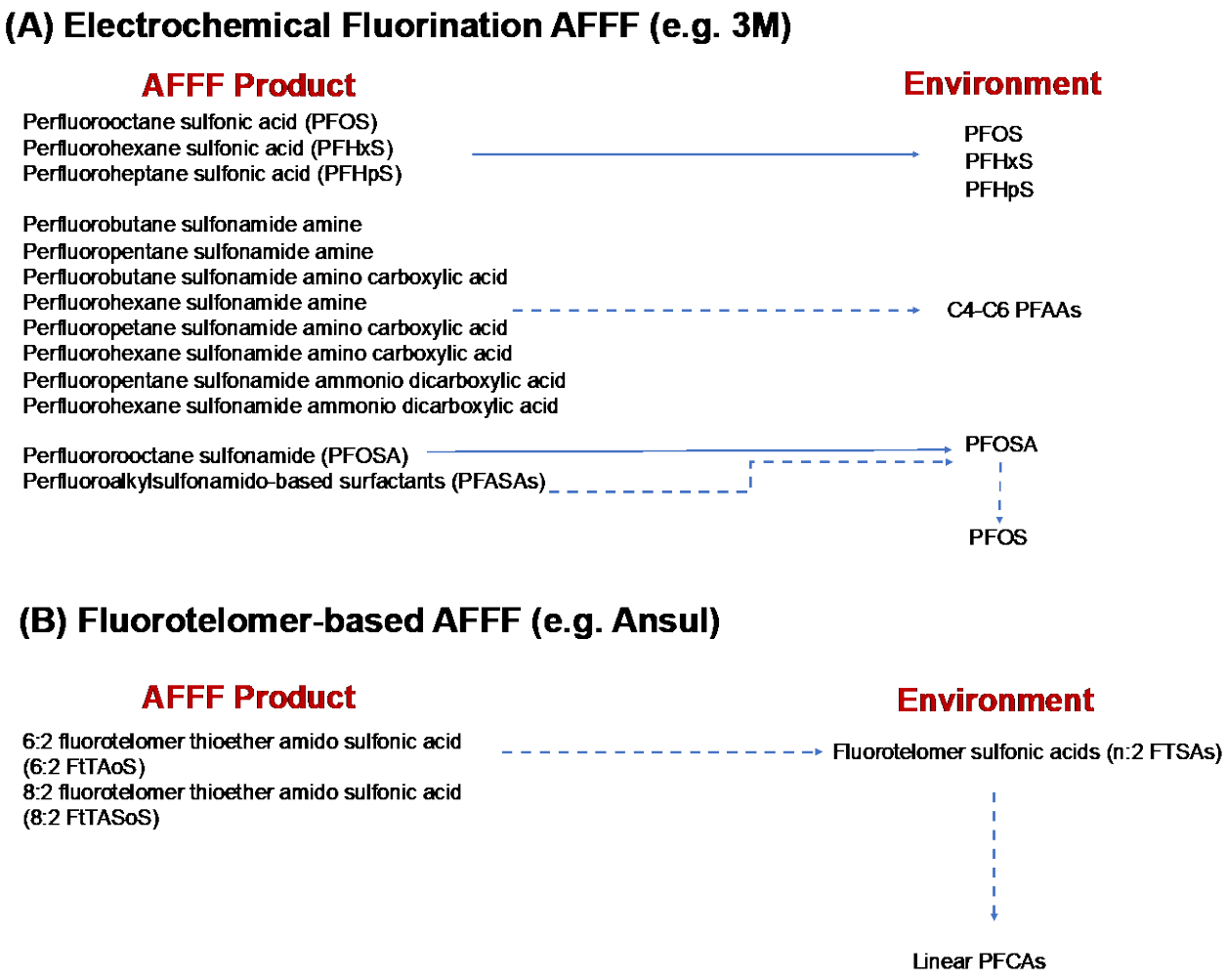

Figure 2-2. Schematic illustration showing potential AFFF product constituents and degradation pathways for (a) electrochemical fluorination based AFFF products and (b) fluorotelomer-based AFFF products.

2.2. Sources, transport and exposure pathways

There have been significant efforts to better understand the sources, transport dynamics and exposure pathways of PFASs (Higgins and Luthy, 2006; Higgins et al., 2007; Sepulvado et al., 2011; Blaine et al., 2013; Guelfo and Higgins, 2013). A schematic illustration representing stressor source emissions, environmental and habitat quality, bioaccumulation in biological receptors, as well direct and indirect impacts to biological receptors (i.e., receptor dysfunction) is shown in Figure 2-3.

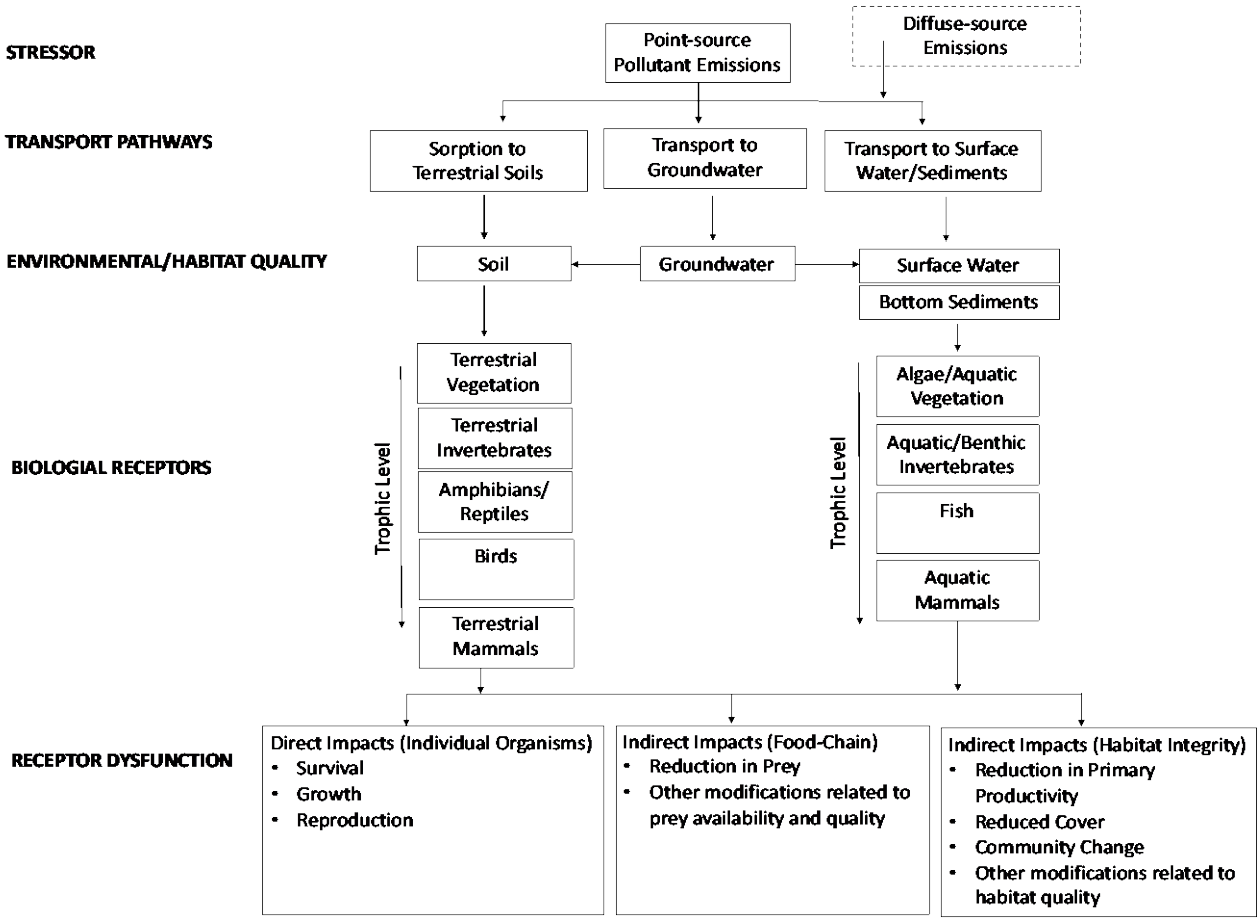

Figure 2-3. Schematic illustration representing stressor source emissions, environmental and habitat quality, bioaccumulation in biological receptors, as well direct and indirect impacts to those biological receptors (i.e., receptor dysfunction).

A key factor governing environmental fate of PFAAs (e.g., PFOS, PFOA etc) is the organic carbon-water partition coefficient (K_{OC}). Site characteristics may also influence transport and exposure assessment of PFASs at AFFF-impacted sites. In particular, information regarding the organic carbon fraction (f_{OC}), pH and surface charge of subsurface solids, as well as the occurrence of non-aqueous phase liquids (NAPL) and other co-occurring contaminants (i.e.,

ionic surfactants) will be useful to effectively assess mobility and bioavailability of PFASs at a given site.

Recent models have been developed and tested for predicting the fate and transport of PFASs in the subsurface (NGWA, 2017, Brusseau et al., 2019). For risk assessments pertaining to AFFF-impacted DoD sites, fate and transport modeling may be a useful tool for providing pertinent information regarding source tracking and source apportionment, with specific knowledge of PFASs from aqueous film forming foam (AFFF) versus non-AFFF sources. Models may also be useful for providing information regarding contaminant composition changes within in a given environmental compartment of interest (e.g., surface water). Effective models will require a comprehensive understanding of the relationship between emission sources and the receptor site, including knowledge of governing chemical transport processes (e.g., atmospheric deposition, groundwater dispersion, land-surface runoff etc.), along with corresponding rates and fluxes.

Fugacity-based multimedia mass-balance models have proven useful for source apportionment, intermedia transport and fate of semi-volatile lipophilic chemicals in aquatic systems (Mackay and Hickie, 2000). For PFASs, chemical activity-based models may be more suitable than fugacity-based models for this purpose. Chemical activity is a more appropriate descriptor for ionizable organic chemicals or IOCs (e.g., PFAS anions), which exhibit an estimated fugacity of zero in the gas phase. The chemical activity approach provides a thermodynamically sound model framework for describing the behavior of neutral and ionizable substances under environmental conditions, with variable pH and ionic strength (Franco and Trapp, 2010; Trapp et al., 2010). Chemical activity-based models have also proven useful for assessing behavior and exposure risks of chemical mixtures, including mixtures of neutral and ionizable substances (Gobas et al., 2017; Gobas et al., 2018).

2.3. Bioaccumulation behavior

The compiled bioaccumulation metric data includes bioconcentration factors (BCFs), bioaccumulation factors (BAFs), biota-sediment accumulation factors (BSAFs), biomagnification factors (BMFs) and trophic magnification factors (TMFs) for various aquatic and terrestrial organisms and food webs. The data consists of 513 laboratory-based and 931 field-based measurements. The compiled data are provided in Electronic Supplementary Material 1 (ESM-1). Based on the compiled data, geometric mean and 95% confidence intervals (95%CI) were calculated for the various bioaccumulation metrics.

Relationships between bioaccumulation potential and membrane-water distribution coefficient (D_{MW}) of PFASs are shown in Figure 2-4 (BCFs, BAFs, BSAFs) and Figure 2-5 (BMFs and TMFs). Based on previous studies, the membrane-water distribution coefficient (D_{MW}) may be a useful property to evaluate bioaccumulation of PFASs, as these compounds have a relatively high affinity for phospholipids compared to neutral storage lipids (Armitage et al., 2012, Armitage et al., 2013).

Whole-body BCFs and BAFs (L/kg) of individual PFASs in aquatic gill-ventilating invertebrates and fish are shown to increase with increasing D_{MW} (Figure 2-4a). Laboratory-based BCF studies in these aquatic biota generally show that long-chain (C12-C14) PFCAs exhibit the highest bioaccumulation potential among the studied PFASs. These compounds have relatively high D_{MW} values (log D_{MW}'s between 5 and 6) and tend to exhibit whole-body BCF values ranging between 18,000-40,000 L/kg. Laboratory-based whole-body BCFs of C8-

C11 PFCAs, which exhibit relatively low D_{MW} values (log D_{MW}'s between 3.5-4.6) are generally much lower (BCF range: 4.0-4,900 L/kg). Similarly, PFOS (log D_{MW} = 4.88) exhibits relatively low laboratory-based whole-body BCFs, generally in the range of 100 to 1,000 L/kg. In general, field-based BAFs tend to be higher than those reported in controlled laboratory studies.

A similar increasing trend is observed for BCFs and BAFs of PFASs in algae and aquatic plants (Figure 2-4b). Conversely, a positive relationship between PFAS BSAF values in sediment-dwelling organisms versus D_{MW} is not apparent (Figure 2-4c).

A plot of TMFs of individual PFASs versus chemical D_{MW} is shown in Figure 2-5. The TMF data are separated by those reported in aquatic food webs (water-respiring organisms only) and food webs containing air-breathing wildlife (i.e., birds and mammals). Similar to observations of the available BCF and BAF data, TMF values tend to be greatest for long-chain (C12-C14) PFCAs.

Field-based studies have shown TMFs of PFCAs and PFSAs in aquatic food webs are relatively low and often < 1, indicating negligible biomagnification. Conversely, TMFs in food webs containing birds and mammals generally exceed 1, indicating biomagnification in air-breathing animals. The highest reported TMFs are for marine mammalian food webs (Kelly et al., 2009; Tomy et al., 2009). For example, the TMF for PFOS in these relatively long food webs containing air-breathing wildlife (e.g., marine birds and mammals) is approximately 20.

The available data indicate that biomagnification of PFAAs primarily occurs in air-breathing wildlife (e.g., birds, mammals), with negligible biomagnification in food webs comprised of water-respiring aquatic organisms (Kelly et al., 2009). This is similar to observations of food-web specific biomagnification of low K_{OW}-high K_{OA} chemicals (Kelly et al., 2007). In particular, PFOS and several other PFASs of concern, which are moderately hydrophobic and poorly metabolizable substances, do not tend to biomagnify in aquatic food webs, due to efficient respiratory elimination to water via gills. Conversely, these substances can biomagnify to a high degree in food webs containing air-breathing animals, as elimination of these substances via lung-air exchange is negligible.

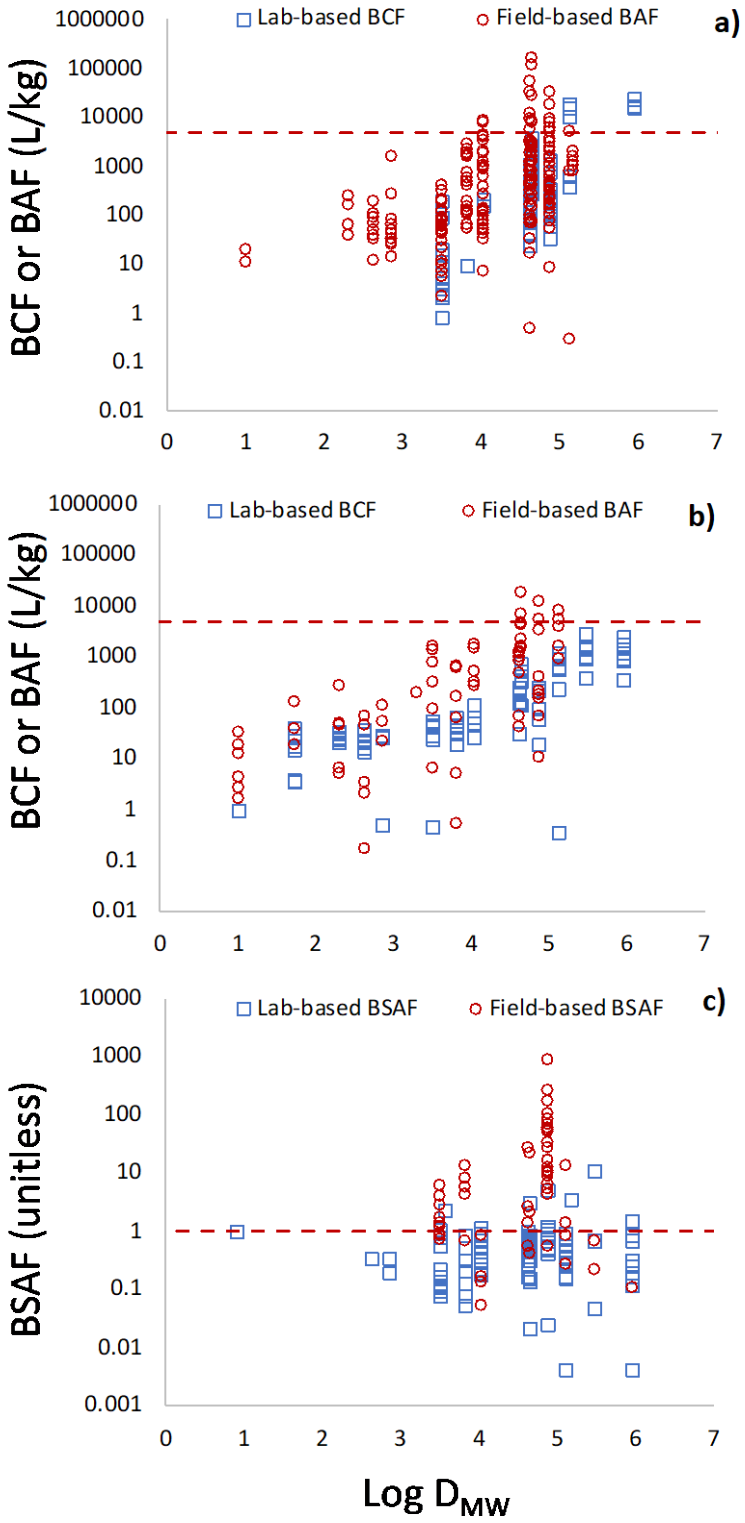

Figure 2-4. Bioaccumulation potential versus membrane-water distribution coefficient (Log D_{MW}) for individual PFASs. Plots correspond to a) laboratory-based bioconcentration factors (BCFs) and field-based bioaccumulation factors (BAFs) of PFASs in aquatic gill-ventilating invertebrates and fish, b) laboratory-based log BCFs and field-based BAFs of PFASs in algae and aquatic plants and c) biota-sediment accumulation factors (BSAFs) of PFASs in sediment-dwelling organisms. Horizontal lines representing a BCF or BAF equal to 5,000 L/kg and a BSAF equal to 1.0 are shown for comparison.

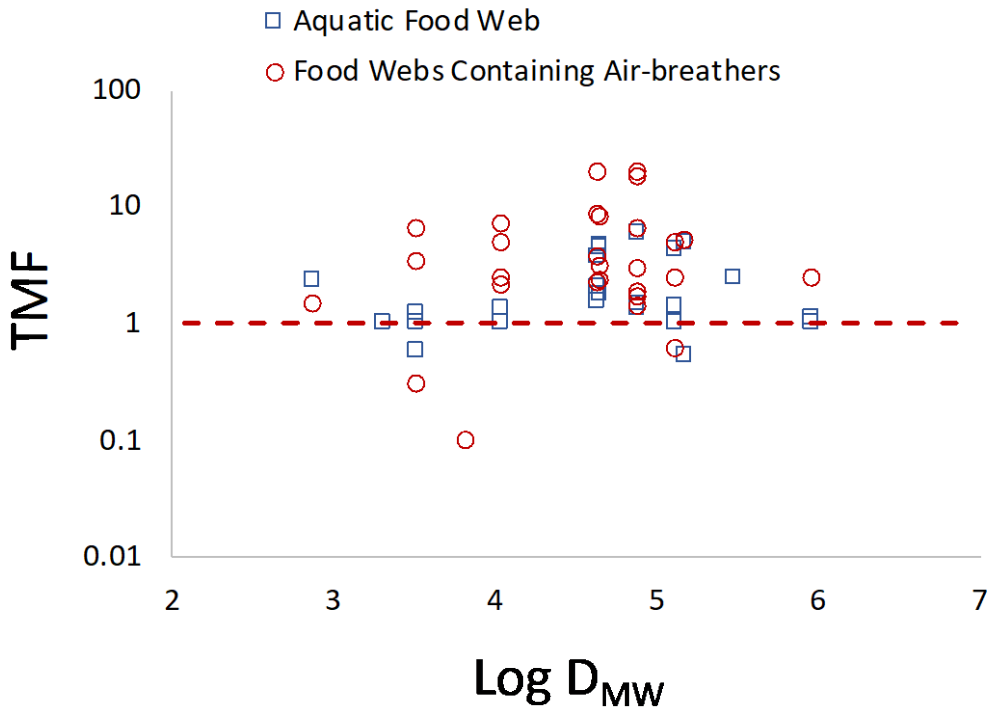

Figure 2-5. Trophic magnification factor (TMF) versus membrane-water distribution coefficient (Log D_{MW}) of individual PFASs. TMFs of PFASs are shown separately for aquatic food webs and food webs containing air-breathing wildlife. A horizontal line representing a TMF equal to 1.0 is shown for comparison.

2.4. Toxicity Data

The compiled PFAS toxicity data and corresponding data sources are provided in Electronic Supplementary Material 2 (ESM-2). The data include reported lethal and sub-lethal effect concentrations (LC_{50}, EC_{50}), as well as available No-Observed and Lowest- Observed Effect Levels (NOEL and LOEL). The compiled PFAS toxicity data was obtained from various sources, including ECOTOX, the US Environmental Protection Agency's Ecotoxicology Knowledgebase, as well as various scientific journal articles. In total, PFAS toxicity data from 119 studies was compiled, including EC50s (45 studies), LC50s (4 studies), LOELs (31 studies) and NOELs (39 studies). These data represent the observed effect levels of individual PFASs in different organisms, including aquatic and terrestrial invertebrates, algae, crustaceans, molluscs, insects, amphibians, plants, fish, birds and mammals. In addition, US Environmental Protection Agency's Toxicity Forecaster (ToxCast) and other in vitro assay results, reported as AC50 values (i.e., nominal concentration, in μM, at which 50% of maximal biological activity is observed), were also compiled for several PFASs.

PFAS toxicity data was categorized into different organism groups. Organisms exposed to PFAS via aqueous media were categorized as aquatic biota. Organisms exposed via sediment were categorized as sediment-dwelling invertebrates. Terrestrial organisms exposed via natural and PFAS amended soils were categorized as terrestrial invertebrates. Terrestrial plants exposed via soil were categorized as terrestrial plants. Air-breathers (birds and mammals) exposed to PFASs via dietary route were grouped into birds and mammals (dietary). Similarly, internal exposure toxicity data, including in vivo studies reporting internal concentrations associated with effects (e.g., serum concentrations), as well as in vitro assay data (e.g., ToxCast AC50 values) were categorized as birds and mammals (internal).

In recent years, there have been significant efforts to advance in vitro and in silico approaches for chemical risk assessment, which is described in detail in the National Research Council's report, "Toxicity Testing in the 21st Century: A Vision and a Strategy" (National Research Council, 2007). These approaches aim to minimize animal use and costs and improve toxicological insights. The US Environmental Protection Agency's Toxicity Forecaster (ToxCast) program (Richard et al., 2016) has generated large numbers of high throughput in vitro assay results, including numerous cell-based assays and whole organism toxicity assays (e.g., zebrafish embryo toxicity assay). Positive tests are reported as an AC50 value (i.e., concentration at which 50% of maximal biological activity is observed). The ToxCast data set consists of concentration-response profiles for each chemical-bioassay pair and provides a determination of whether or not the chemical was active in each bioassay. For the purpose of the present study, we compiled all the available AC50 values reported in the ToxCast database.

Currently, ToxCast includes data for eleven PFASs (i.e., N-Et-PFOSA, PFBS, PFDA, PFHpA, PFHxA, PFHxS, PFNA, PFOA, PFOS, PFOSA and PFUnA) comprising 1409 high-throughput screening assays in various test formats (see ESM-2). The various assays include cell-based: 881, protein single and complex: 439, physicochemical: 12 and others: 77. Overall, PFASs were biologically active in total of 1,290 ToxCast bioassays. A summary of the results is as follows for various species tested (assay type, active number); Mammals: Bovinae (protein-based, 7), *Cavia porcellus* (protein-based, 14), *Cricetulus griseus* (cell-based, 2), *Homo sapiens* (cell-based, 948 and protein-based, 226), *Mus musculus* (protein-based, 3), *Ovis aries* (protein-based, 1), *Pan troglodytes* (protein-based, 1) and *Rattus* (cell-based, 10 and protein-

based, 11); Bird: *Gallus gallus domesticus* (cell-based, 11); Fish embryo: *Danio rerio* (cell-based, 48).

For the purpose of the present study, we converted concentration-based ToxCast AC50 values (μM) of various PFASs into activity (*a*, unitless), following the approach proposed by Armitage et al., (2014). This approach involves estimation of the freely dissolved chemical concentration in the aqueous phase of the in vitro assay system by applying a simple mass balance model that accounts for analyte sorption to the various constituents. A key factor in this approach is the amount of fetal bovine serum (% FBS) utilized for a given assay protocol. The % FBS utilized is often available for ToxCast and other in vitro assays protocols. For the present study, we only calculated PFAS activities for ToxCast assay results that provided information regarding % FBS in the protocol. See Electronic Supplementary Material 3 (ESM-3) for the calculations used to determine PFAS activities in the various in vitro assays.

2.5. PFAS concentrations in environmental and biological media at DoD sites

PFASs are widespread ubiquitous contaminants, commonly detected in environmental media and biota within aquatic and terrestrial ecosystems. Several PFASs (PFBS, PFHxS, PFOS, PFHpA, PFOA and PFNA) are included in EPAs Third Unregulated Contaminant Monitoring Rule (UCMR 3). Thus, there are numerous measurements of these compounds in drinking water resources across the United States. Also, in recent years there have been significant efforts to evaluate the occurrence and levels of PFASs in drinking water resources at DoD installations. Anderson et al., (2016) reported measurements of several PFASs in groundwater, surface water, sediments and soils at ten U.S. Air Force AFFF release sites. The extent of the PFAS problem in groundwater and drinking water at DoD installations is summarized in the recent report to the U.S. Congress (United States Department of Defense, 2017), which shows that 139 DoD installations exhibit PFOS/PFOA levels above the US EPA lifetime health advisories in drinking and groundwater (i.e., > 70 ng/L).

Figure 2-6 illustrates a series of Geographic Information Systems (GIS) generated maps representing measured PFAS concentrations in different environmental media and biota within the continental US. Reported concentrations of PFASs were compiled for ground water (n = 940), surface water (n = 1,062), soil (n = 66), sediment (n = 266), birds (n = 13), harbor seals (n = 13), fish (n = 198), crustaceans (n = 13) and bivalves (n = 12). The compiled concentration data used to generate these maps are provided in Electronic Supplementary Material 4 (ESM-4).

Reported environmental concentrations of PFASs range widely. The highest concentrations reported in abiotic media include 10,970 μg PFOA+PFOS/L in groundwater, 19 μg PFOS/L in surface water, 32 μg PFOA/kg in soil and 4,280 μg PFOS/kg in sediment (Figure 2-6a and 2-6b). Concentrations in ground water and surface water often exceed EPAs lifetime health advisory level for human exposure for drinking water of 70 ng/L, PFOS) and/or PFOA in single or combined. Extensive PFAS contamination in ground water at several sites is evident, with eleven regions exhibiting concentrations over 1,000 μg/L (Figure 2-6a).

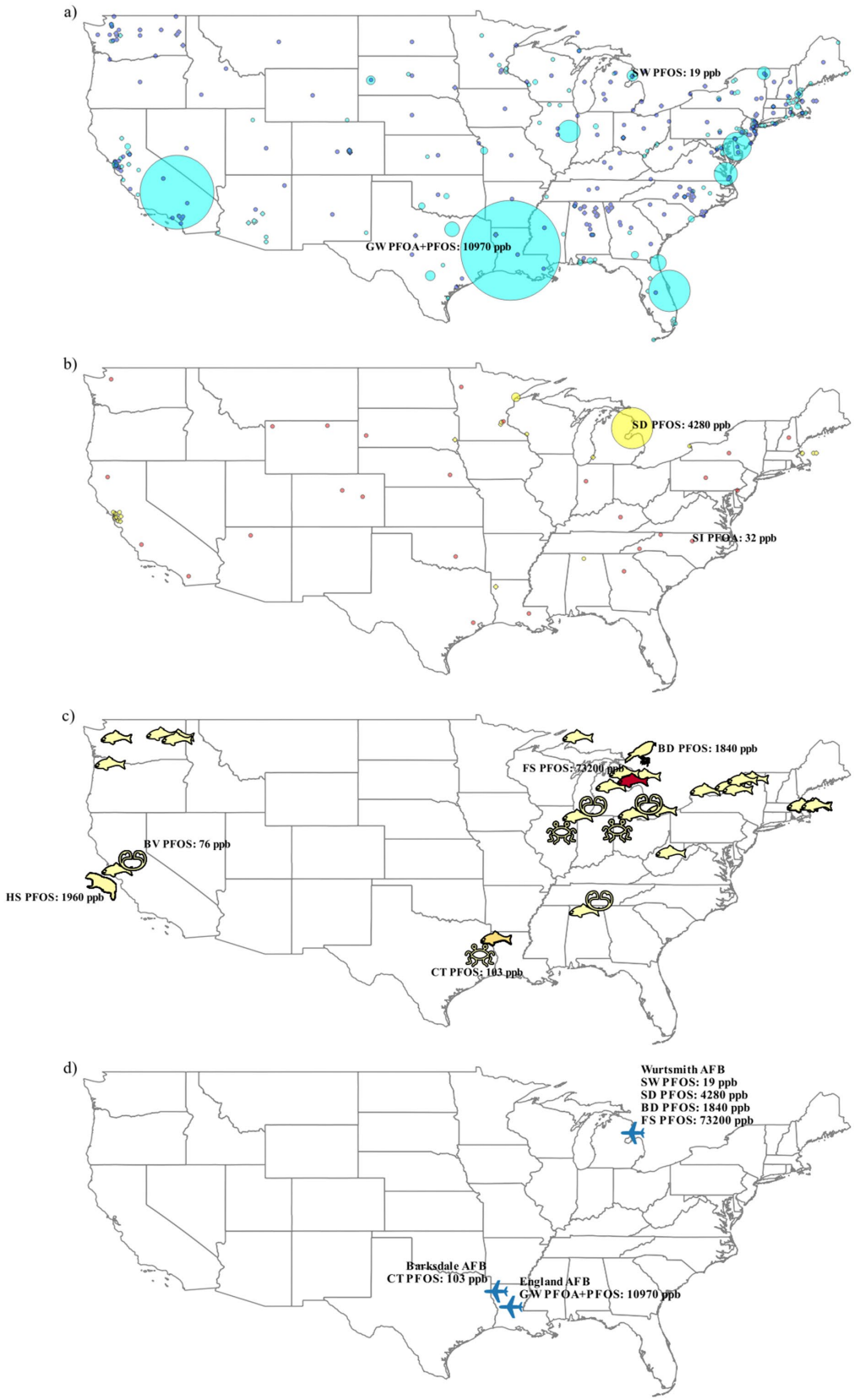

Figure 2-6. GIS-based maps illustrating PFAS concentrations in different environmental and biological media across the continental United States. The GIS maps show data related to a) PFOS or PFOA+PFOS concentrations in groundwater (cyan) and surface water (blue), b) PFOS concentrations in soil (red) and sediments (yellow) and c) PFOS concentrations in biota. Plot d illustrates DoD sites exhibiting the highest PFAS concentrations in different media. PFAS concentrations are shown in parts per million (ppb). Circle

size represents relative diffrences in concentrations in environmental media. For biota (plot b), a color gradient (orange to red) is used to represent relative differences in concentrations in biota. Maps were generated using QGIS. Abbreviations used to identify sample type include: Surface water (SW), ground water (GW), sediment (SD), soil (SI), harbor seal (HS), bird (BD), fish (FS), crustacean (CT), bivalve (BV). AFB refers to US Air Force Base.

PFOS is typically the dominant PFAS detected in environmental and biological samples, which can be explained by the extensive historical use of PFOS and its precursors, which are ultimately transformed to PFOS, an end stage metabolite/degradation product (Giesy and Kannan, 2002; Moody et al., 2003; Barzen-Hanson and Field, 2015). The highest reported concentration of PFOS in fish is 73,200 ng/g. The highest reported PFOS concentrations in other biota include 1840 ng/g in birds, 1,960 ng/g in harbor seals, 103 ng/g in crustaceans and 76 ng/g in bivalves (Figure 2-6c).

For comparison, Figure 2-6d illustrates the highest reported PFOS concentrations in environmental and biological samples at specific DoD installations. These data include PFOS concentrations in surface water, sediments, fish and birds at former Wurtsmith AFB, groundwater at England AFB and crustaceans at Barksdale AFB.

It is important to note that Anderson et al., (2016) reported median and maximum PFAS concentrations in different environmental media at ten active USAF installations. The highest PFOS concentrations reported in environmental samples at these ten sites included 4,300 µg/L in groundwater, 8,970 µg/L in surface water, 190,000 µg/kg in sediments, 9,700 µg/kg in surface soil and 1,700 µg/kg in subsurface soil. As the names and locations of these sites were not provided in the study, these data are not included in Figure 2-6.

2.6. PFAS risk assessments at DoD sites

There have been numerous studies to assess the bioaccumulation potential and exposure risks of PFOS, PFOA and other PFAAs, including contaminated site investigations related to PFAS manufacturing and fire-training areas. However, to date there have been relatively few comprehensive ecological risk assessments of PFASs related to AFFF-impacted DoD sites. Two recently conducted studies of PFAS risks to aquatic organisms and birds have been reported for Former Wurtsmith AFB in Michigan (Larson et al., 2018) and Barksdale AFB in Louisiana (Salice et al., 2018), respectively.

Larson et al., (2018) estimated the total daily intake (TDI) of seven PFASs (PFHxA, PFOA, PFNA, PFDA, PFHxS, PFOS, and PFDS) in aquatic-dependent birds at AFFF-impacted sites. For some of the exposure scenarios evaluated, the estimated TDI in birds exceeded the derived Avian TRV, based on a laboratory-based NOEL value of 0.77 mg/kg BW/d. In particular, PFAS exposure was found to be highest for spotted sandpiper (*Actitis macularia*), a diving invertivore, with apparent RQs (i.e. DI/NOEL) ranging between approximately 5 and 10. The authors also highlighted that sediments (rather than surface water) was likely a major route of PFAS exposure for these aquatic birds.

Salice et al., (2018) compared measured surface water concentrations from multiple sites at Barksdale AFB with several PFOS chronic toxicity benchmarks for freshwater aquatic organisms, including the lower 95 % confidence limit value of the HC5 (0.42 µg/L) for a species sensitivity distribution (SSD) using available chronic toxicity data for a range of

aquatic organisms. The authors reported the probability of exceeding the lower 95% confidence limit of the HC5 ranged between less than 0.001 at a reference site to approximately 0.51 at the site with relatively high PFOS concentrations in surface water. The results suggest there is some potential for adverse effects in aquatic organisms at this AFFF-impacted DoD site.

2.7. T&E species at DoD sites

The recently reported PFAS risk assessments for AFFF-impacted DoD sites (Larson et al., 2018; Salice et al., 2018) did not explicitly include assessments of potential bioaccumulation and adverse impacts in T&E species at those sites. The evaluation of exposure pathways to T&E species of concern was highlighted as a critical priority area in the recent SERDP and ESTCP workshop SERDP and ESTCP Workshop on Research and Demonstration Needs for Management of AFFF-Impacted Sites in Washington, D.C. (https://www.serdp-estcp.org/Featured-Initiatives/Per-and-Polyfluoroalkyl-Substances-PFASs/2017-Workshop-Report-on-Per-and-Polyfluoroalkyl-Substances).

Based on information provided by the DoD Natural Resources Program (http://www.dodnaturalresources.net), the department manages and protects approximately 400 federally-listed species and over 550 species at-risk. Some of the T&E species that are routinely managed by DoD include red-cockaded woodpeckers, desert tortoises, San Clemente loggerhead shrikes, California least terns, western snowy plovers and humpback whales.

For the purpose of the present study, we utilized the U.S. Fish & Wildlife Service Environmental Conservation Online System (ECOS) to identify T&E species with habitat ranges that overlap AFFF-impacted DoD lands containing legacy PFAS residues. Specifically, we utilized this database to determine the various T&E species listed in the County of a given DoD installation. T&E species information was compiled for a total of fourteen DoD installations (Table 2-1). Habitat ranges and species composition information are shown graphically in Figure 2-7.

Pearl Harbor, Camp Pendleton and Homestead AFB are associated with substantially more T&E species than other sites, which is highlighted by the darker grey color regions on the GIS-based map (Figure 2-7). Specifically, T&E species listed for Honolulu County (Pearl Harbor) includes 132 flowering plants, 17 insects, 13 birds, 12 ferns and allies, 3 reptiles, 1 mammal and 1 snail. T&E species in San Diego County (Camp Pendleton) includes 21 flowering plants, 7 birds, 4 mammals, 3 crustaceans, 3 reptiles, 3 fishes, 2 insects and 1 amphibian. The gradient of grey color was generated by overlaying equal weighted regional shapefiles of individual T&E species in contact within one county.

There are several T&E species with ≥ 2 sharing counties. A list of these species is provided in Appendix II. The dots on the GIS-based map represent T&E species sharing their habitat ranges with at least two counties (Figure 2-7). It is important to note that risk assessment of T&E species with habitat ranges spanning multiple counties may be more complex, due to uncertainties with site-specific interaction.

Table 2-1. T&E species listed at the county-level for select DoD installations.

DoD Installation		County	State	Total Number of Listed T&E Species	T&E Species Organism Class
1.	Pease Air Force Base	Rockingham	New Hampshire	7	Birds (3), Flowering Plants (1), Mammals (1)
2.	Barksdale Air Force Base	Bossier Parish	Louisiana	4	Birds (2), Fish (1), Mammals (1)
3.	Wurtsmith Air Force Base	Iosco	Michigan	5	Birds (2), Flowering Plants (1), Mammals (1), Reptiles (1)
4.	Naval Auxiliary Landing Field Fentress	Virginia Beach	Virginia	9	Birds (3), Mammals (1), Reptiles (5)
5.	Peterson Air Force Base	El Paso	Colorado	6	Birds (1), Fish (1), Flowering Plants (2), Insects (1), Mammals (1)
6.	Joint Base McGuire-Dix-Lakehurst	Burlington	New Jersey	7	Birds (1), Flowering Plants (4), Mammals (1), Reptiles (1)
7.	Marine Corps Base Camp Pendleton	San Diego	California	44	Amphibians (1), Birds (7), Crustacean (3), Fish (3), Flowering Plants (21), Mammals (4), Reptiles (3)

8. Naval Station Pearl Harbor	Honolulu	Hawaii	179	Birds (13), Fern and Ally (12), Flowering Plants (132), Insects (17), Mammals (1), Reptiles (3), Snails (1)
9. Naval Air Station Pensacola	Escambia	Florida	19	Amphibians (1), Birds (3), Clams (6), Fish (1), Mammals (2), Reptiles (6)
10. Homestead Air Force Base	Miami-Dade	Florida	43	Birds (11), Ferns and Ally (1), Flowering Plants (18), Mammals (3), Reptiles (5), Snails (1)
11. Eglin Air Force Base	Okaloosa	Florida	20	Amphibians (1), Birds (4), Clams (4), Fish (2), Lichen (1) Mammals (2), Reptiles (6)
12. Avon Park Air Force Range	Polk and Highlands	Florida	32	Birds (6), Flowering Plants (19), Lichen (1), Mammals (3), Reptiles (3)
13. Naval Air Station Whidbey Island	Island	Washington	8	Birds (4), Fish (1), Flowering Plants (1), Insect (1), Reptiles (1)
14. Joint Base Lewis-McChord	Pierce	Washington	16	Amphibians (1), Birds (4), Fish (1), Flowering Plants (3), Insect (1), Mammals (6)

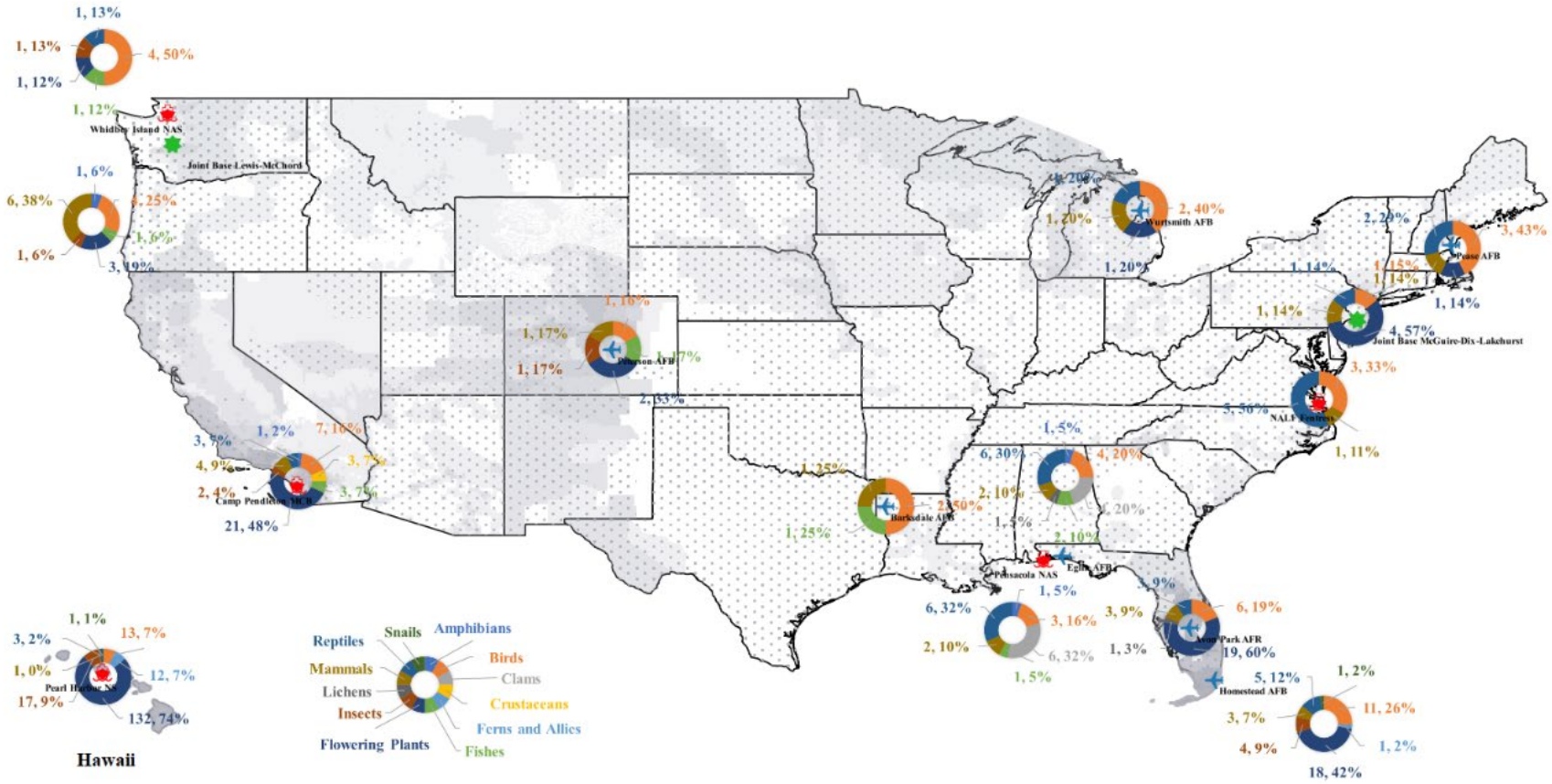

Figure 2-7. Map showing the listed threatened and endangered species in fourteen selected DoD sites. Diversity and habitat range of threatened and endangered species in county level for 14 DoD sites are mapped using GIS shapefiles (US Fish and Wildlife). The gradient of grey color was generated by overlaying equal weighted regional shapefiles of individual threatened and endangered species in contact with 1 county. The dotted spots represent threatened and endangered species sharing their habitat ranges with at least 2 counties. Pie charts indicate taxonomic groups threatened and endangered species.

3. Proposed Framework for Risk Assessment of T&E Species Potentially Exposed to PFASs at AFFF-impacted Sites

3.1. Overview of the proposed risk assessment framework

A recently completed guidance document "Guidance for Assessing the Ecological Risks of PFASs to Threatened and Endangered Species at Aqueous Film Forming Foam-Impacted Sites", SERDP Project ER18-1614, provides key recommendations and information to support quantitative ecological risk assessment (ERA) for threatened and endangered (T&E) species of 18 commonly occurring PFASs at aqueous film forming foam (AFFF)-impacted sites (https://www.serdp-estcp.org/content/download/49882/491435/file/ER18-1614%20Guidance%20Document.pdf). This guidance document outlines key aspects of risk assessment approaches for T&E species potentially exposed to PFASs at AFFF-impacted DoD facilities, discussions related to ecological conceptual site models, PFAS exposure assessments, risk evaluation and interpretation. The framework proposed in the present study is consistent with the recommended approach outlined in this guidance document.

Ecological risk assessments are inherently data-driven, requiring a range different data including measured and model-predicted contaminant concentrations in environmental and biological media for exposure characterization and corresponding concentrations that are associated with adverse effects in organisms. It is important to note, that EPAs pesticide program office have developed robust approaches for assessing impacts in T&E species potentially exposed to pesticides in the United States (U.S. Environmental Protection Agency, 2004, U.S. Environmental Protection Agency, 2008). These studies included risk assessments of pesticide exposure in T&E species such as California red-legged frog (*Rana aurora draytonni*) and Alameda whipsnake (*Masticophis lateralis euryxanthus*). Contaminant-related impacts in T&E species are typically of heightened concern, due to possible catastrophic population level impacts in these vulnerable populations. Forbes et al., (2015) recently conducted a critical review of ecological risk assessment practices for T&E species potentially exposed to pesticides, with particular focus on utilizing population models. The extensive knowledge and experience gained through past risk assessments of pesticide exposure in T&E species provides useful guidance for current initiatives related to PFAS risk assessment for T&E species at AFFF-impacted DoD sites.

Figure 3-1 is a schematic illustration of a proposed framework for assessing risks to T&E species potentially exposed to PFASs at AFFF-impacted DoD sites. The first component of the framework, characterization of PFAS exposure in T&E species, involves formulation of an approach utilizing a combination of site-specific measurements and food web modeling to estimate PFAS exposure in various organisms of concern. A key factor affecting exposure is the identification and quantification of PFAS exposure pathways and predator-prey relationships for a given species of concern. Also, site characteristics and PFAS physicochemical properties will influence phase distribution, bioavailability and bioaccumulation behavior. Evaluation of empirical data and mechanistic models will help to identify the governing mechanisms and key parameters related to fate, transport and food web bioaccumulation of PFASs. The extent of biomagnification of a given PFAS can be substantially different between different organisms (water-respiring, sediment- and soil-dwelling, air-breathing), due to inherent physiological differences (Kelly et al., 2007). Thus, employing organism- and food web-specific models will be required. Bioaccumulation models

should incorporate simulation of multi-residue exposure and toxicokinetics, as well as quantification of PFAA precursor transformation processes and contributions.

The second component of the framework involves characterization of PFAS effects in T&E species, which generally involves evaluation of the available toxicity data to derive suitable TRVs for T&E species of concern. In particular, existing NOEL and LOEL estimates for suitable surrogate species can be evaluated and extrapolated for T&E species. TRVs are derived separately for water-respiring, sediment- and soil-dwelling and air-breathing organisms.

The last component in the framework, risk estimation, involves evaluation of the PFAS exposure and effects data and associated uncertainties to generate a quantifiable level of risk posed to T&E species at AFFF impacted sites. Ultimately, it is desirable to incorporate all the various sources of uncertainty in the final risk estimate. Thus, while risk quotients (RQs) can be determined, a probabilistic approach involving probability density functions or confidence intervals is preferred. Utilizing species-sensitivity distributions (SSDs) can provide information regarding threshold effect levels in different organisms, ranging across various trophic levels, which may therefore help to incorporate assessment of indirect effects (e.g., impacts on prey).

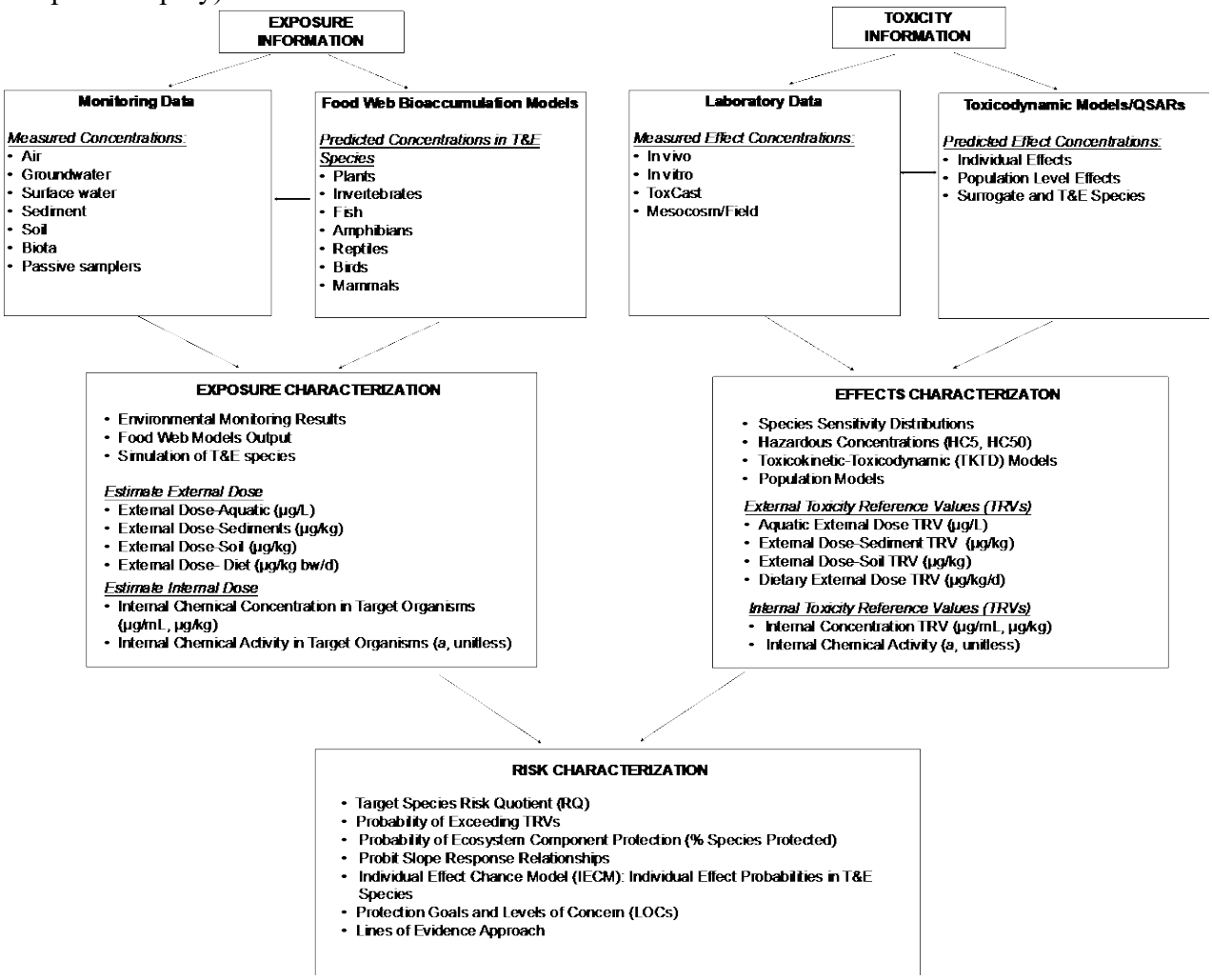

Figure 3-1. Schematic illustration of a proposed framework for assessing potential impacts in T&E species potentially exposed to PFASs at AFFF-impacted DoD sites.

3.2. Chemical activity-based risk assessment

Chemical activity has proven useful for exposure and effects characterization of organic contaminants, including assessing bioaccumulation and exposure risks of various neutral and ionizable organic chemicals (Gobas et al., 2017; Gobas et al., 2018). This approach may be particularly useful for PFASs, as it provides a common measure (activity) for the various classes of PFASs, including ionic compounds (anionic, cationic and zwitterionic compounds), as well as neutral PFASs such as perfluoroalkane sulfonamido compounds.

The chemical activity-based approach provides a thermodynamically sound model framework for describing the behavior of neutral and ionizable substances under environmental conditions, with variable pH and ionic strength (Franco and Trapp, 2010; Trapp et al., 2010). Chemical activity in aqueous media is related to the chemical's concentration by the equation:

$$a = \gamma \times C_W$$

where γ is the activity coefficient (dimensionless), which represents the deviation from the ideal solution (i.e., pure water). Chemical activity (a, unitless) in a given medium can be estimated by the ratio of molar concentration (C, mol/m^3) and the apparent solubility (S, mol/m^3) for the chemical in that medium (i.e., $a = C/S$). Thus, chemical activity in water (a_W, unitless) is equivalent to the ratio of the freely dissolved aqueous concentration and the water solubility of the chemical (i.e., $a_W = C_W/S_W$). Similarly, activity-based internal exposure in biota can be estimated as ratio of the internal molar concentration and the apparent solubility of the chemical in the organism (whole body) or in a specific tissue/compartment of the organism (i.e., $a_B = C_B/S_B$). The apparent solubility of organic compounds in organisms is related to the solubility in key biological constituents, including neutral lipids, phospholipids, proteins and carbohydrates. Thus, key parameters for this modeling approach include neutral lipid-water distribution coefficients ($D_{NL\text{-}W}$), phospholipid-water distribution coefficients ($D_{PL\text{-}W}$), protein-water distribution coefficients (D_{PW}) and carbohydrate-water distribution coefficients (D_{CW}). See Electronic Supplementary Material 5 (ESM-5) for details regarding calculations of apparent PFAS solubility in different environmental and biological media.

Figure 3-2 illustrates the key steps involved in a chemical-activity based risk assessment for PFASs. The first step involves determining PFAS activity in ambient environmental media (e.g. a_{water}, $a_{sediments}$, a_{soils}), which can be estimated using a simple activity calculator, which utilizes measured or estimated chemical concentrations (i.e., monitoring data) and the estimated chemical solubility in those media.

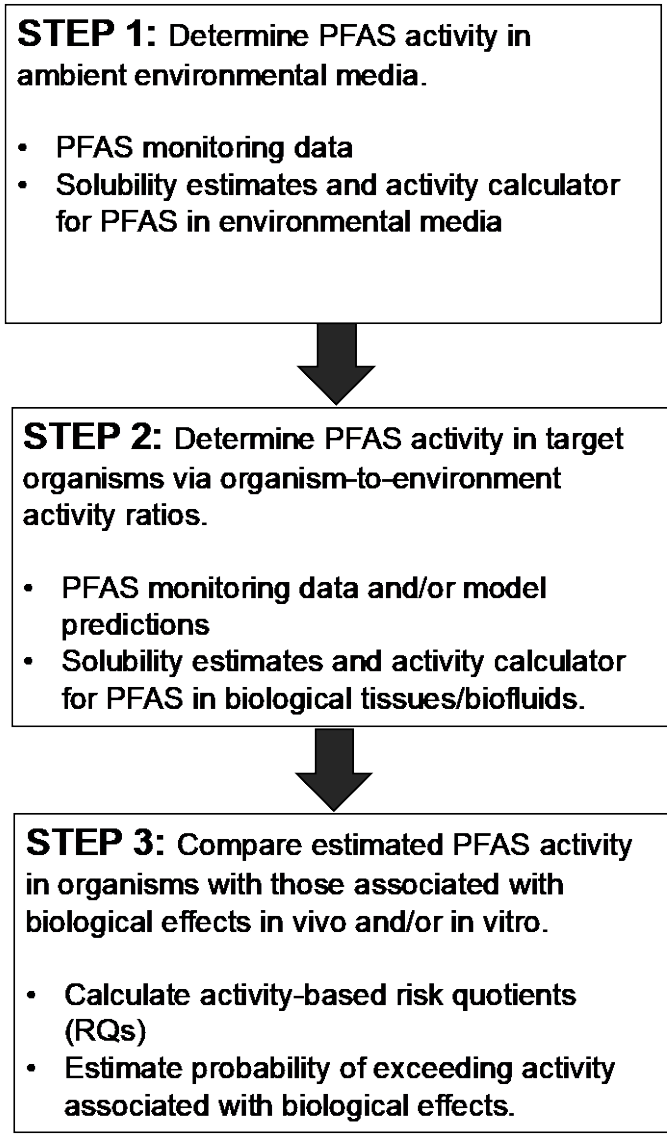

Figure 3-2. Schematic illustration of the key steps involved in a chemical-activity based risk assessment.

The second step involves determining PFAS activity in different organisms of concern. The internal activity in organisms can also be determined using a simple activity calculator, based on chemical concentrations in tissues/biofluids (biomonitoring data) and the estimated chemical solubility in those matrices. Internal PFAS activity may also be determined by application of organism-to-environment activity ratios (e.g., $a_{organism}/a_{water}$), which can be derived from field data or via mechanistic modeling. For example, as PFOS and other PFAAs are not expected to biomagnify in aquatic organisms and food webs, the activity of these compounds in aquatic water-respiring organisms can be assumed to be equal to the activity in surface water.

The final step in this activity-based approach is to compare the estimated internal activity with chemical activities related to biological effects. In particular, the estimated chemical activity in organisms can be compared to activities associated with biological effects in vivo and/or in vitro. Thus, risk estimation can be characterized using activity-based RQs, with RQs > 1 indicating a relatively high degree of risk. Also, if sufficient exposure data are available (i.e.,

distributions rather than point-estimates), it is possible to determine a probability of exceeding chemical activities associated with biological effects.

The strengths of utilizing a chemical-activity based approach for PFAS risk assessment include (i) the approach is based on sound thermodynamic theory, (ii) data for all environmental compartments (i.e., multi-media) can be incorporated into the risk assessment by using a single unifying measure (i.e., *a*, unitless), (iii), ToxCast and other in vitro assay data can be incorporated into the risk assessment, which is consistent with the National Research Council's vision and strategy related to toxicity testing in the 21st Century, (iv) mixture toxicity is easily incorporated, as chemical activities are additive, (v) chemical equilibrium and disequilibrium can be evaluated and (vi) there is no presumption regarding mode of toxic action. Further, this approach may effectively facilitate coordination of research efforts by different research groups, which may help to strengthen future PFAS risk assessments. In particular, chemical activity provides a common measure that can be utilized in different components of risk assessment, including environmental and biological monitoring, bioaccumulation behavior/exposure assessment and toxicity.

One limitation of this approach is that solubility estimates and hence calculated activities are based on numerous assumptions regarding physicochemical properties, phase partitioning, protein-binding and toxicokinetics. However, uncertainty factors may be used to effectively account for this uncertainty. Also, chemical activity is an unusual concept and communication of results can therefore be challenging.

3.3. PFAS Exposure Characterization

3.3.1. Site and ecosystem characteristics

Exposure characterization ultimately involves employing a range of techniques and tools to gain insight into the environmental concentrations and uptake of chemicals of concern in different organisms interacting with a given contaminated site. Thus, it is important to understand site and ecosystem characteristics in the early stages of a risk assessment. In particular, understanding geographic scale and watershed boundaries are crucial for defining a risk assessment study area and/or remedial management area. Having a clear understanding of the contamination zone (relative to background levels) is crucial for accurately quantifying lifetime exposure in resident species vs. intermittent interactions of migratory non-resident species. For example, T&E bird species such as red knot (*Calidris canutus rufa*), piping plover (*Charadrius melodus*) and roseate tern (*Sterna dougallii dougallii*), migratory non-resident species that potentially interact with AFFF-impacted sites, will likely have a much lower lifetime exposure profile compared to local resident species.

Understanding ecosystem characteristics such as food web structure and function is also an important first step in exposure assessment. This includes understanding interactions and linkages for resident vs. non-resident species. Stable isotope analyses have proven useful for understanding trophodynamics of aquatic and terrestrial ecosystems, as well as evaluating bioaccumulation behavior of environmental contaminants in food webs (Broman et al., 1992; Fisk et al., 2001; Kidd et al., 2001; Campbell et al., 2005; Kelly et al., 2007; Kelly et al., 2009; Lavoie et al., 2010; Coelho et al., 2013). The stable isotope ratio of nitrogen ($^{15}N/^{14}N$) has large trophic fractionation and is commonly used to determine organism trophic level (TL), while the stable isotope ratio of carbon ($^{13}C/^{12}C$) has relative unchanging trophic fractionation and is most often used to identify sources of dietary carbon. δ15N and δ13C values are determined

using isotope ratio mass spectrometry (IRMS). Generated stable isotope data for AFFF-impacted sites will aid determination of exposure pathways, which is critical for accurate determination of dietary dose (i.e., estimated daily intakes) for a given organism.

3.3.2. Environmental and Biological Monitoring

For effective risk assessment of AFFF- impacted sites, it is important to provide an accurate assessment of PFAS residue concentrations at a given site. Monitoring of contaminant concentrations typically involves analysis of environmental media and/or biota at different locations over time. The generated contaminant concentration data is typically the most important component of exposure characterization.

PFAS monitoring data typically consists of measured concentrations of PFOS and other PFASs of concern in surface water (ng/L), bottom and suspended sediments (ng/g), phytoplankton, zooplankton and other aquatic invertebrates (ng/g), fish and other wildlife tissues or biofluids (ng/g, ng/mL). PFAS concentrations in soil and plant tissues are useful for risk assessments involving terrestrial T&E species. For PFAS risk assessment, liquid-chromatography tandem mass spectrometry (LC-MS/MS) based methods will likely be the main analytical approach used for generating PFAS concentration data at AFFF-impacted sites.

There have been numerous studies involving the development and testing of LC-MS/MS based analytical methods for quantitative determination of individual PFASs in various environmental and biological samples. EPA has issued as technical brief for PFAS analysis in environmental samples (https://www.epa.gov/sites/production/files/2020-01/documents/pfas_methods-sampling_tech_brief_7jan2020-update.pdf). EPA's methods for analyzing PFASs in environmental media are in various stages of development and validation. Suitable analytical methods for groundwater, surface water, wastewater, and solids, including soils, sediments, biota, and biosolids are currently being assessed. These and other validated analytical methods will be essential for maintaining effective risk assessments of T&E species at AFFF-impacted DoD sites in the future. Further recommendations regarding field sampling strategies, analytical methods and quality assurance/quality control of laboratory generated PFAS concentration data are provided in the SERDP guidance document, "Guidance for Assessing the Ecological Risks of PFASs to Threatened and Endangered Species at Aqueous Film Forming Foam-Impacted Sites", SERDP Project ER18-1614 (https://www.serdp-estcp.org/content/download/49882/491435/file/ER18-1614%20Guidance%20Document.pdf).

3.3.3. Passive sampling devices

The recent 2017 SERDP and ESTCP Workshop on Management of Aqueous Film Forming Foam (AFFF)-Impacted Sites highlighted the importance of developing robust passive sampling approaches that can provide repeatable and environmentally relevant measures of a range of PFASs at AFFF-impacted sites. The recent SERDP statement of need, ERSON-20-C2, "Development of Passive Sampling Methodologies for Per- and Polyfluoroalkyl Substances", aims to advance development and testing of suitable passive sampling devices for PFASs at AFFF-impacted DoD sites.

A range of passive sampling techniques have been developed to determine time-weighted average ($C_{W,TWA}$) concentrations of organic contaminants in air, water, and soil. These samplers generally require knowledge of compound-specific sampling rates (R_S), typically

derived using a linear uptake model. Alternatively, rapidly equilibrating passive samplers (e.g., thin film solid phase microextraction) can be used to determine ambient concentrations at equilibrium ($C_{W,Eq}$). These equilibrium type samplers rely on knowledge of compound-specific partition coefficients (log K) or distribution coefficients (log D) that represent the equilibrium distribution ratios between the sampler media (e.g., polymer films) and a given environmental media (e.g., water).

Semipermeable membrane devices (SPMDs), most often consisting of triolein encased in low density polyethylene tubing, are commonly used as passive samplers for hydrophobic organic chemicals such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), (Vrana and Schuurmann, 2002). Solid phase microextraction (SPME) techniques have been developed using polymer coatings such as polydimethylsiloxane (PDMS), ethylene vinyl acetate (EVA), plasticized polyvinyl chloride (PVC) and silicon rubber (Harner et al., 2003; Farrar et al., 2005; Golding et al., 2008). Polar organic chemical integrative samplers (POCIS) have been used to sequester hydrophilic organic chemicals such as pharmaceuticals and herbicides from aqueous environments (Alvarez et al., 2004; Arditsoglou and Voutsa, 2008).

Passive samplers for PFASs have mainly involved quantification of neutral precursor compounds (Loewen et al., 2008; Chaemfa et al., 2010; Kim et al., 2012; Makey et al., 2017; Dixon-Anderson and Lohmann, 2018; Guan et al., 2018). PFAAs have been monitored in aquatic systems using commercially available or modified polar organic chemical integrative samplers (POCIS), (Cerveny et al., 2016; Cao et al., 2018; Cerveny et al., 2018). One limitation of this approach is that it is based on adsorption rather than absorption and the solid-phase sorbent can act as an infinite sink for target analytes. This adsorptive process does not effectively represent uptake of contaminants in tissues of aquatic biota (e.g., partitioning into cell membranes). Further, solid-phase sorbents utilized in POCIS are often customized for a specific class of compounds. For example, POCIS utilized for sequestering PFAAs (PFOS, PFOA etc.) are based on weak anion exchange (WAX) sorbents, which may not effectively sequester neutral or cationic PFASs.

For equilibrium type passive samplers (e.g., polymeric thin films), freely dissolved water concentrations of PFASs are determined from the observed concentration in the passive sampling device (C_S) and the sampler-water distribution coefficient (D_{SW}) for the target compound. For time integrated type passive samplers, time-weighted average concentrations in water ($C_{W,TWA}$) are determined as:

$$C_{W,TWA} = C_S M_S / R_S t$$

where C_S is the analyte concentration in sampling device sorbent, M_S is the mass of the sorbent, and t is time in days.

Passive sampling devices have proven useful for providing effective assessments of spatial and temporal variability of chemical contaminants at a given site. Equilibrium partitioning based passive samplers can be characterized as a biomimetic accumulation process and hence can provide important information regarding bioavailability and internal exposure levels in organisms (Parkerton et al., 2000; McGrath et al., 2005; Redman et al., 2012; Redman et al., 2014; Butler et al., 2016; Redman et al., 2018; Redman et al., 2018). Currently, studies involving the development and validation of passive sampling methods for PFASs are limited. It is anticipated that ongoing passive sampler studies funded under SERDP will help to close this knowledge gap. In particular, development and validation of effective samplers for PFASs may aid future PFAS risk assessment initiatives by providing a better understanding PFAS

bioavailability and bioaccumulation potential, as well as the spatial and temporal variability associated with PFAS concentrations at AFFF-impacted sites.

3.3.4. Assessing exposure using chemical activity

Following the chemical activity-based risk assessment approach shown in Figure 3-2, PFAS activity in ambient environmental media (e.g. a_{water}, $a_{sediments}$, a_{soils}) can be estimated from measured or estimated chemical concentration (i.e., via monitoring data) and the estimated chemical solubility in a given medium This approach can also be used to determine PFAS activity in organisms ($a_{organism}$), using concentrations in tissues/biofluids and the estimated solubility in a given sample matrix. For the present study, observed PFAS concentrations and estimated solubilities are used to determine PFAS activity in water, sediments and biota at several DoD sites.

Table 3-1 shows the apparent solubility values of PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnA, PFDoA and PFOSA in water, sediments, whole fish, fish muscle/fillet, plasma, eggs (oviparous organisms) and liver tissue. With the exception of PFOSA, the studied PFASs exhibit relatively high water solubilities (S_W, mol/m$^{3)}$. For example, PFHxS, PFOS, PFOA, PFNA and PFDA exhibit $S_W \geq 1$ mol/m^3. The apparent solubility of PFASs in biological tissues and biofluids is dependent on physicochemical properties and the relative fractions of various macromolecules (e.g., neutral lipids, phospholipids, proteins etc.). Solubility in liver (S_L) and eggs (S_{Egg}) is shown exhibit the highest solubility values. For example, the estimated solubility of PFOS in liver and eggs is 7,069 and 3,258 mol/m^3, respectively. PFAS activity in a given sample is easily calculated as the ratio of the molar chemical concentration and corresponding solubility for a given sample (i.e., $a_{plasma} = C_{plasma}/S_{plasma}$). See Electronic Supplementary Material 5 (ESM-5) for details regarding PFAS solubility calculations.

Table 3-1. Estimated solubilities (S, mol/m^3) of several PFASs in different environmental and biological media.

	Water Solubility (S_W, mol/m^3) [a]	**Solubility in Sediments (S_S, mol/m^3)** [b]	**Solubility in Whole Fish (S_F, mol/m^3)** [c]	**Solubility in Fish Muscle/Fillet (S_M, mol/m^3)** [d]	**Solubility in Plasma (S_P, mol/m^3)** [e]	**Solubility in Eggs (S_{EGG} mol/m^3)** [f]	**Solubility in Liver (S_L, mol/m^3)** [g]
PFHxS	12.3	3.4	6,732	1,414	5,528	13,949	17,431
PFOS	1.36	15	2,120	570	1,618	3,258	7,069
PFOA	10.4	36	9,046	1,764	7,499	19,700	21,723
PFNA	3.1	13	2,507	530	2,037	5,085	6,524
PFDA	0.9	4.26	826	220	626	1263	2,709
PFUnA	0.28	1.48	233	63	167	310	763
PFDoA	0.08	0.50	143	44	95	133	533
PFOSA	0.00048	0.46	2.56	0.47	1.28	2.10	4.69

[a] Based on measured or estimated water solubility values (See Appendix 1)
[b] Solubility in dry sediment solids. Dry sediment solids assumed to exhibit organic carbon content of 3%.
[c] Whole fish assumed to be comprised of 4% neutral lipids, 12% structural protein, 5% transporter protein, 1% phospholipid, 78% water.
[d] Fish muscle tissue assumed to be comprised of 0.4 % neutral lipids, 20% structural protein, 0.4 % phospholipid, 79% water.
[e] Blood plasma assumed to be comprised of 1% neutral lipids, 6% transporter protein, 0.8 % phospholipid, 92% water.
[f] Eggs assumed to be comprised of 2% neutral lipids, 15% transporter protein, 1% phospholipid, 68% water.
[f] Liver tissue assumed to be comprised of 2% neutral lipids, 10% structural protein, 15% transporter protein, 5% phospholipid, 68% water.

3.3.5. Development and application of food web bioaccumulation models

In addition to monitoring data, bioaccumulation models can provide estimated exposure concentrations in organisms, as well as external/dietary and internal dose estimates. The recently completed guidance document "Guidance for Assessing the Ecological Risks of PFASs to Threatened and Endangered Species at Aqueous Film Forming Foam-Impacted Sites, SERDP Project ER18-1614 (https://www.serdp-estcp.org/content/download/49882/491435/file/ER18-1614%20Guidance%20Document.pdf), proposes an empirical modeling approach. This approach utilizes best estimates of PFAS bioaccumulation metric values (e.g., BAFs) to determine concentration and dose estimates in various organisms, based on ambient environmental concentrations (e.g., surface water).

An alternative approach is to develop and apply mechanistic bioaccumulation models. This approach has proven useful for assessing bioaccumulation and exposure risks of numerous organic contaminants, including legacy pollutants such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). However, unlike those neutral lipophilic chemicals, PFAAs are ionizable organic compounds (IOCs) that are more associated with proteins and phospholipids, rather than storage lipids (e.g., fat reserves). Chemical K_{OW}, which has proven extremely useful for modeling the bioaccumulation of lipophilic contaminants, is not an effective descriptor for bioaccumulation behavior IOCs like PFAAs. Other properties such as D_{MW} and D_{PW} have been proposed as important parameters for describing bioaccumulation behavior of IOCs such as PFAAs (Armitage et al., 2012; Armitage et al., 2013; Ng and Hungerbuhler, 2013; Ng and Hungerbühler, 2014). It has also been demonstrated that different proteins (structural vs. transporter proteins) can exhibit substantially different sorptive capacity for PFASs and other IOCs (Henneberger et al., 2016; Henneberger et al., 2016). In particular, these studies show that anionic compounds (such as PFAAs) can exhibit muscle protein-water distribution coefficients ($D_{MP\text{-}W}$) that are orders of magnitude lower than serum albumin-water distribution coefficients (e.g., $D_{BSA\text{-}W}$) for a given chemical.

Physiologically-based toxicokinetic (PBTK) models have been developed to assess toxicokinetics of PFOS and PFOA in various animal models and humans (Andersen et al., 2006;Tan et al., 2008; Fabrega et al., 2014; Fabrega et al., 2016). PBTK models may be useful for high resolution modeling of PFAS bioaccumulation in T&E species. PBTK models have also been developed and evaluated for PFOS and PFOA in fish (Consoer et al., 2014,Consoer et al., 2016; Khazaee and Ng, 2018). PBTK models may be particularly useful to assess the influence of membrane transporters, renal secretion and reabsorption mechanisms.

For the purpose of the present study, we modified existing modeling approaches used for predicting food web bioaccumulation of organic chemicals (Gobas, 1993;Arnot and Gobas, 2004;Gobas et al., 2003;Kelly et al., 2007), making appropriate modifications to model equations and parameters. This follows the approach proposed by Armitage et al., (2013) for modeling bioaccumulation of IOCs. Further, we utilized a chemical activity-based approach for estimating distribution coefficients and rate constants for the model. In addition to prediction of internal PFAS concentration in organism tissues, the model also calculates internal PFAS activity. For the purpose of the present study, the developed PFAS food web bioaccumulation model was used to predict PFAS concentrations and activities in different organisms (including listed T&E species) at the select DoD sites. Details regarding model equations and parameters, as well as base input concentrations for select DoD sites are provided in Appendices III-VI.

Figure 3-3 is a schematic illustration of the developed PFAS food web bioaccumulation model, which includes several key organisms of concern, comprising typical aquatic and terrestrial food webs. The organisms included in the aquatic component of the model include phytoplankton, zooplankton, benthic invertebrates, bivalves, benthic-feeding fish, mid-trophic level fish, upper trophic-level fish, amphibians, aquatic reptiles, insectivorous birds, piscivorous birds and amphibious mammals. For the terrestrial food web component, PFAS concentrations and activities are calculated for plants, herbivorous insects, insectivorous birds, insectivorous mammals (e.g., bats), terrestrial reptiles, herbivorous mammals, upper trophic carnivorous mammals and humans. Protein-water and membrane-water distribution coefficients (D_{PW}, D_{MW}) are key parameters in this model.

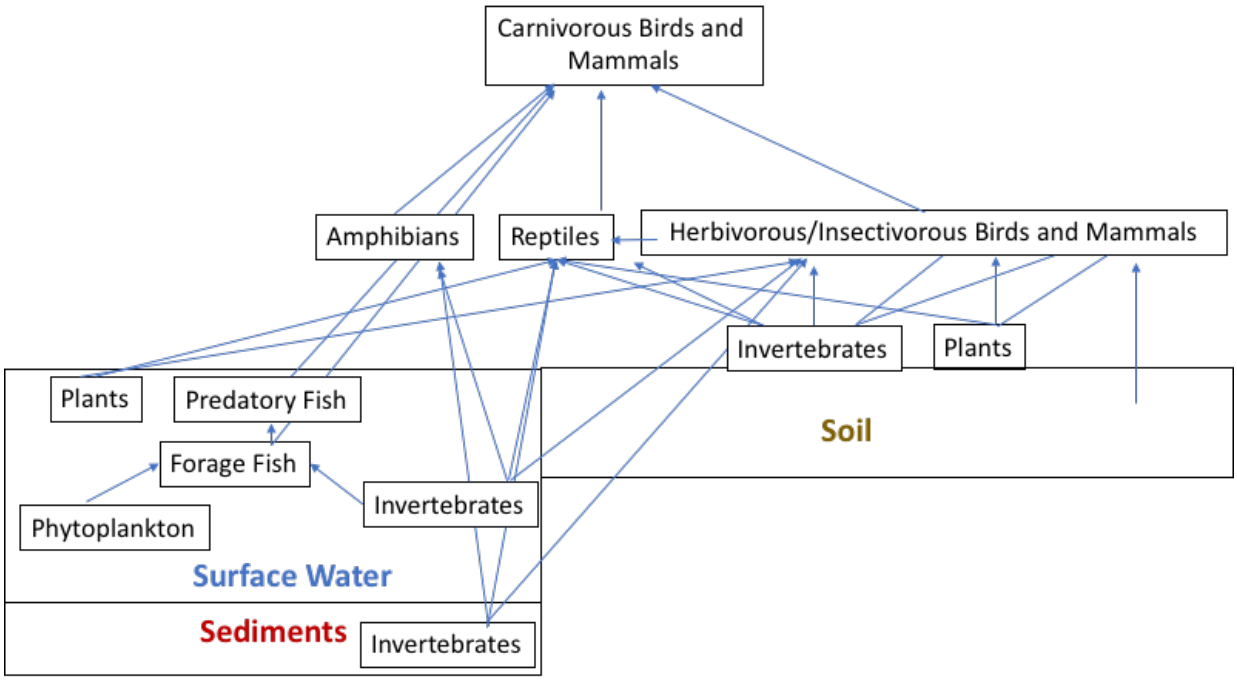

Figure 3-3. Schematic illustration of generic food web bioaccumulation model comprising various aquatic and terrestrial organisms.

Figure 3-4 illustrates model predictions of steady-state PFOS activity in aquatic and terrestrial biota at a hypothetical site exhibiting PFOS concentrations in surface water, sediments and soils equal to 340 ng/L, 10 ng/g dry wt. and 0.24 ng/g dry wt., respectively. The model outputs demonstrate that steady-state activities of PFOS in the majority of organisms in the aquatic food web, at all trophic levels, are equivalent to those in surface water, indicating equilibrium rather than a disequilibrium state (i.e. biomagnification). Exceptions include benthic invertebrates and benthic fish. Benthic invertebrates are shown to exhibit PFOS activity equal to that in sediment porewater.

For birds, mammals and reptiles, the modeling results indicate that PFOS activities are elevated above those in ambient environmental media (i.e., soil) and prey organisms, primarily due to efficient gastrointestinal uptake and negligible elimination via key depuration pathways (i.e., urinary, biliary, respiratory, biotransformation). This food web specific behavior is consistent with our previous observations of biomagnification of low K_{OW}-high K_{OA} substances in food webs containing air-breathing animals (Kelly et al., 2007).

Model predicted PFOS activities and corresponding concentrations in biota at select DoD sites (Wurtsmith AFB, Barksdale AFB, Former Pease AFB, Joint Base McGuire-Dix-Lakehurst and Peterson AFB) are provided in Appendix VII and VIII, respectively. Figure 3-5 shows a plot of observed versus model predicted activity (unitless) for three PFASs (PFOS, PFHxS and PFOSA) in biota at DoD sites with available biomonitoring data. The data include model predictions and observations for Joint Base McGuire-Dix-Lakehurst, Former Wurtsmith AFB and Barksdale AFB. Based on these preliminary model simulations, the model is shown to predict internal PFAS exposure levels at DoD sites reasonably well, with model predicted values generally within a factor of three of the observed field data.

PFAS monitoring data for Wurtsmith AFB includes concentrations of PFSAs, PFCAs and PFOSA in surface water, sediments, various fish species (pumpkinseed, mouth bass, perch, and golden shiner), as well as plasma and eggs of birds (tree swallows). Model predicted PFOS concentrations (ng/g wet weight) in biota at this site are relatively close to the observed values. For example, predicted concentration in pelagic fish was 322 ng/g (95%CI: 32-3,220), which is similar to observed values (geometric mean:184 ng/g; 95%CI: 11.2-3,050). Similarly, model predicted concentrations of PFOS in tree swallow plasma (780; 95%CI: 260-2,340) and eggs (1970 ng/g; 95%CI: 660-5,910) are within the range of the observed concentrations. These model predictions are based on model input values of 1,710 ng/L (95%CI: 140- 20,954) for surface water, 4,280 ng/g dry wt. for sediment and 0.24 (95%CI: 0.08-0.74) for soils. It is important to note that the PFOS concentrations used for soils represent levels observed in background reference soils from North America.

Preliminary evaluation of the developed PFAS food web bioaccumulation model indicates this mechanistic modeling approach may be useful for PFAS risk assessments at AFFF-impacted DoD sites. However, further development and testing of this modeling approach is needed. This modeling approach is highly dependent on PFAS physicochemical properties such as aqueous solubility, transporter protein-water distribution coefficients ($D_{TP,W}$), structural protein-water distribution coefficients ($D_{SP,W}$) and membrane-water distribution coefficients (D_{MW}). Empirical measurements and/or in silico estimation of these and other properties of PFASs would be beneficial to future modeling efforts.

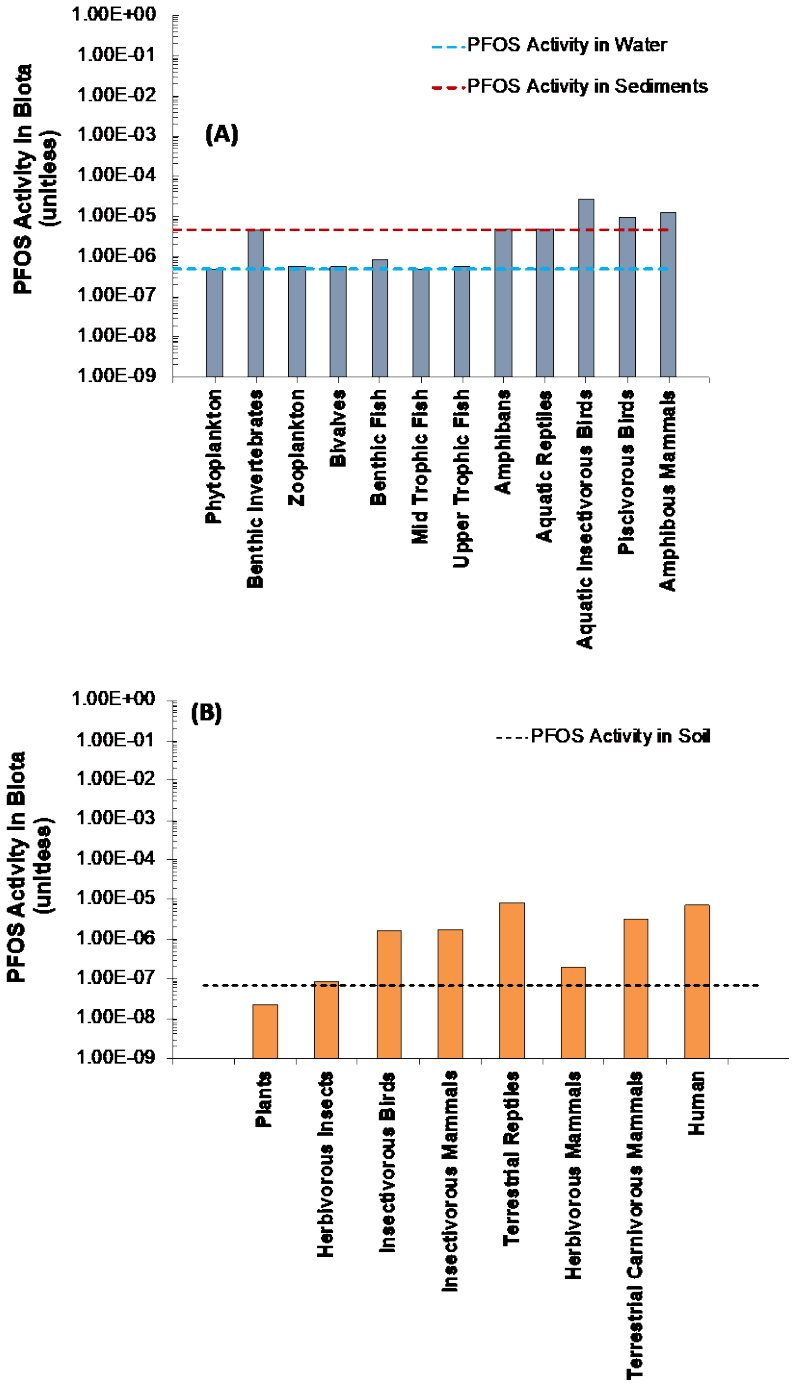

Figure 3-4. Model predicted activity of PFOS in the (a) aquatic food web and (b) terrestrial food web for a simulation with input concentrations of PFOS in surface water and sediment concentrations of 340 ng/L and 10 ng/g dry wt., respectively.

Information regarding active uptake and/or depuration mechanisms for specific PFAAs is needed to resolve apparent differences in bioaccumulation behavior of some of these compounds. In particular, the available data regarding PFOA bioaccumulation in aquatic organisms suggests the relatively low bioaccumulation potential of this compound (reported laboratory BCF values between approximately 1 and 100 L/kg) may be related to a facilitated renal elimination mechanism involving membrane transporter proteins (Consoer et al., 2014; Consoer et al., 2016). Currently, the model employed in the present study overpredicts PFOA bioaccumulation, likely due to the fact the model does not incorporate an active renal

elimination mechanism. Measurement or estimation of renal clearance rate constants via membrane transporter proteins may help to resolve uncertainties regarding bioaccumulation behavior of PFOA and other PFASs. In addition, laboratory-based measurement of protein-water and membrane-water partitioning behavior of PFOA and other PFASs would aid parameterization of mechanistic PFAS food web bioaccumulation models.

Information regarding biotransformation half-life values ($t_{1/2}$, d) and corresponding biotransformation rate constants (k_M, d^{-1}) for PFAA precursor compounds in different organisms is also needed to effectively assess the contribution of PFAA precursors towards PFAA tissue residues in exposed organisms. Biotransformation in the PFAS model employed in the current study is only included for model simulations of PFOSA ($t_{1/2}$ = 10 d). PFAAs are assumed to be non-metabolizable terminal products in PFAS biotransformation pathways, hence k_M is set to zero for these compounds.

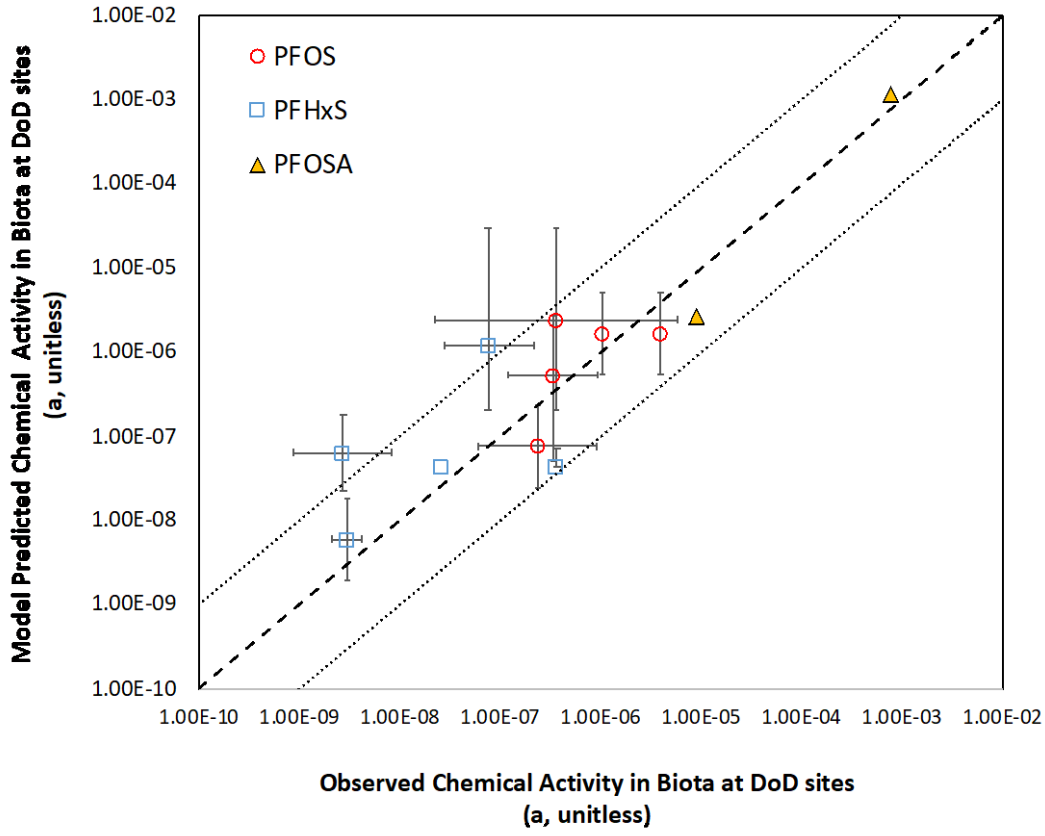

Figure 3-5. Plot showing observed versus model predicted activities of different PFASs (PFOS, PFHxS and PFOSA) in biota at DoD sites. Dashed lines represent 1:1 (perfect model agreement) and 10 times above and below 1:1. Error bars represent 95% confidence limits of observed monitoring data and model simulations based on lower and upper 95% confidence limit values as model input for surface water and sediment concentrations.

3.3.6. External and internal exposure estimates

The various techniques and tools described above are utilized for the purpose of understanding external PFAS concentrations and activities in the ambient environment, as well as internal concentrations and activities in various organisms within the studied environment. The ultimate goal of exposure characterization is to provide knowledge of the anticipated exposure and/or dose estimates for a range of organisms potentially exposed to PFASs at a given site. This approach typically includes deriving estimates based on external and internal exposure levels. For risk assessment of T&E species potentially exposed to PFASs at AFFF-impacted DoD sites, determining multiple exposure estimates (external and internal measures) would provide a more robust characterization of exposure.

A key external-based exposure estimate for aquatic organisms is the estimated environmental concentration (EEC), which can comprise a single-point estimate or distribution of concentrations in surface water (ng/L) and/or sediments (µg/kg dry wt.). A similar approach using soil concentrations may be utilized to assess external exposure in soil-dwelling organisms and/or terrestrial plants. In addition to external concentrations, external dose via the diet, often denoted as daily intake (DI, µg/kg BW/d), is determined for a given organism. This latter exposure metric is typically only utilized for exposure assessment of birds and mammals.

Conversely, internal exposure estimates involve measured or modelled contaminant concentrations in whole organisms and/or specific tissues or biofluids such as liver, muscle and plasma. Similarly, for an activity-based exposure assessment, internal activity (*a*, unitless) of the chemical may be used as an additional exposure metric for exposed organisms.

3.4. PFAS Effects Characterization

3.4.1. Surrogate species for assessing toxicity in T&E species

Direct toxicity testing in T&E species is not permitted under the U.S. Congress's 1973 Endangered Species Act. Thus, toxicity information related to suitable surrogate species is often utilized to represent these species. Table 3-2 shows PFOS toxicity data for test species comprising various taxa. The data highlight the large variations in PFOS sensitivities within a given taxon. For example, the median NOELs of PFOS reported in different fish species is 30 µg/L, with minimum and maximum values of 3.1 and 16,000 µg/L.

There are no standard protocols for selection of surrogate species (Banks et al., 2010; Banks et al., 2014). Surrogate species are often chosen on the basis of similar physiology, phylogeny or life history (Andelman and Fagan, 2000; Andelman et al., 2004; Wiens et al., 2008; Murphy et al., 2011; Romeis et al., 2013). However, selecting surrogates based on these comparisons may not always be suitable and may be insufficient to represent the corresponding listed species in toxicological risk assessment, as no single species is the most sensitive to all contaminants (Dwyer et al., 2005; Dwyer et al., 2005; Banks et al., 2014). Several approaches have been used to extrapolate toxicity data between surrogate and T&E species, including employing uncertainty factors (UFs), (Chapman et al., 1998; Sappington et al., 2001), SSDs (Mineau et al., 2001) and interspecies correlation estimation (ICE) models (Awkerman et al., 2008; Willming et al., 2016).

Table 3-2. Compiled PFOS toxicity data, shown as median values (minimum, maximum) for various taxa and exposure routes.

Taxonomic Group	Aquatic Biota External Aqueous Exposure	Terrestrial Invertebrates External Exposure via Soil	Birds and Mammals Dietary Exposure	Birds and Mammals In Vitro Assay Exposure
	NOEL (µg/L)	LOEL (µg/kg)	NOEL (µg/kg/d)	AC50 (µg/L)
Algae	35,000 (30,000, 40,000)	-	-	-
Birds	-	-	44,700 (580, 230,000)	32,700 (21700, 57,500)
Crustaceans	15,200 (313, 100,000)	-	-	-
Fish	30 (3.1, 16,000)	-	-	-
Insects	21.7 (2.3, 94.9)	-	-	-
Invertebrates	-	50,000 (250, 160,000)	-	-
Mammals	-	-	-	13,900 (6.5, 14,300)
Molluscs	100,000 (3,000, 200,000)	-	-	-
Plants	-	150,000 (100,000, 200,000)	-	-

3.4.2. PFAS mixture toxicity

AFFF products typically consist of complex PFAS mixtures, including recalcitrant PFAAs (e.g., PFHxS, PFOS), as well as several precursor compounds (e.g., polyfluorinated fluorotelomer based substances, perfluoroalkane sulfonamido substances) that may be transformed to PFAAs in the environment or in vivo. Currently, there is a lack of information regarding mixture toxicity of PFASs.

Figure 3-6 shows the cumulative probability distributions of estimated activity of individual PFASs in various ToxCast in vitro assays. Chemical activity in these in vitro assay systems were calculated following the approach developed by Armitage et al., (2014). The data indicate that PFAAs generally exhibit a specific mode of toxic action, generally in the same activity range (activities between 10^{-6} to 10^{-3}). PFOSA, a PFOS precursor compound is shown to exhibit non-specific baseline toxicity ($a > 0.01$). The ToxCast results for N-Et-FOSA activities exceeded the maximum possible value of 1.0, which represents the chemical's solubility in the medium. These results indicate ToxCast assays for N-Et-FOSA may have been conducted at saturated dosage levels that exceeded the chemical's solubility or exhibit significant analytical errors (Gobas et al., 2017; Gobas et al., 2018). While not conclusive, the available ToxCast AC50 data provide some insight regarding PFAS mixture toxicity.

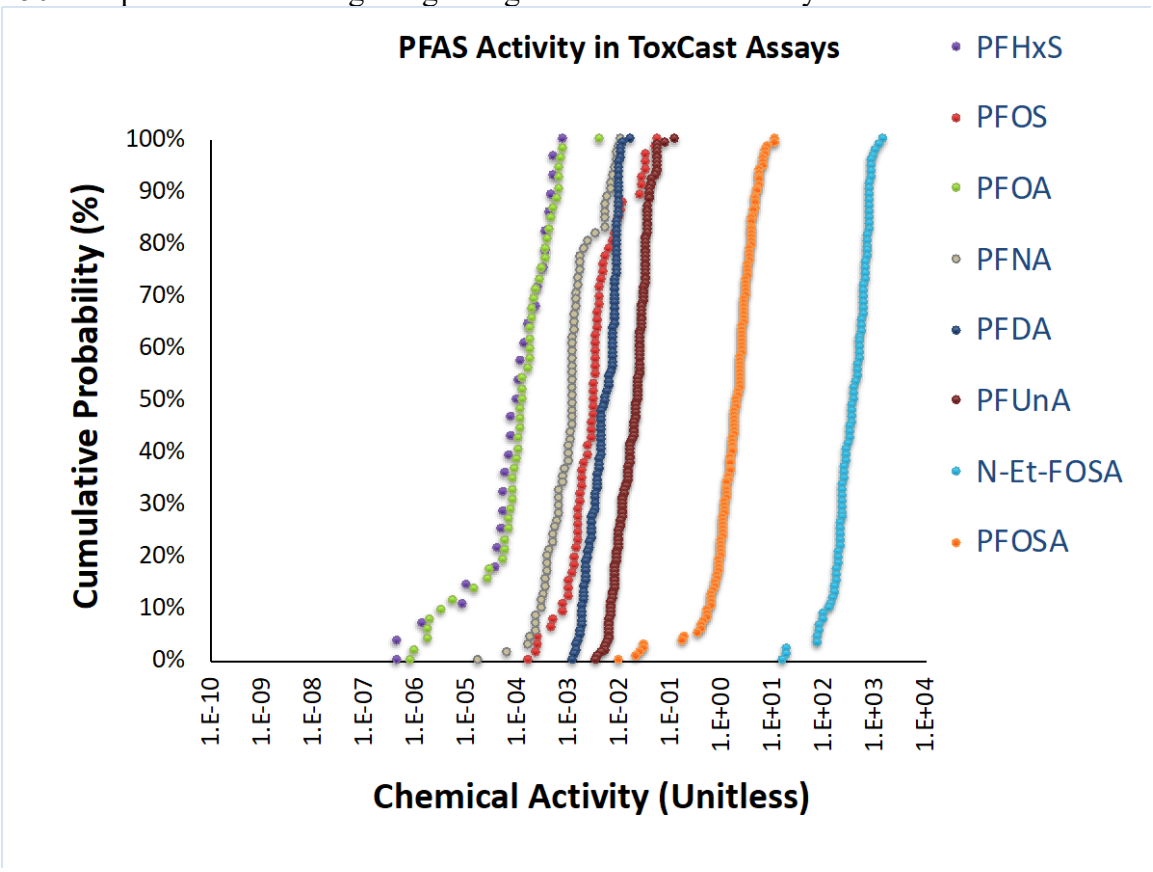

Figure 3-6. Cumulative probability of activity-based AC50 values (a, unitless) of different PFASs reported in cell-based ToxCast assays.

Figure 3-7 shows the percent contribution of individual perfluoroalkyl acids (PFAA) to total PFAA activity (*a*, unitless) observed in fish (muscle/fillet), tree swallow plasma and tree swallow eggs at DoD sites (See ESM-4 for PFAS monitoring data). The activity of PFOS in fish muscle/fillet is approximately 99% of the total PFAA activity. The relative contribution of

PFOS to the total PFAA activity in tree swallow plasma and eggs is approximately 92% and 95%, respectively.

The available data suggest that a simple additivity approach may be used for assessing PFAS exposure risks at AFFF-impacted sites. This approach involves summation of concentrations or activities of individual PFAAs to determine a total PFAA exposure level (C_{TOTAL}). Larson et al., (2018) used this approach and utilized ΣPFAAs (sum of PFOS, PFOA, PFDA, PFNA, PFHxA, PFDS, and PFHxS) to assess the PFAS risk to birds at AFFF-impacted sites. It is important to note that this approach is best suited for use with observed or predicted internal chemical activities rather than external activities in a given environmental compartments (e.g., surface water), as the former estimates inherently account for solubility and bioaccumulation potential differences of the various target substances.

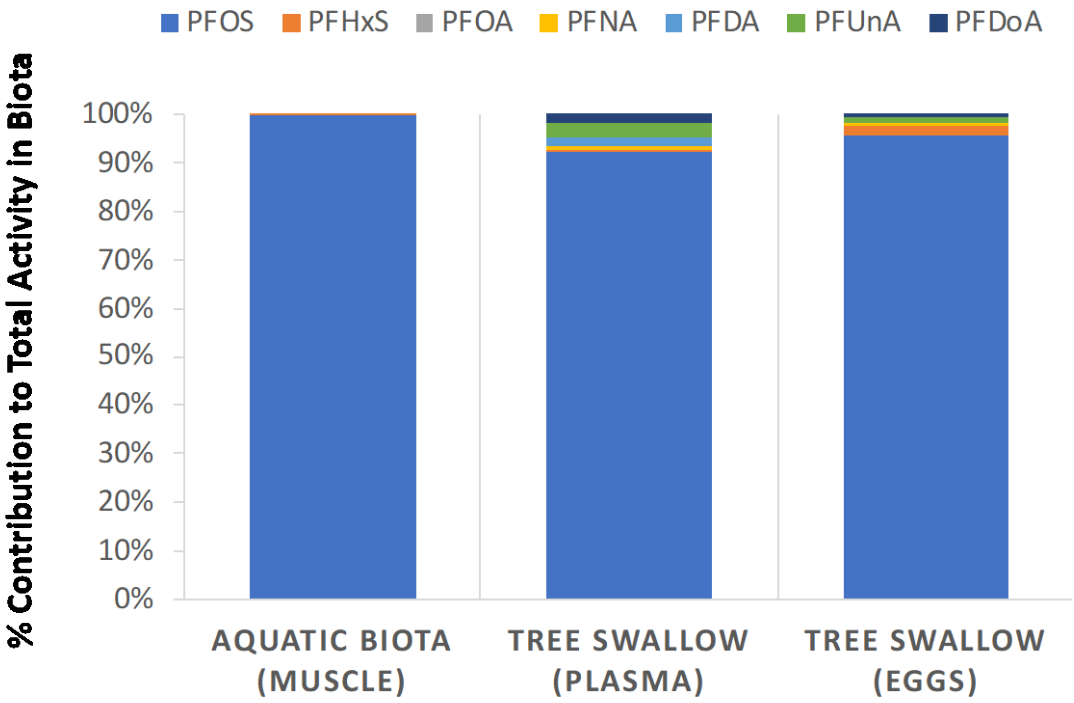

Figure 3-7. Percent contribution of individual perfluoroalkyl acids (PFAA) to total PFAA activity (a, unitless) observed in aquatic biota (muscle), tree swallow plasma and tree swallow eggs at DoD sites.

3.4.3. Species-sensitivity distributions (SSDs)

SSDs were developed for four different animal groups (i.e., aquatic biota, terrestrial invertebrates, birds and mammals (dietary) and birds and mammals (internal), (Figure 3-8). SSDs for sediment-dewlling organisms was not possible due to lack of available PFAS toxicity data for these organisms. SSDs were used to estimate the hazardous concentrations corresponding to 5% of affected species (HC5s), along with lower and upper 95% confidence limit values.

SSDs are often utilized to assess the variartion in toxic effects of a given toxicant to multiple exposed species in an ecosystem. SSDs can be combined with available exposure data to estimate the fraction of potentially affected species in a given ecosystem (Suter, 1993). For the present study, SSDs were developed using PFAS toxicity data for the various animal groups characterized in Section 2.4. SSDs were developed for animal groups/endpoints only if there was sufficient data and regression fitting results (Appendix IX).

The lowest estimated HC5 is 3.2 µg PFOS/L for aquatic biota using the NOEL dataset (Appendix X). This result is similar to the value of 1.1 µg PFOS/L that was previously derived using both LOEL and NOEL datasets (Salice et al., 2018). The somewhat lower HC5 previously reported is due to incorporation of a LOEL value of 0.6 µg PFOS/L in *Danio rerio* (Keiter et al., 2012). In contrast, the lowest NOEL value of 2.3 µg PFOS/L for *Chironomus tentans*, previously reported by MacDonald et al., (2004), was used in the present study. The estimated HC5s for PFNA and PFOA exposure in aquatic organisms are similar, 23,400 and 27,000 µg/L, respectively (Figure 3-8a).

For terrestrial soil-dwelling invertebrates, the HC5 is determined to be approximately 1,800 µg PFOS/kg using the available LOEL dataset (Appendix X). The SSDs using the available LOEL and NOEL for dietary intake (i.e., external dietary exposure) in birds and mammals is shown in Figure 3-8c. The estimated HC5s for birds and mammals was found to be approximately 1,790 and 738 µg/kg BW/d, respectively.

SSDs based on internal concentration data in birds and mammals (AC50 results from in vitro assays) are shown to be similar among different PFASs (i.e., N-Et-PFOSA, PFDA, PFHpA, PFNA and PFOS), (Figure 3-8d). The estimated HC5s for these in vitro assay data range between approximately 1,160 to 3,290 µg/L. N-Et-PFOSA exhibits the lowest HC5 value, while PFNA exhibits the highest. The HC5 for PFOS NOEL results from in vitro assays is appoximately 1,590 µg/L. Also, the HC5 for PFOS based on internal tissue residue concentration (µg/kg) was found to be 7.7 µg/kg. The majority of these data consist of reported NOEL values from in ovo tests.

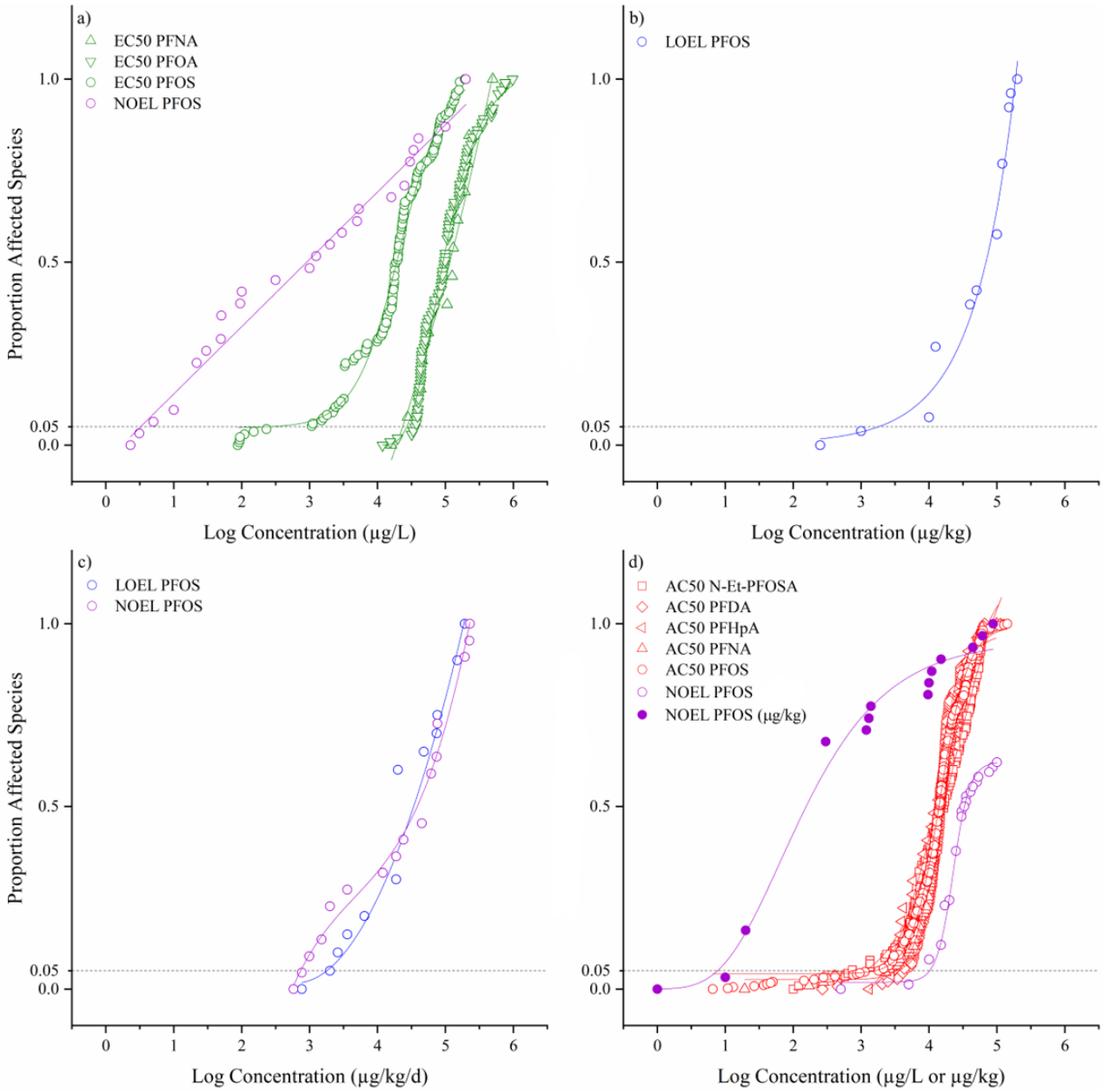

Figure 3-8. Species-sensitivity distributions (SSDs) are shown for a) aquatic biota, b) terrestrial invertebrates, c) birds and mammals (dietary) and d) birds and mammals (internal). The SSDs were used to estimate the hazardous concentrations (HCs) at 5% of affected species (black dotted horizontal lines).

3.4.4. Derivation of TRVs for water-respiring, sediment- and soil-dwelling and air-breathing organisms

Table 3-3 shows the derived TRVs for the present study. TRVs were derived based on HC5s or lower 95% of HC5s of the developed SSDs, or alternatively using the lowest reported toxicity value for a given organism/exposure scenario (see Appendix XI).

Employing uncertainty factors (UFs) to derive TRVs is a common approach in ecological risk assessment. Specifically, UFs between 1 and 10 are typically applied to the effect concentration or dose (e.g., LOEL) to account for (i) toxicological endpoint extrapolation (e.g., LOEL to NOEL), (ii) interspecies extrapolation, (iii) exposure duration differences (i.e. lab exposure vs. field exposure). UF values can be applied in series and is typically based on professional judgment and/or best practices. For the present study, we generally applied a UF of 10 when deriving TRVs based on lowest single-point toxicity. Following Beach et al., (2006), we applied a UF of 36 to derive the PFOS TRV for birds and mammals. This resulting TRV for birds and mammals was equal to 20.5 µg/kg BW/d. This value is similar to the single LOEL value for birds (770 µg/kg BW/d) divided by 36 (i.e., 21.4 µg/kg BW/d), (Beach et al., 2006).

Among the various PFASs, PFOS tends to exhibit the lowest TRV for exposure in aquatic organisms, followed by PFOSA, PFHxS, PFNA and PFOA (Table 3-3). Conversely, TRVs in terrestrial invertebrates are lowest for PFOA and PFHxS (100 µg/kg) compared to PFOS (1,800 µg/kg). TRVs of PFOS and PFOA for dietary exposure in birds and mammals are substantially different, with PFOS TRV equal to 20.5 µg/kg BW/d and the PFOA TRV equal to 10,000 µg/kg BW/d.

Activity-based TRVs are also shown in Table 3-3. The activity-based TRVs for aquatic biota (1.6×10^{-6}) was determined by converting the concentration-based TRV into chemical activity, using the corresponding water solubility (Sw, mol/m^3) of a given PFAS. Other activity-based TRVs were determined using observed in vitro assay data or using the developed PFAS food web bioaccumulation model. For example, the activity-based TRV for birds and mammals (3×10^{-7}) is based on internal LOEL values from reproductive studies conducted with Northern Bobwhites (*Colinus virginianus*) exposed to PFOS in the diet (Newsted et al., 2005). Specifically, the activity-based TRV for PFOS in birds and mammals is based on the reported LOEL for serum of those exposed birds (8.7 µg/mL). After application of a UF of 36, the concentration-based TRV in serum is 0.25 µg/mL (or 5×10^{-4} mol/m^3). This molar concentration in serum is converted to activity using the estimated solubility of PFOS in serum (1,618 mol/m^3, Table 3-1), (i.e., $a_{serum} = C_{serum} \div S_{serum} = 5 \times 10^{-4}$ mol/m^3 ÷ 1,618 mol/m^3 $= 3 \times 10^{-7}$).

Table 3-3. Toxicity reference values (TRVs) for different PFASs derived from available toxicity data.

Chemical/ TRV type	Exposure Media	Metric	Units	TRV (Concentration-based)	TRV (Activity-based, *a*, unitless) [d]
PFHxS					
Aquatic Biota	Aqueous	AC50	µg/L	397 [a*]	8.1×10^{-5}
Terrestrial Invertebrate	Soil	NOEL	µg/kg	100 [a*]	1.3×10^{-5}
Birds and Mammals	In vitro assay	AC50	µM	0.0082 [a*]	4.5×10^{-7}
PFOS					
Aquatic Biota	**Aqueous**	**NOEL**	**µg/L**	**1.1** [b]	**1.6×10^{-6}**
Terrestrial Invertebrates	**Soil**	**LOEL**	**µg/kg**	**1,800** [c]	**3.6×10^{-4}**
Birds and Mammals	**Dietary Dose**	**LOEL**	**µg/kg BW/d**	**20.5** [c**]	**-**
Birds and Mammals	**Serum**	**LOEL**	**µg/mL (serum)**	**0.25** [a**]	**3.0×10^{-7}**
Birds and Mammals	In vitro assay	AC50	µM	0.54 [a*]	1.7×10^{-5}
PFOSA					
Aquatic Biota	Aqueous	AC50	µg/L	20.0 [a*]	2.8×10^{-3}
Birds and Mammals	In vitro assay	AC50	µM	0.014 [a*]	1.0×10^{-3}
PFHxA					
Birds and Mammals	In vitro assay	AC50	µM	2.4×10^{-5} [a*]	3.9×10^{-11}
PFHpA					
Birds and Mammals	In vitro assay	AC50	µM	0.76 [a*]	3.2×10^{-6}

PFOA					
Aquatic Biota	Aqueous	EC50	µg/L	25,800 [b]	6.0×10^{-3}
Terrestrial Invertebrate	Soil	NOEL	µg/kg	100 [a*]	7.3×10^{-6}
Birds and Mammals	Dietary Dose	NOEL	µg/kg/d	10,000 [a*]	-
Birds and Mammals	In vitro assay	AC50	µM	0.022 [a*]	8.2×10^{-8}
PFNA					
Aquatic Biota	Aqueous	EC50	µg/L	23,400 [c]	1.6×10^{-2}
Birds and Mammals	In vitro assay	AC50	µM	0.037 [a*]	1.8×10^{-6}
PFDA					
Birds and Mammals	In vitro assay	AC50	µM	0.07 [a*]	1.3×10^{-4}

[a] = Lowest reported value
[b]= Lower 95% confidence limit of the HC5
[c] = HC5 value
[d] = Chemical activity based on the concentration-based TRV value and the corresponding chemical solubility in the exposure media (i.e., $a = C/S$). See Electronic Supplementary Material 5 (ESM-5) and Table 3-1 for details regarding PFAS solubility values for activity calculations. See Electronic Supplementary Material 3 (ESM-3) for details regarding calculation of chemical activity for in vitro assay results.
* = Uncertainty factor (UF) of 10, following Beach et al., (2006)
* *= Uncertainty factor (UF) of 36, following Beach et al., (2006)

3.4.5. Integration of passive sampler data with target lipid and chemical activity-based models for effects characterization

Recent ecotoxicology studies of complex petroleum mixtures have utilized passive sampling devices coupled with target lipid model (TLM) and chemical activity (CA) models to assess exposure and effects in aquatic organisms (Parkerton et al., 2000; McGrath et al., 2005; Redman et al., 2012; Redman et al., 2014; Butler et al., 2016; Redman et al., 2018; Redman et al., 2018). The developed approach, combining passive sampling devices and mechanistic modeling, may allow for rapid, cost-effective assessment of PFASs at AFFF-impacted sites.

For this approach, measured contaminant concentrations in passive sampling devices are typically converted to a lipid-based concentration and expressed in terms of toxic units (TU), assuming additive effects for non-polar narcosis at the site of toxic action (Redman et al., 2014; Redman et al., 2018). TLM and CA models are then utilized to assess bioaccumulation potential (e.g., BCFs) and internal exposure levels.

The TLM is based on the fact that the ability of organic chemicals to cause adverse effects in aquatic organisms is highly dependent on the partitioning potential and chemical activity at a particular target site, which is typically the organic phase of the organism (e.g. membranes). In particular, the TLM assumes toxicity occurs when the concentration of organic chemicals (individual compounds or multi-residue mixtures) in lipids (C_{LIPID}) exceed a toxicological threshold, referred to as the critical target lipid body burden (CTLBB), as a result of lipid-water partitioning. Employing CA based models have been show to complement the TLM approach for assessing toxicity of complex petroleum mixtures (Butler et al., 2016).

To our knowledge, this integrated approach using passives sampler data and TLM/CA models for effects characterization has not been applied to PFASs. Laboratory and field-based studies will be required to evaluate and compare partitioning behavior of PFASs in passive sampling device media (e.g., SPME fibres, polyacrylate films etc.) and target lipids (e.g., phospholipids). It is anticipated that ongoing passive sampler studies funded under SERDP will provide important information that will aid efforts to integrate passive sampler data with mechanistic models for PFAS exposure and effects characterization.

3.5. PFAS Risk Estimation

3.5.1. Risk Quotients

The simplest approach for comparing data generated in the exposure and effects characterization phase is the quotient method, which involves calculation of risk quotients (RQs), which are typically expressed as the ratio of the estimated exposure concentration (EEC) divided by a threshold effects concentration or a derived TRV. For example, based on a TRV of 1.1 µg/L for PFOS, the RQ for aquatic organisms within a waterbody exhibiting PFOS concentrations equal to EPAs lifetime health advisory level of 70 ng/L is determined as RQ = EEC/TRV = 0.06.

RQs can be determined using a range of exposure and effects data types, including surface water concentrations, as well as concentrations in sediments, soils or internal tissue residue concentrations in organisms. For the chemical activity-based approach, RQs are determined using chemical activity values (unitless), which are calculated from molar concentration and corresponding solubilities for a given medium ($a = C/S$). Chemical activity-based RQs may be

particularly useful for characterizing risk on the basis of internal dose, as toxic effects are related to a chemical's activity at a given target site. RQs can also be determined on an external dose basis, which involves the ratio of estimated daily intake (DI, µg/kg body wt./day) and a reference dose value (RfD) or external dietary-based TRV, determined from laboratory studies in suitable animal models. The quotient method is particularly useful for screening level risk assessments of large numbers of chemicals and/or sites.

3.5.2. Probabilistic Approaches that Incorporate Variability in Exposure and/or Effects

In addition to RQ determinations using single-point estimates, probabilistic approaches that incorporate variability in exposure and effects data are also common practice in ecological risk assessment. In particular, cumulative distribution functions (CDFs) and probability density functions (PDFs) can generate a probability associated for a given scenario of exposure and/or effect levels (U.S. Environmental Protection Agency, 1998). For example, when the variability of exposure data is known, a single-point estimate of effects (e.g., TRV) can be compared with a cumulative exposure distribution. This approach provides a statement of likelihood that the effects point estimate exceeds the exposure point estimate. Further, when sufficient data allows for quantification of the variability in exposure and effects then many different risk estimates can be calculated. For example, a common risk estimation technique is to compare upper limit of the exposure distribution (e.g., 95th percentile of surface water concentrations) with the lower limit of the effects data (e.g., HC5). Using this approach, a probability of exceeding a derived TRV can be determined.

SSDs derived from the distribution of effects data in multiple organisms allow for determination of the percentage of species likely effected under a given exposure scenario. This method is often used to compare the estimated risk to potentially exposed species to the benchmark goal of protecting 95% of the species within a particular ecosystem of concern.

For previous risk assessments of T&E species potentially exposed to pesticides, EPA has recommended using available probit dose response relationships as a tool for providing additional information on the potential for acute direct effects to individual listed species and aquatic animals that may indirectly affect the listed species of concern (U.S. Environmental Protection Agency, 2004). This technique involves employing an Individual Effect Chance Model, which allows for determination of probability and risk level (e.g., 1 in 1,000,000 chance of acute lethal effect) based on the mean probit slope estimate (and the 95% confidence limits of that estimate) and the acute RQ.

3.5.3. Assessing direct vs. indirect effects

Direct effects of contaminant exposure in a given organism are determined using single-point RQs or probabilistic approaches that compare distributions of exposure and effects in the organism of interest. In addition to assessment of direct effects, indirect effects are often evaluated for more robust risk assessment.

Past risk assessments of T&E species potentially exposed to pesticides have included indirect effects assessments, including evaluating the potential for impacts in prey species and designated critical habitat. For example, risk assessments of Linuron and 2,4-D in federally threatened California red-legged frogs (*Rana aurora draytonii*) included a comprehensive

assessment of direct exposure risks, as well as assessments of these contaminants on potential reduction in their prey (aquatic and terrestrial invertebrates, fish, frogs, non-vascular plants and small mammals), reduction in habitat and/or primary productivity and reduction in terrestrial plant communities (U.S. Environmental Protection Agency, 2008; U.S. Environmental Protection Agency, 2009).

Risk assessments of T&E species potentially exposed to PFASs at AFFF-impacted DoD sites should follow this approach, incorporating risk estimates for both direct and indirect effects. This will involve determination of RQs and/or probabilistic risk estimates for specific T&E species of concern, as well as similar risk estimates for prey species and critical habitat features. It is important to note that risk assessments utilizing SSDs that incorporate a wide range of organisms comprising the food web of target T&E species may inherently incorporate such indirect effects. For example, derived TRVs based on comprehensive SSDs may include prey species of a given T&E species of concern. Regardless, risk analysis and risk estimation involving direct and indirect effects of PFASs in T&E species would be undoubtedly provide more robust risk assessments.

3.5.4. Levels of concern (LOC) and protection goals

For risk assessments of T&E species potentially exposed to PFASs at AFFF-impacted DoD sites, it will be important to establish protection goals and determine specific levels of concern (LOC) for effective risk estimation. For example, calculated risk quotients should be compared to pre-defined LOC. A low LOC (e.g., LOC = 0.01) indicates a robust, risk-averse position, while a relatively high LOC (e.g., LOC = 1) represents a higher level of risk acceptance. For PFAS risk assessments involving T&E species, employing a relatively low LOC is recommended.

Defining an acceptable level of risk is also important. For example, EPAs approach utilizing the Individual Effect Chance Model is used to determine a specific level of risk that is acceptable for a given assessment (i.e., chance of acute lethal effect), based on the LOC, the LC50 or LD50 and probit slope response value. For example, an acceptable risk level may be determined to be 1 in 1,000,000 chance of acute lethal effects. Another common protection goal proposed by EPA involves the 95% species protection criterion (U.S. Environmental Protection Agency, 1998). This approach typically utilizes the 5% hazardous concentration effect level (HC5) associated with a given SSD, which exhibits an effect level that theoretically protects 95% of species. This approach can inherently incorporate indirect effects on a given target organism, as this protection goal aims to protect potential prey species of upper trophic wildlife with a given ecosystem. A limitation of this approach is that it is possible that an important prey or related species falls within the 5% of species affected. Thus, caution and professional judgment are required when utilizing this criterion.

4. Application to Select DoD Sites

4.1. Overview of PFAS risks to T&E species at select DoD sites

4.1.1. Risk estimation based on measured environmental concentrations and external dose

For the purpose of the present study, PFAS exposure risks are evaluated solely on PFOS exposure and effects characterization. While the available toxicity data indicate exposure to other PFAAs (e.g., PFHxS, PFOA, PFNA etc) may be additive, the occurrence of these compounds at the studied AFFF-impacted DoD sites is typically negligible compared to those of PFOS.

RQs for PFOS were determined for aquatic biota and piscivorous birds at the five selected DoD sites (Table 4-1). For this assessment, birds were assumed to have a diet consisting of 100% fish from a given site, with an assumed food consumption rate of 5 % of body weight per day and a body weight of 5 kg. The results indicate that RQs for PFOS exposure in aquatic biota are often < 1 at these DoD sites, but can exceed TRVs in some cases. For example, an RQ for aquatic biota equal to 19 was calculated based on upper 95% confidence levels in surface water at Wurtsmith AFB. Similarly, RQs for piscivorous birds at these sites are generally ≤ 1, but can exceed the TRV in some cases. For example, RQ for PFOS exposure in piscivorous birds at Barksdale AFB was determined to be 1.7 (95%CI: 0.6-4.9).

RQs for soil-dwelling organisms are shown in Table 4-2. The RQ values for soil-dwelling organisms were determined based on typical background contamination levels for North America (Vedagiri et al., 2018), as well as RQs based on recently reported PFAS levels at ten active USAF bases (Anderson et al., 2016). In addition to RQs for soil-dwelling invertebrates at these exposure levels, Table 4-2 shows estimated RQs of PFOS exposure in upper trophic wildlife based on chronic effects, as well as the estimated probability of individual acute effects in upper trophic terrestrial wildlife at these sites. The PFAS food web bioaccumulation model was use to estimate dietary intake in upper trophic wildlife (e.g., terrestrial reptiles). RQs for chronic effects were based on the TRV of 20.5 µg/kg BW/d. The risk probability associated with acute lethal effects was determined using a previously reported LD50 and probit slope value of 150 mg/kg BW/d and 3.655, respectively.

The results for exposure risks via soils (shown in Table 4-2) indicate that potential for effects related to PFOS exposure in terrestrial invertebrates is relatively low, except at the highest levels at active USAF bases (i.e., Max PFOS concentration of 9,700 µg/kg). Conversely, PFOS exposure risks for upper trophic terrestrial wildlife is relatively high (RQs > 1), even for sites exhibiting concentrations observed in background reference soils.

he relatively high PFOS concentrations in soils from the ten active USAF bases are expected to result in relatively high exposure risks for terrestrial wildlife. For example, model predicted exposure levels in terrestrial reptiles at a site with PFOS soil concentrations of 52.5 ng/g dry weight, the median level at active USAF bases, correspond to a chronic RQ value of 791 and a risk probability for individual acute effects equal to 1 in 4,900.

Table 4-1. Calculated risk quotients (RQs) for PFOS exposure in aquatic biota and piscivorous birds at select DoD sites using external concentrations (surface water) for aquatic biota and dietary intake estimates for piscivorous birds. RQ estimates include values based on geometric mean concentrations or dietary concentrations, along with estimates using lower and upper 95% confidence limit values (values shown in brackets).

DoD Installation	RQs for Aquatic Biota [a]	RQs for Piscivorous Birds [b]
Wurtsmith AFB	1.5 (0.13-19.0)	0.5 (0.03-7.5)
Barksdale AFB	0.3 (0.08-1.5)	1.7 (0.6-4.9)
Joint Base McGuire-Dix-Lakehurst	0.05 (0.02-0.15)	0.1 (0.03-0.4)
Pease AFB	1.0 (0.3-4.1)	-
Peterson AFB	0.02 (0.002-0.1)	-

[a] RQ values determined using measured PFOS concentrations reported in surface water at a given DoD site, along with the derived TRV for PFOS exposure in aquatic organisms (1.1 µg/L).
[b] RQ values determined using measured PFOS concentrations reported in aquatic biota at a given DoD site. Dietary intake estimates were based on an assumed feeding rate of 0.25 kg/d and body weight of 5 kg.

Table 4-2. Risk quotients for PFOS exposure in soil-dwelling organisms based on median and maximum background contamination levels in soils from North America, as well as contamination levels observed at active USAF bases.

Site Characteristics		PFOS Concentration in Soil (ng/g dry weight) [a]	RQs for Terrestrial Invertebrates [b]	Daily Intake (DI) for Upper Trophic Terrestrial Wildlife (mg/kg BW/d) [c]	RQs for Chronic Effects Upper Trophic Terrestrial Wildlife [d]	Probability of Individual Acute Effects in Upper Trophic Wildlife (Estimated using Probit Slope Response and LD50) [e]
Background Reference Soils	Median	0.27	1.5×10^{-4}	0.083	4.07	1 in 1.6×10^{33}
	Maximum	2.55	1.4×10^{-3}	0.79	38.4	1 in 2.5×10^{16}
Active USAF Bases Soils	Median	52.5	0.03	16.2	791	1 in 4,900
	Maximum	9,700	5.4	2,990	1.46×10^{5}	100% mortality (DI > LD50)

[a] Reported median and maximum concentrations in background reference soils and soils at active USAF bases.
[b] RQs in terrestrial invertebrates determined using concentration in soil divided by TRV for terrestrial invertebrates (see Table 3-3).
[c] Estimated daily intake (DI) in upper trophic wildlife feeding on soil invertebrates. Dietary intake estimates were based on an assumed feeding rate of 0.25 kg/d and body weight of 5 kg. Concentrations in terrestrial invertebrates were determined using the PFOS food web model, which assumes soil-dwelling invertebrates are in equilibrium with ambient soil (i.e., equivalent chemical activity, $a_{organism} = a_{soil}$).
[d] RQs for upper trophic wildlife were determined by dividing the estimated daily intake by avian/mammalian TRV of 0.02 mg/kg BW/d (see Table 3-3).
[e] Probit slope and LD50 values used were 3.655 and 150 mg/kg BW/d, respectively.

4.1.2. Risk estimation based on chemical activity and internal dose

Figure 4-1 shows the cumulative probability of estimated PFOS activity in water associated with adverse effects in aquatic organisms during laboratory toxicity tests, along with estimated PFOS activity in surface water and bottom sediments at several DoD sites. The data for DoD sites represent monitoring data for Former Wurtsmith AFB, Barksdale AFB, Former Pease AFB, Joint Base McGuire-Dix-Lakehurst, Peterson AFB and 114th Fighter Wing, South Dakota Air National Guard Joe Foss Field.

The toxicity data in Figure 4-1a suggests that many of the reported PFOS toxicity studies in aquatic organisms are related to non-specific baseline toxicity (i.e., narcosis), which tends to occur when chemical activity exceeds 0.01. The majority of the reported toxicity data are well below the 0.01 threshold level, indicating PFOS exhibits a specific mode of action at relatively low concentrations. The results also indicate that estimated PFOS activity in surface water and sediments DoD sites (shown in Figure 4-1b) can exceed EPAs lifetime health advisory level of 70 ng/L (activity equivalent = 1×10^{-7}), as well as the chemical activity-based TRV for aquatic organisms of 1.6×10^{-6} (corresponding to PFOS concentration of 1.1 μg/L). Also, estimated PFOS activity in sediments at a few sites approach the baseline toxicity (narcosis) threshold activity of 0.01.

Figure 4-2 shows the cumulative probability of estimated PFOS activity related to in vitro cell-based toxicity assays (ToxCast), (Figure 4-2a), along with estimated PFOS activity in aquatic biota and birds at several DoD sites (Figure 4-2b). The represented DoD sites are the same as those above with surface water and sediments. PFOS activity in birds was estimated based on measured PFOS concentrations in field-collected samples of eggs (1,220 ng/g) and plasma (1,840 ng/g) of tree swallows (*Tachycineta bicolor*) at Former Wurtsmith AFB.

The ToxCast data shown in Figure 4-2a suggests that the majority of the reported PFOS ToxCast assay results (AC50s) occur below the level related to narcosis ($a < 0.01$), with the lowest observed AC50 occurring at an activity of 1.7×10^{-4}. The PFOS activities observed in ToxCast assays are comparable to those observed in in vivo toxicity tests, which suggests toxicity threshold effects of PFOS may be in the same range for different organisms (i.e., fish, birds, mammals).

The data suggest estimated internal PFOS activity in aquatic biota at the studied DoD sites (shown in Figure 4-2b) can exceed the activity-based TRVs for aquatic biota (1.6×10^{-6}). It is important to note that estimated PFOS activity in aquatic biota ranges between approximately 7.6×10^{-9} to 7.3×10^{-5}, which is similar to PFOS activity in surface water (range: 2.9×10^{-11} to 2.8×10^{-5}). This indicates that equilibrium partitioning and bioconcentration is likely the governing mechanism of PFOS bioaccumulation (i.e., $a_B = a_W$). Also, the available data for tree swallows at DoD sites indicates PFOS activity in birds at an AFFF-impacted DoD site (~ 1×10^{-6}) exceeds the activity based TRV for birds (3×10^{-7}).

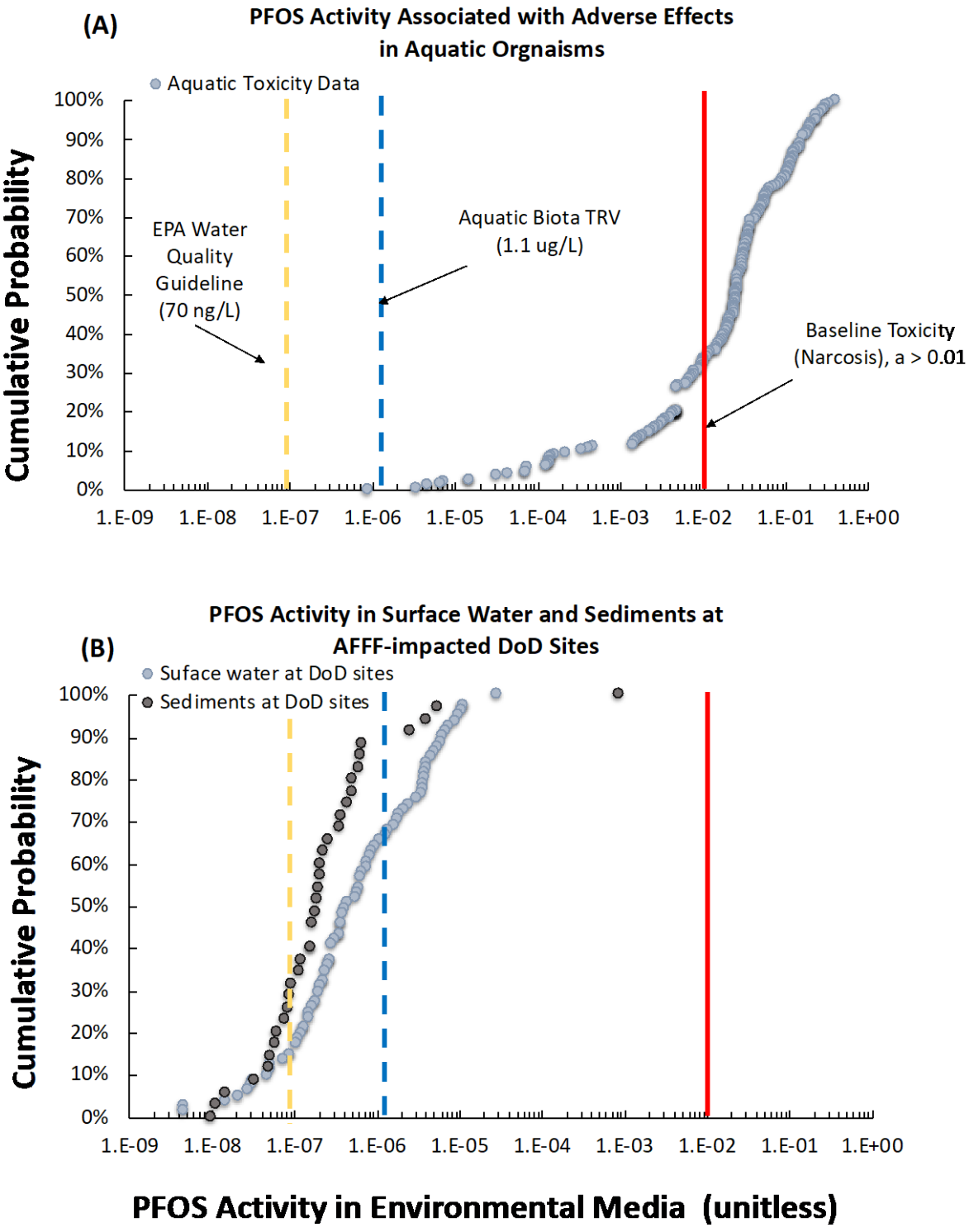

Figure 4-1. Cumulative probability of PFOS activity (a, unitless) in (A) water associated with adverse effects in aquatic organisms during laboratory toxicity tests and (B) PFOS activity in surface water and bottom sediments at several DoD sites. Vertical lines representing PFOS activity corresponding to EPAs lifetime health advisory level of 70 ng/L (a = 1 × 10^{-7}), the derived aquatic TRV of 1.1 µg/L (a = 1.6 × 10^{-6}), as well as non-specific baseline toxicity, i.e., narcosis (a > 0.01) are plotted for comparison.

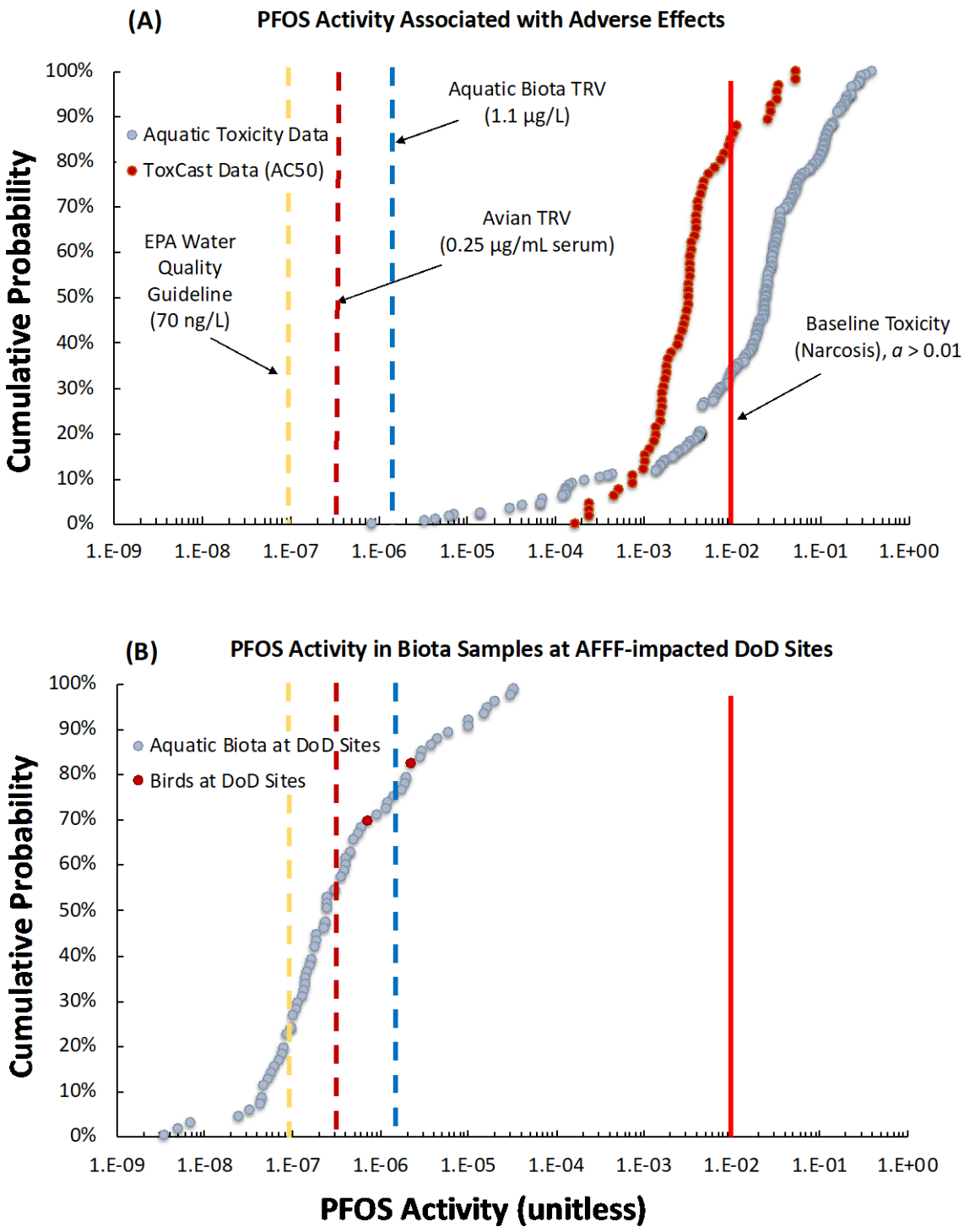

Figure 4-2. Cumulative probability of PFOS activity (a, unitless) in (A) in vitro cell-based toxicity assays (ToxCast AC50 values) and (B) aquatic biota and birds at DoD sites. Vertical lines representing PFOS activity corresponding to EPAs lifetime health advisory level of 70 ng/L ($a = 1 \times 10^{-7}$), the derived aquatic TRV ($a = 1.6 \times 10^{-6}$), avian TRV ($a = 3 \times 10^{-7}$), as well as non-specific baseline toxicity, i.e., narcosis ($a > 0.01$) are plotted for comparison.

4.1.3. Risk estimation based on bioaccumulation model predictions

Figure 4-3 shows model predicted activities (*a*, unitless) of PFOS in aquatic and terrestrial food webs at the five selected DoD sites. Horizontal lines representing PFOS activity corresponding to EPAs lifetime health advisory level of 70 ng/L, the derived activity-based TRVs for aquatic biota and avian/mammalian species, as well as non-specific baseline toxicity (i.e., narcosis) are plotted for comparison. The predicted PFOS activities and corresponding tissue residue concentrations (ng/g wet wt.) of the aquatic and terrestrial organisms at the select DoD sites are provided in Appendix VII and VIII, respectively.

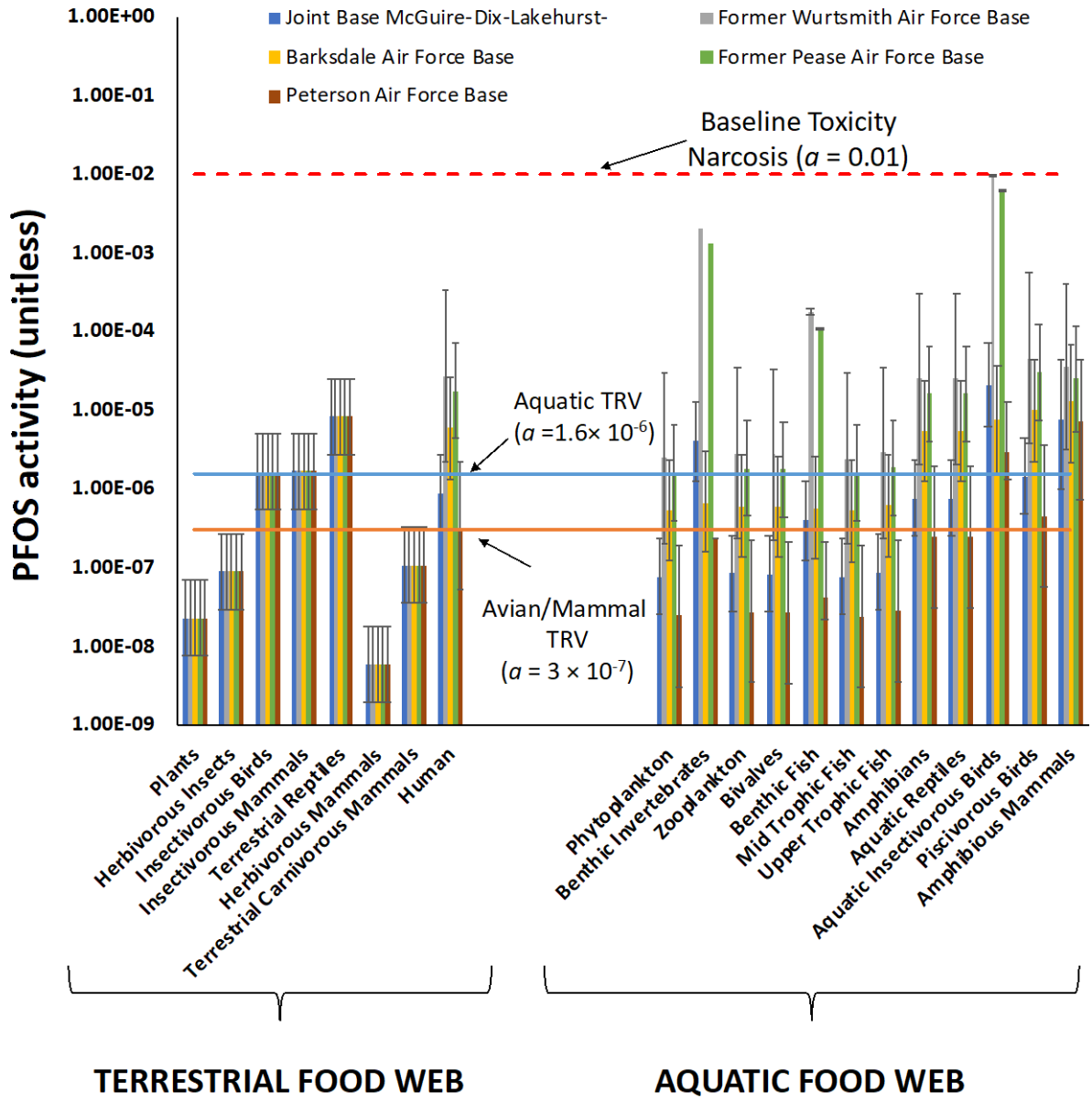

Figure 4-3. Model predicted activities (a, unitless) of PFOS in aquatic and terrestrial food webs at the five selected DoD sites. Horizontal lines representing the derived aquatic and avian TRVs, as well as non-specific baseline toxicity (i.e., narcosis) are plotted for comparison.

The modeling results indicate that PFOS activities in water-respiring organisms at these sites are generally below the activity-based TRV of 1.6×10^{-6}. The exception is benthic invertebrates and benthic-feeding fish, which exhibit elevated PFOS activity due to higher exposure via sediments. Air-breathing wildlife within both the terrestrial food webs (e.g., terrestrial reptiles, insectivorous mammals) and the aquatic food webs (e.g., amphibians, piscivorous birds, amphibious mammals) in some cases exhibit PFOS activities that exceed the activity-based TRV for birds and mammals (3×10^{-7}). Plants, herbivorous insects and herbivorous mammals are shown to exhibit relatively low PFOS activities, generally well below the TRV.

Figure 4-4 shows the calculated RQs based on model predicted activities (*a*, unitless) of PFOS in aquatic and terrestrial food webs at the five selected DoD sites. RQ values for PFOS exposure in specific T&E species at the studied DoD sites are shown in Table 4-3. The results show that RQs for PFOS exposure in benthic invertebrates and benthic fish can exceed 1.0 at some sites. Similarly, RQs for aquatic insectivorous birds (feeding on benthic invertebrates) are also relatively high, with RQ values generally exceeding 1.0. RQs for pelagic fish are generally lower, but can exceed the LOC value of 0.1 at some sites.

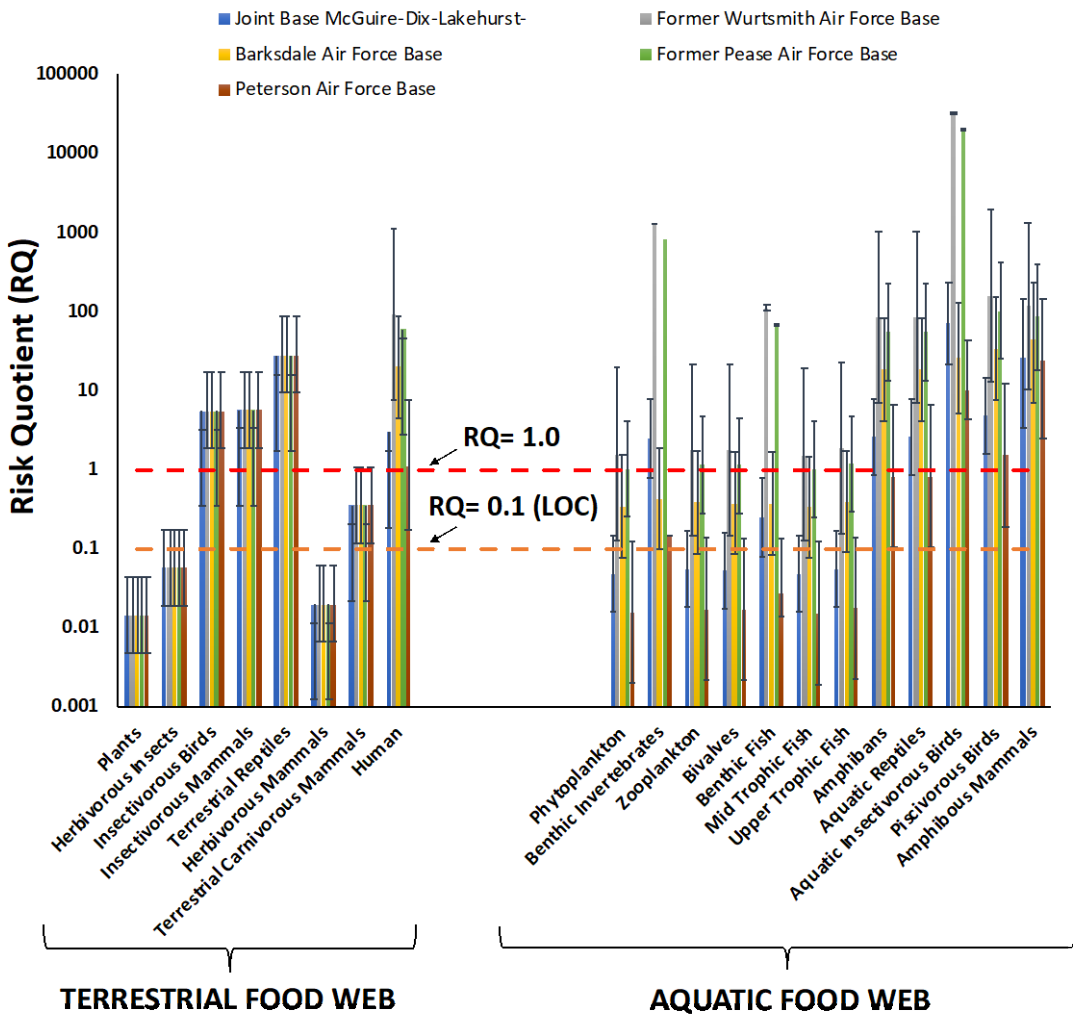

Figure 4-4. Risk quotients (RQs) based on model predicted activities (a, unitless) of PFOS in aquatic and terrestrial food webs at the five selected DoD sites. Horizontal lines represent a RQs equal to 1.0 and 0.1 (defined as the level of concern, LOC, for purposes of the present study).

Table 4-3. Risk quotients for PFOS exposure in T&E species at select DoD sites using model predicted activities and activity-based TRVs. RQ estimates include values based on geometric mean PFOS activities, along with estimates using lower and upper 95% confidence limit values.

Taxonomic Group	Common Name	Activity-Based Risk Quotient (RQ)
Wurtsmith AFB (Iosco County, Michigan)		
Flowering Plants	Pitcher's thistle	0.01 (0.005-0.04)
Birds	Kirtland's Warbler	5.52 (1.84-17.0)
Birds	Red Knot	3.2×10^4
Mammals	Northern Long-Eared Bat	5.63 (1.88-17.4)
Reptiles	Eastern Massasauga Rattlesnake	27.6 (9.2-85.1)
Barksdale AFB (Bossier Parish, Louisiana)		
Fishes	Pallid sturgeon	0.33 (0.08-1.48)
Birds	Whooping crane	33.7 (7.6-150)
Birds	Red-cockaded woodpecker	5.52 (1.84-17.0)
Birds	Least tern	25.7 (5.1-125)
Mammals	Louisiana black bear	19.8 (4.49-87.5)
Mammals	Northern Long-Eared Bat	5.63 (1.88-17.4)
Pease AFB (Rockingham County, New Hampshire)		
Flowering Plants	Small whorled Pogonia	0.01 (0.005-0.04)
Birds	Piping Plover	2.1×10^4
Birds	Roseate Tern	2.1×10^4
Birds	Red Knot	2.1×10^4
Reptiles	Hawksbill Sea Turtle	54.8 (13.5-221)
Reptiles	Leatherback Sea Turtle	54.8 (13.5-221)
Mammals	Northern Long-Eared Bat	5.63 (1.88-17.4)
Joint Base McGuire-Dix-Lakehurst (Burlington, New Jersey)		
Flowering Plants	Knieskern's Beaked-Rush	0.01 (0.005-0.04)
Flowering Plants	Sensitive Joint-Vetch	0.01 (0.005-0.04)
Flowering Plants	Swamp Pink	0.01 (0.005-0.04)
Flowering Plants	American Chaffseed	0.01 (0.005-0.04)
Birds	Red Knot	70.0 (21.0-238)
Reptiles	Bog Turtle	2.59 (0.85-7.88)
Mammals	Northern Long-Eared Bat	5.63 (1.88-17.4)
Peterson Air Force Base (El Paso County, Colorado)		
Flowering Plants	Ute Ladies'-Tresses	0.01 (0.005-0.04)
Flowering Plants	Western Prairie Fringed Orchid	0.01 (0.005-0.04)
Insects	Pawnee Montane Skipper	0.06 (0.019-0.17)
Fishes	Greenback Cutthroat Trout	0.02 (0.002-0.14)
Fishes	Peppered Chub	0.02 (0.002-0.14)
Birds	Whooping Crane	1.52 (0.19-12.2)
Birds	Bald Eagle	1.52 (0.19-12.2)
Birds	American Peregrine Falcon	0.35 (0.12-1.08)
Birds	Mexican Spotted Owl	0.35 (0.12-1.08)
Birds	Southern White-tailed Ptarmigan	5.52 (1.84-17.03)

Reptiles	Desert Massasauga Rattlesnake	27.6 (9.2-85.1)
Mammals	Preble's Meadow Jumping Mouse	5.63 (1.88-17.4)
Mammals	North American Wolverine	0.35 (0.12-1.10)

The results also demonstrate that air-breathing wildlife within both the terrestrial food web (e.g., terrestrial reptiles, insectivorous mammals) and the aquatic food web (e.g., amphibians, piscivorous birds, amphibious mammals) exhibit relatively high PFOS exposure risks, with RQ values often exceeding 1.0. The elevated exposure risk in these animals is primarily due to the high degree of biomagnification in these organisms. The findings demonstrate the current need to better understand PFAS concentrations in surface soils and subsequent biomagnification and exposure risks for terrestrial organisms at AFFF-impacted DoD sites .

It is important to note that information regarding PFAS concentrations in soils at specific DoD sites is lacking. Consequently, it was not possible to calculate RQ values based on measured PFAS concentrations in soils at the five select DoD sites investigated in the present study. However, using the developed PFAS food web bioaccumulation model, PFOS activities in terrestrial organisms were predicted based on reported concentrations in background soils in North America (Vedagiri et al., 2018), as well as concentrations recently reported in soils sampled at several active USAF bases (Anderson et al., 2016). The results are shown graphically in Figure 4-5. Model predicted activities of PFOS in upper trophic terrestrial wildlife based on soil concentrations at the tested USAF bases are shown to exceed the TRV based on dietary dose (20.5 μg/kg BW/d), as well as the activity-based internal TRV of 3×10^{-7}, and ultimately may levels associated with narcosis ($a > 0.01$). Model predicted PFOS activities in terrestrial organisms based on background reference soil concentrations are much lower, but in some cases can also exceed TRVs.

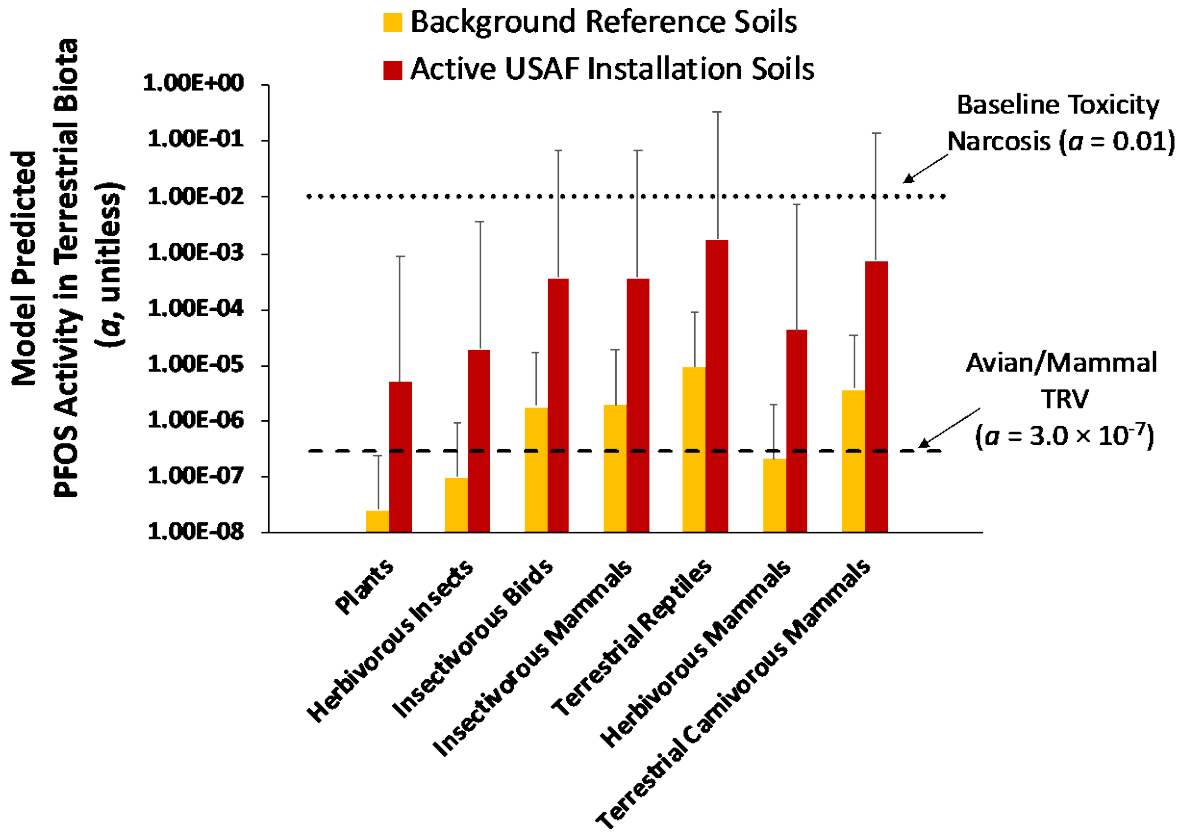

Figure 4-5. Model predicted PFOS activity (a, unitless) in different organisms of the terrestrial food web resulting for simulations based on average background reference soil concentrations, as well as reported concentrations at ten active USAF installations. Bars represent simulations using median PFOS soil concentrations, while the value of the positive error bar represents simulations using maximum PFOS soil concentrations. Horizontal lines representing the activity-based TRV for PFOS exposure in birds and mammals and the activity related to non-specific baseline toxicity (i.e., narcosis) are plotted for comparison.

Figure 4-6 shows a plot of model predicted PFOS activity versus RQ in select T&E species, assuming median concentrations of PFOS in the ambient environment. Based on the available monitoring data for all DoD sites, median PFOS concentrations in surface water, sediments and soils are equal to 350 ng/L, 0.85 ng/g dry wt. and 52.2 ng/g dry wt., respectively.

Based on these exposure levels, the anticipated risk is generally shown to be highest for terrestrial wildlife and lowest for aquatic water-respiring organisms. For example, based on a median soil concentration of 52.2 ng/g dry wt., the predicted internal PFOS activity and RQ for eastern massasauga rattlesnake is 1.8×10^{-3} and 6,040, respectively. Other terrestrial species such as red-cockaded woodpeckers and northern long eared bats also exhibit a relatively high degree of exposure risk, as PFOS exposure in those organisms are also highly related to ambient soil concentrations.

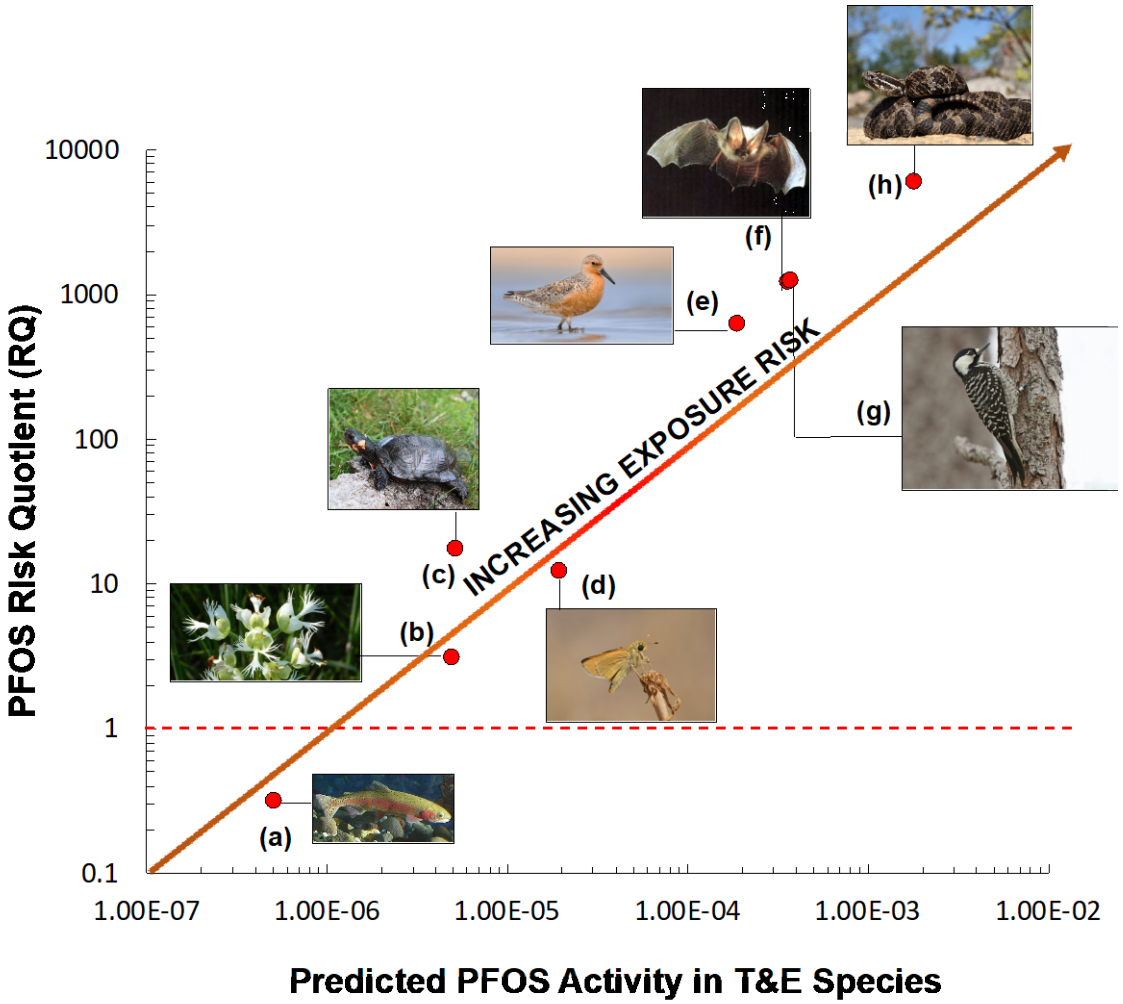

Figure 4-6. Model predicted PFOS activity (a, unitless) and corresponding risk quotients (RQs) for select T&E species at a hypothetical site exhibiting median PFOS concentrations found at DoD sites. Specifically, model predicted activities and RQs are shown for (a) upper trophic fish (greenback cutthroat trout), b) terrestrial plants (western prairie fringed orchid, (c) aquatic reptiles (bog turtle), (d) terrestrial insects (Pawnee Montane skipper), (e) aquatic insectivorous birds (red knot), (f) terrestrial insectivorous mammals (northern long-eared bats), (g) terrestrial insectivorous birds (red-cockaded woodpecker) and (h) terrestrial reptiles (eastern massasauga rattlesnakes). Model predictions are based on median concentrations of PFOS in surface water (350 ng/L), sediments (0.85 ng/g dry wt.) and soils (52.2 ng/g dry wt.).

Insectivorous birds (e.g., red knot), assumed to feed on a mixture of sediment-dwelling and terrestrial invertebrates, also exhibit a relatively high exposure risk. The estimated activity and RQ value for red knot for this exposure scenario are is 1.9×10^{-4} and 630, respectively. Conversely, the estimated PFOS exposure risk for aquatic T&E species such as greenback cutthroat trout is orders of magnitude lower (RQ < 1).

The results further highlight that PFAS exposure risks are generally higher in air-breathing wildlife (reptiles, birds, mammals) compared to aquatic water-respiring organisms, which is consistent with our mechanistic understanding of moderately hydrophobic, non-metabolizable organic chemicals exhibiting high chemical K_{OA} (Kelly et al., 2007). Moreover, air-breathing wildlife exposed to PFASs via the soil exposure pathway (e.g. soil → terrestrial invertebrate → small mammal → terrestrial reptile) may particularly be vulnerable at AFFF-impacted DoD sites, given that PFAS concentrations in soils at those sites can attain levels substantially higher than those at background reference sites. It would be prudent to focus future PFAS risk assessment efforts on these most vulnerable species. In particular, information regarding the transfer of PFAS residues from soil to upper trophic wildlife is a major knowledge gap limiting the efficacy of T&E species risk assessments at AFFF-impacted DoD sites.

4.2. Former Wurtsmith Air Force Base

Former Wurtsmith AFB is located in Iosco County, Michigan (Appendix XII). PFOS concentrations in surface water at this DoD site (1,710 ng/L; 95%CI: 140- 20,954) slightly exceed the TRV for aquatic biota (1,100 ng/L), with an RQ based on this external exposure approach equal to 1.5 (0.13-19.0). Results using the chemical activity-based approach to assess aquatic biota exposure are similar. The T&E species listed within this County include pitcher's thistle, Kirtland's warbler, red knot, northern long-eared bats and eastern massasauga rattlesnakes. The model predicted RQs for PFOS exposure in these T&E species are shown in Table 4-3. With the exception of the listed plant species (pitcher's thistle), RQ values can exceed 1.0 at this DoD site.

The estimated RQ in red knot, assuming exposure to PFOS via consumption of aquatic and sediment-dwelling invertebrates at Wurtsmith AFB, is substantially greater than 1.0 at this site ($RQ = 3.2 \times 10^4$). The high RQ for these birds is due relatively high PFOS concentration in sediments (observed level = 4,280 ng/g) and hence concentrations in benthic invertebrates (model predicted concentration = 980 μg/g) at this site. The results are consistent with previous risk assessment of PFOS exposure in birds at this site (Larson et al., 2018).

The RQ for eastern massasauga rattlesnakes at this site is also relatively high (RQ = 27.6; 95%CI: 9.2-85.1), primarily due to biomagnification of PFOS residues from soils in the terrestrial food web (i.e., soil → invertebrates → small mammals → reptiles). Lower trophic terrestrial wildlife (Kirtland's warbler and northern long-eared bats) exhibit somewhat lower RQs, due to reduced exposure levels in prey species (i.e., insects).

It is important to note that RQs for eastern massasauga rattlesnakes and other terrestrial wildlife are based on background reference soil concentrations of PFOS. For the present study, estimated RQs of PFOS for terrestrial wildlife are equivalent at the different DoD sites, as background reference concentrations were used as model inputs at all sites. The results indicate that current levels of PFOS in typical North American soils (median = 0.27 ng/g dry wt.; max = 2.55 ng/g dry wt.) are potentially hazardous for terrestrial wildlife, especially higher trophic

predators. The observed PFOS concentrations in soils at AFFF-impacted DoD sites (median = 52.2 ng/g dry wt.; max = 9,700 ng/g dry wt.) suggest exposure levels related to these relatively more contaminated soils may be 200 to 4,000 times higher compared to those related to background reference soils. Future research efforts should aim to better quantify concentrations in soils around specific DoD installations and the extent to which T&E species and their prey interact with these environments.

4.3. Barksdale Air Force Base

Barksdale AFB is located in Bossier Parish, Louisiana (Appendix XII). PFOS concentrations in surface water at this DoD site (368 ng/L; 95%CI: 83- 1,630) are generally below the TRV for aquatic biota (1,100 ng/L), with an RQ based on this external exposure approach equal to 0.33 (0.08-1.5). Results using the chemical activity-based exposure assessment approach are similar. The results are consistent with previous risk assessment findings for PFOS exposure in aquatic biota at this site (Salice et al., 2018).

The T&E species listed within this County include Pallid sturgeon, whooping cranes, red-cockaded woodpeckers, least terns, Louisiana black bears, northern long-eared bats. The results show PFOS RQ values for these T&E species are typically greater than the 1.0, with the exception of the listed fish species (Pallid sturgeon), which exhibits an RQ of 0.33. Whooping crane and least terns exhibit the highest RQ values at this site.

The estimated RQ for PFOS exposure in least terns at Barksdale AFB is based on the assumption that these aquatic insectivorous birds consume benthic invertebrates at this DoD site. As the measured PFOS concentrations in sediments at this site are relatively low (1.4 ng/g dry wt.; 95%CI: 0.33-6.4), the RQ for least terns at this site is much lower than for similar birds at other sites. For example, the RQ for PFOS exposure in least terns at Barksdale AFB is orders of magnitude lower than the RQ estimated for aquatic insectivorous birds such as red knots at Wurtsmith AFB, which exhibits much higher sediment PFOS concentrations.

4.4. Former Pease Air Force Base

Former Pease AFB is located in Rockingham County, New Hampshire (Appendix XII). PFOS concentrations in surface water at this DoD site (1,105 ng/L; 95%CI: 273- 4,462) are similar to the TRV for aquatic biota (1,100 ng/L), with an RQ based on this external exposure equal to 1.0 (0.3-4.1). Results using the chemical activity-based exposure assessment approach are similar. This DoD site is located within a county with seven listed T&E species, including small whorled pogonia, piping plovers, roseate terns, red knots, hawksbill sea turtles, leatherback sea turtles and northern long-eared bats. Similar to findings for Wurtsmith AFB, aquatic insectivorous birds assumed to feed on benthic invertebrates (e.g., piping plover, roseate tern, red knot) are shown to exhibit very high RQs (2.1×10^4), due to relatively high sediment PFOS concentrations at this site (2,780 ng/g dry wt.).

With the exception of the listed flowering plant (small whorled pogonia), other T&E species at this site exhibit RQs that exceed the LOC of 0.1. The estimated PFOS RQs for hawksbill and leatherback sea turtles (54.8; 95%CI: 13.5-221) are based on model simulations that assumed consumption of prey organisms (phytoplankton, pelagic invertebrates) exposed to concentrations equivalent to those reported in surface water samples near this DoD site (i.e., 1,100 ng/L). It is important to note that the estimated RQs for these coastal marine animals are

highly uncertain and should be viewed with caution. Future research efforts to assess PFAS transport dynamics from DoD sites to coastal marine ecosystems would be useful to better understand the potential risk of PFASs to these and other important marine wildlife species.

4.5. Joint Base McGuire-Dix-Lakehurst

Joint Base McGuire-Dix-Lakehurst is located in Burlington County, New Jersey (Appendix XII). PFOS concentrations in surface water at this DoD site (52.2 ng/L; 95%CI: 17.2- 159) are much lower than the TRV for aquatic biota (1,100 ng/L). The PFOS RQ for aquatic biota based on external exposure is equal to 0.05 (0.02-0.15). Results using the chemical activity-based exposure assessment approach are similar.

In addition to red knots, bog turtles and northern long-eared bats, five flowering plants are listed as T&E species within this County. Based on the assumption that this site exhibits PFAS concentrations similar to background reference soils, the RQs for plant species are relatively low (0.01; 0.005-0.04). Similar to other sites, aquatic insectivorous birds (i.e., red knot) exhibits the highest RQ among the listed T&E species (RQ = 70.0; 95%CI: 21.0-238), due to elevated exposure via consumption of benthic invertebrates. While PFOS sediment concentrations at this site are not very high (8.5 ng/g dry wt.; 95%CI: 2.7- 26.9) compared to other sites, the estimated exposure in aquatic birds feeding on sediment-dwelling invertebrates results in RQs > 1. The estimated PFOS RQ for bog turtles at this site is 2.59 (95%CI: 0.85-7.88). While PFOS is expected to biomagnify in these organisms, the estimate RQ in these aquatic reptiles are still low due to relatively low compared to other sites, due to relatively low surface water concentrations of PFOS at this site.

4.6. Peterson Air Force Base

Peterson AFB is located in El Paso County, Colorado (Appendix XII). PFOS concentrations in surface water at this DoD site are the lowest among the studied DoD sites (16.6 ng/L; 95%CI: 2.1- 133). The PFOS RQ for aquatic biota based on external exposure is equal to 0.02 (95%CI: 0.002-0.1). Results using the chemical activity-based exposure assessment approach are similar.

The T&E species listed in El Paso County include two flowering plant species (Ute ladies'-tresses, western prairie fringed orchid), one insect species (Pawnee montane skipper), two fish species (greenback cutthroat trout, peppered chub), five bird species (whooping crane, bald eagle, American peregrine falcon, Mexican spotted owl and southern white-tailed ptarmigan), one reptile (desert massasauga rattlesnake) and two mammals (Preble's meadow jumping mouse and North American wolverine). Among the listed species at this site, the desert massasauga rattlesnake exhibits the highest RQ value (27.6; 95%; CI: 9.2-85.1). Several other terrestrial wildlife species of concern at this site (Preble's meadow jumping mouse, American peregrine falcon, Mexican spotted owl) are also shown to exhibit relatively high PFOS RQ values. The estimated RQs in these terrestrial species are based on background reference soil concentrations of PFOS. An assessment based on relatively more contaminated soils such as those investigated by Anderson et al., (2016) would undoubtedly result in much higher PFOS RQ values for these T&E species.

5. Summary and Conclusions

Assessing the potential risks of PFAS exposure to T&E species is currently a critical priority for implementing effective management of AFFF-Impacted DoD sites in the United States. The objective of the present SERDP project was to develop a framework for conducting robust, scientifically defensible risk assessments of PFASs in T&E species at AFFF-impacted DoD installations.

The present study provides a comprehensive literature review of physicochemical properties, bioaccumulation metrics and environmental concentrations. The proposed approach generally follows conventional methods employed for ecological risk assessment, including exposure characterization, effects characterization and risk estimation. The proposed risk assessment approach utilizes a combination of field-based measurements and bioaccumulation modeling to evaluate exposure in T&E species. TRVs are derived from available PFAS toxicity data. A chemical activity-based approach is presented which may aid exposure and effects characterization of PFASs. A mechanistic modeling approach is also presented for assessing PFAS bioaccumulation and exposure levels in aquatic and terrestrial food webs. The developed food web model was tested for several DoD sites where PFAS monitoring has been recently conducted. Below are some key findings, uncertainties and knowledge gaps, as well as recommendations for future research efforts:

- Based on the available aquatic toxicity data, a concentration based TRV for PFOS exposure in aquatic biota was determined to be 1.1 µg/L in surface water. Using the chemical activity approach, this corresponds to a chemical activity-based TRV for aquatic biota equal to 1.6×10^{-6}. The available PFOS monitoring data and model predictions for several DoD sites indicates that PFOS exposure in aquatic organisms may, in some cases, exceed TRVs.

- Based on the available toxicity data for birds and mammals, an external dietary dose based TRV for PFOS was determined to be 20.5 µg/kg BW/d. Also, based on observed PFOS serum concentrations related to reproductive impacts in birds, a serum concentration of 0.25 µg/mL was derived as an internal concentration-based TRV for birds and mammals. Using the chemical activity approach, this corresponds to a chemical activity-based internal TRV for birds and mammals equal to 3×10^{-7}. The available monitoring data and model estimates indicate that PFOS exposure levels in wildlife at AFFF-impacted DoD sites may, in some cases, exceed these derived TRVs.

- In the proposed framework, we demonstrate an alternative approach that characterizes PFAS exposure and effects in terms of chemical activity. Chemical activity (a, unitless) in a given medium is determined as the ratio of the concentration (C, mol/m^3) and the corresponding solubility (S, mol/m^3) of the chemical for a given phase or compartment (i.e., $a = C/S$). This activity-based risk assessment approach is relatively simple, consisting of three key steps, including (i) determining PFAS activities in environmental media, (ii) determining internal PFAS activities in target organisms of concern and (iii) comparison of activities in organisms with those related to biological effects in vivo.

- The chemical activity-based risk assessment approach also aims to incorporate ToxCast AC50 and other in vitro assay data. This approach is consistent with the goal of

minimizing animal use and costs related to toxicity testing, highlighted in the National Research Council's vision and strategy related to toxicity testing in the 21st Century. It is important to note that ToxCast and other in vitro toxicity assay results should not generally be used to derive TRVs for ecological risk assessment, as in vitro toxicological endpoints (AC50 values) may not accurately represent threshold effect levels related to chronic exposure and ecological relevant impacts (e.g., growth, development, reproduction) in wildlife. However, using the chemical activity-based approach subcellular responses in vitro can be considered in a risk assessment alongside individual and population level responses.

- Evaluation of the available ToxCast data for individual PFASs indicates that commonly detected perfluoroalkyl acids (PFAAs) such as PFOS, PFHxS, PFOA, PFNA and PFDA exhibit a specific mode of toxic action, generally occurring in the chemical activity range between 10^{-6} to 10^{-3}, which is below levels related to narcosis ($a \geq 0.01$). PFAA activities associated with effects in vitro (i.e., ToxCast AC50 values) are generally comparable to activities associated with toxic effects in vivo. ToxCast data for PFAA precursors (N-Et-FOSA and PFOSA) suggests these relatively more hydrophobic compounds tend to exhibit baseline toxicity, with effect levels occurring in the range known to be associated with nonpolar narcosis ($a \geq 0.01$).

- As PFAAs exhibit toxic effects in the same chemical activity range, a simple additivity approach may be adopted to incorporate mixture effects of PFAAs at AFFF-impacted sites. However, as PFOS is typically the predominant PFAA (> 95%), contribution of other PFAAs is likely negligible in most cases. Thus, risk assessments based solely on PFOS may adequately represent the overall PFAS risk at a given site, especially if PFAAs are the main PFAS class of concern.

- A mechanistic PFAS food web bioaccumulation model was developed to predict internal exposure levels (chemical concentrations and activities) and external exposure (daily intake, µg/kg BW/d) of individual PFASs in various aquatic and terrestrial organisms. The model was parameterized and applied to simulate PFAS bioaccumulation at several DoD sites that have existing PFAS monitoring data. The model was shown to predict internal PFAS exposure levels in biota at DoD sites reasonably well, with model predicted values generally within a factor of three of the observed field data.

- Model simulations of PFAA bioaccumulation in aquatic in terrestrial food webs demonstrate that these compounds are expected to preferentially biomagnify in food webs containing air-breathing wildlife (birds, mammals, terrestrial reptiles). Conversely, PFAA concentrations in aquatic water-respiring organisms are expected to reach a chemical equilibrium with concentrations in ambient surface water (i.e., PFAS activity in aquatic biota is equivalent to that in ambient surface water, hence $a_B = a_W$).

- Model predictions indicate that at some sites with elevated PFAS concentrations in sediments, concentrations in benthic invertebrates can attain levels similar to those expected to induce acute effects in aquatic organisms. Biomagnification of PFASs in aquatic insectivorous bird species (feeding on benthos) at these sites may therefore result in an increased risk to these organisms.

- PFAS concentrations in soils were found to be very important for the potential risk to numerous T&E species within terrestrial food webs, including several terrestrial reptiles and birds (e.g., eastern massasauga rattlesnake, Kirtland' warblers). PFAS exposure risks to upper trophic terrestrial wildlife are evident, even for sites exhibiting PFAS concentrations in soil equivalent to background reference soils, with RQs often exceeding the LOC of 0.1. Moreover, sites that exhibit elevated PFAS concentrations in soils, such as those at several active USAF sites, estimated exposure risks to terrestrial organism may be very high. For example, at sites exhibiting relatively high exposure levels in soils (e.g., Median PFOS = 52.5 μg/kg in soils at monitored USAF sites), the resulting probability of acute individual effects in terrestrial wildlife is 1 in 4,900. For the extreme case, (e.g., Maximum PFOS = 9,700 μg/kg in soils at monitored USAF sites), the estimated daily intake via diet (DI, mg/kg BW/d) of PFOS for terrestrial wildlife is expected to exceed the PFOS LD50 value of 150 mg/kg BW/d.

- Risk estimates for potentially exposed T&E species in the present study are based on scenarios that assume PFAS exposure occurs via concentrations at the studied DoD sites. The extent of interaction of T&E species (along with their prey) with AFFF-impacted soils and surface waters is a major knowledge gap in the present assessment of PFAS exposure risks a the studied DoD sites.

- Future research efforts should include investigations to better understand the frequency and duration of various T&E species (and their prey) at AFFF-impacted DoD sites. In particular, studies to determine PFAS concentrations in prey and relative prey consumption rates would be useful.

- Other important research needs include investigations to better understand PFAS residue concentrations in sediments and soils residue at DoD sites and the corresponding risk to upper trophic terrestrial wildlife.

- The developed PFAS food web bioaccumulation model indicates this mechanistic modeling approach may be useful for future risk assessments of T&E species potentially exposed to PFASs at AFFF-impacted DoD sites. However, further development and testing of the model is still needed.

- This study demonstrates the potential and merit of a chemical activity-based approach for assessing the risk of PFAS exposure to T&E species of concern. A limitation of this approach is that apparent solubility values used to estimate chemical activities are based on numerous assumptions regarding physicochemical properties, phase partitioning, protein-binding and toxicokinetics. Currently, there is a need for further laboratory-based measurements of PFAS solubilities (S, mol/m^3) in different environmental and biological media, as well as media-water distribution coefficients for different transporter proteins ($D_{TP,W}$), structural proteins ($D_{SP,W}$), phospholipids ($D_{PL,W}$), neutral lipids ($D_{NL,W}$), carbohydrates (D_{CW}) and organic carbon (D_{OC}). Accurate estimates of solubility and distribution coefficient values will undoubtedly strengthen the reliability of the activity-based risk assessment approach. This will also aid PFAS bioaccumulation modeling efforts, as the various distribution coefficients are key parameters within the proposed mechanistic food web bioaccumulation model.

6. References

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APPENDICES

Appendix I. Physicochemical properties of per- and polyfluoroalkyl substances.

Chemical Name	Acronym	CAS	MW (g/mol) [a]	S_W (mg/L) [a,b]	VP (Pa) [a]	H_N (Pa·m^3/mol) [a]	pK_a [c]	Log $K_{OW,N}$ [a,d]	Log $K_{OA,N}$ [e]	Log D_{OW} [f]	Log D_{MW} [g,h]	Log D_{PW} [i,j]
Perfluorobutane sulfonic acid	PFBS	29420-49-3	300	510 [b]	1.50×10^{-6}	8.82×10^{-7}	0.14	3.91 [d]	9.11	-3.43	2.63 [g]	3.86 [i]
Perfluorohexane sulfonic acid	PFHxS	355-46-4	400	4,926 [b]	3.09	2.51×10^{-1}	0.14	4.95 [d]	7.15	0.06	3.82 [g]	3.9 [i]
Perfluorooctane sulfonic acid	PFOS	1763-23-1	500	680 [b]	0.32	2.33×10^{-1}	0.14	5.93 [d]	9.95	2.83	4.88 [g]	4.1 [i]
Perfluorobutanoic acid	PFBA	375-22-4	214	3.5×10^5 [a]	132	8.07×10^{-2}	0.08	3.24 [d]	6.62	-0.96	1.0 [g]	2.5 [j]
Perfluoropentanoic acid	PFPeA	2706-90-3	264	1.3×10^5 [a]	339	7.05×10^{-1}	-0.16	3.69 [d]	6.35	-0.29	1.73 [g]	3.4 [i]
Perfluorohexanoic acid	PFHxA	307-24-4	314	3.9×10^4 [a]	120	9.63×10^{-1}	-0.16	4.10 [d]	6.88	0.38	2.31 [g]	4.05 [i]
Perfluoroheptanoic acid	PFHpA	375-85-9	364	1.3×10^4 [a]	128	3.59	-0.15	4.58 [d]	6.98	1.05	2.87 [g]	3.5 [i]
Perfluorooctanoic acid	PFOA	335-67-1	414	4,300 [b]	12	1.16	3.8	5.10 [d]	8.42	2.25	3.51 [g]	4.14 [i]
Perfluorononanoic acid	PFNA	375-95-1	464	14.6 [a]	3.47	1.10	-0.17	5.56 [d]	8.90	2.46	4.04 [g]	4.05 [i]
Perfluorodecanoic acid	PFDA	335-76-2	514	471 [a]	1.01	1.10	-0.17	5.99 [d]	9.33	2.89	4.63 [g]	3.86 [i]
Perfluoroundecanoic acid	PFUnA	2058-94-8	564	156 [a]	0.26	9.29	-0.17	6.47 [d]	9.89	3.37	4.65 [h]	3.7 [i]
Perfluorododecanoic acid	PFDoA	307-55-1	614	51.6 [a]	0.085	1.01	-0.17	6.93 [d]	10.3	3.83	5.12 [h]	3.3 [i]
Perfluorotridecanoic acid	PFTrA	72629-94-8	664	16.7 [a]	0.015	6.15	-0.17	7.28 [d]	10.9	4.18	5.47 [h]	4.8 [j]
Perfluorotetradecanoic acid	PFTeA	376-06-7	714	5.54 [a]	0.01	1.29	-0.17	7.76 [d]	11.0	4.66	5.96 [h]	5.1 [j]
Perfluorooctane sulfonamide	PFOSA	754-91-6	499	0.24 [a]	0.079	165	6.24	5.80 [a]	6.97	4.97	5.17 [h]	4.0 [j]
N-Methyl perfluorooctane sulfonamide	MeFOSA	31506-32-8	514	2.6×10^{-3} [a]	35.7	7.05	6.24	6.27 [a]	2.81	5.44	5.65 [h]	4.3 [j]
N-Ethyl perfluorooctane sulfonamide	EtFOSA	4151-50-2	527	8.1×10^{-3} [a]	5.70×10^{-5}	37.1	6.24	6.76 [a]	8.58	5.93	6.14 [h]	4.5 [j]
N-Methyl perfluorooctane sulfonamidoethanol	MeFOSE	24448-09-7	557	0.72 [a]	0.002	1.54	6.24	5.51 [a]	8.71	4.68	4.88 [h]	3.8 [j]
N-Ethyl perfluorooctane sulfonamidoethanol	EtFOSE	1691-99-2	571	0.15 [a]	0.50	1,920	6.24	6.0 [a]	6.10	5.17	5.37 [h]	4.1 [j]
N-Methyl perfluorooctane sulfonamidoethyl acrylate	MeFOSEA	25268-77-3	611	1.9×10^{-4} [a]	0.03	9.65×10^4	6.24	6.87 [a]	5.27	6.04	6.25 [h]	4.6 [j]
N-Ethyl perfluorooctane sulfonamidoethyl acrylate	EtFOSEA	423-82-5	625	0.89 [a]	0.002	1.41	6.24	7.36 [a]	10.6	6.53	6.75 [h]	4.9 [j]
2-(N-Methylperfluorooctanesulfonamido) Acetic Acid	MeFOSAA	2355-31-9	571	0.05 [a]	4.3×10^{-3}	49.1	1.1	5.4 [a]	7.10	2.30	3.57 [h]	3.8 [j]
2-(N-Ethylperfluorooctanesulfonamido) Acetic Acid	EtFOSAA	2991-50-6	585	2.4×10^{-3} [a]	4.3×10^{-3}	1,050	1.21	5.14 [a]	5.51	2.04	3.31 [h]	3.6 [j]

6:2 Fluorotelomer sulfonic acid	6:2 FTS	27619-97-2	428	10.97 [a]	0.11	70.93	4.5	2.66 [a]	4.20	0.26	0.92	2.2 [j]
8:2 Fluorotelomer sulfonic acid	8:2 FTS	39108-34-4	528	0.18 [a]	0.06	1,960	4.5	4.00 [a]	4.09	1.60	2.28	3.0 [j]

[a] Estimated value obtained from EPI Suite physicochemical property estimation software Version 4.1.
[b] Measured value obtained from TOXNET HSDB, U.S. National Library of Medicine (https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm)
[c] Estimated value obtained from SPARC physicochemical property estimation software (http://www.archemcalc.com/sparc.html)
[d] Estimated value obtained from COSMOtherm physicochemical property software, COSMOtherm– 2011 (C2.1 release 01.10) as reported in Armitage et al., 2013 and Wang et al., 2011. $K_{OW,N}$ values shown are on a "wet octanol basis". These values were determined using the COSMOtherm predicted values generated on a "dry octanol" following the approach employed by Armitage et al., 2013.
[e] Octanol-air partition coefficient of neutral species ($K_{OA,N}$) calculated as $K_{OW,N} \div K_{AW,N,}$ where $K_{AW,N}$ is determined as HN/RT, where R is the gas constant, 8.314 $Pa \cdot m^3 \cdot K^{-1} \cdot mol^{-1}$ and T is temperature in Kelvin. All values calculated at 20 °C (293 K).
[f] Octanol-water distribution coefficient (D_{ow}) at pH 7 was determined as D_{ow} (pH 7)= $f_N \times K_{OW,N} + f_I \times K_{OW,I}$, where f_N and f_I are fraction of neutral and ionic species at pH 7, respectively, as predicted from the Henderson Hasselbach equation; $K_{OW,N}$ and $K_{OW,I}$ are the octanol-water partition coefficients (K_{ow}) of the neutral and ionic species, respectively. $K_{OW,I}$ was determined by assuming $K_{OW,N}$ was approximately 3 log units higher (i.e., $\Delta_{OW} = 3.1$).
[g] Measured membrane-water distribution coefficient (log D_{MW}) value (pH 7) using solid supported lipid membranes (Droge, 2019).
[h] Estimated D_{MW} value (pH 7) determined following the approach outlined by Escher et al., 2009. Specifically, the liposome-water partition coefficient was determined as Log $K_{lipw} = 0.90 \times$ Log $K_{OW} + 0.52$. Subsequently, D_{MW} was determined as, D_{MW} (pH 7) = $f_N \times K_{lipw,N} + f_I \times K_{lipw,N} \times 0.1$ where f_N is the fraction of chemical in neutral form and f_I is the fraction of chemical in charged form at pH 7, as predicted by the Henderson-Hasselbalch equation.
[i] Measured protein-water partition coefficient (K_{PW}) value, as reported by Bischel et al., 2011.
[j] Estimated protein-water partition coefficient (K_{PW}) value, determined using the regression equation, Log $K_{PW} = 0.57 \times$ Log $K_{ow} + 0.69$, presented by Debruyn and Gobas, 2007.

Appendix II. List of threatened and endangered (T&E) species at 14 select DoD installations (See Table 2-1 for DoD installation names and locations).

DoD Installation	Group	Common Name	Scientific Name	Status
1	Flowering Plant	Small whorled pogonia	*Isotria medeoloides*	Threatened
2	Fish	Pallid sturgeon	*Scaphirhynchus albus*	Endangered
3	Flowering Plant	Pitcher's thistle	*Cirsium pitcheri*	Threatened
	Reptile	Eastern Massasauga (=rattlesnake)	*Sistrurus catenatus*	Threatened
4	-	-	-	-
5	Bird	Mexican spotted owl	*Strix occidentalis lucida*	Threatened
	Fish	Greenback Cutthroat trout	*Oncorhynchus clarkii stomias*	Threatened
	Flowering Plant	Ute ladies'-tresses	*Spiranthes diluvialis*	Threatened
	Flowering Plant	Western prairie fringed Orchid	*Platanthera praeclara*	Threatened
	Insect	Pawnee montane skipper	*Hesperia leonardus montana*	Threatened
	Mammal	Preble's meadow jumping mouse	*Zapus hudsonius preblei*	Threatened
6	Flowering Plant	Knieskern's Beaked-rush	*Rhynchospora knieskernii*	Threatened
	Flowering Plant	Sensitive joint-vetch	*Aeschynomene virginica*	Threatened
	Flowering Plant	Swamp pink	*Helonias bullata*	Threatened
	Flowering Plant	American chaffseed	*Schwalbea americana*	Endangered
	Reptile	Bog turtle	*Clemmys muhlenbergii*	Threatened
7	Amphibian	Arroyo (=arroyo southwestern) toad	*Anaxyrus californicus*	Endangered
	Bird	Yuma clapper rail	*Rallus longirostris yumanensis*	Endangered
	Bird	Light-footed clapper rail	*Rallus longirostris levipes*	Endangered
	Bird	Least Bell's vireo	*Vireo bellii pusillus*	Endangered
	Bird	Western snowy plover	*Charadrius nivosus nivosus*	Threatened
	Bird	Coastal California gnatcatcher	*Polioptila californica californica*	Threatened
	Bird	Southwestern willow flycatcher	*Empidonax traillii extimus*	Endangered
	Crustacean	Riverside fairy shrimp	*Streptocephalus woottoni*	Endangered

Crustacean	Vernal pool fairy shrimp	*Branchinecta lynchi*	Threatened
Crustacean	San Diego fairy shrimp	*Branchinecta sandiegonensis*	Endangered
Fish	Unarmored threespine stickleback	*Gasterosteus aculeatus williamsoni*	Endangered
Fish	Desert pupfish	*Cyprinodon macularius*	Endangered
Fish	Tidewater goby	*Eucyclogobius newberryi*	Endangered
Flowering Plant	San Diego thornmint	*Acanthomintha ilicifolia*	Threatened
Flowering Plant	San Diego ambrosia	*Ambrosia pumila*	Endangered
Flowering Plant	Del Mar manzanita	*Arctostaphylos glandulosa ssp. crassifolia*	Endangered
Flowering Plant	Coastal dunes milk-vetch	*Astragalus tener var. titi*	Endangered
Flowering Plant	Nevin's barberry	*Berberis nevinii*	Endangered
Flowering Plant	Thread-leaved brodiaea	*Brodiaea filifolia*	Threatened
Flowering Plant	Orcutt's spineflower	*Chorizanthe orcuttiana*	Endangered
Flowering Plant	Otay tarplant	*Deinandra (=Hemizonia) conjugens*	Threatened
Flowering Plant	Willowy monardella	*Monardella viminea*	Endangered
Flowering Plant	San Bernardino bluegrass	*Poa atropurpurea*	Endangered
Flowering Plant	Salt marsh bird's-beak	*Cordylanthus maritimus ssp. maritimus*	Endangered
Flowering Plant	San Diego button-celery	*Eryngium aristulatum var. parishii*	Endangered
Flowering Plant	California Orcutt grass	*Orcuttia californica*	Endangered
Flowering Plant	San Diego mesa-mint	*Pogogyne abramsii*	Endangered
Flowering Plant	Encinitas baccharis	*Baccharis vanessae*	Threatened
Flowering Plant	Spreading navarretia	*Navarretia fossalis*	Threatened
Flowering Plant	Otay mesa-mint	*Pogogyne nudiuscula*	Endangered
Flowering Plant	Mexican flannelbush	*Fremontodendron mexicanum*	Endangered
Flowering Plant	Slender-horned spineflower	*Dodecahema leptoceras*	Endangered
Flowering Plant	Gambel's watercress	*Rorippa gambellii*	Endangered
Flowering Plant	Vail Lake ceanothus	*Ceanothus ophiochilus*	Threatened
Insect	Quino checkerspot butterfly	*Euphydryas editha quino (=E. e. wrighti)*	Endangered
Insect	Laguna Mountains skipper	*Pyrgus ruralis lagunae*	Endangered
Mammal	Stephens' kangaroo rat	*Dipodomys stephensi (incl. D. cascus)*	Endangered

	Mammal	Pacific pocket mouse	*Perognathus longimembris pacificus*	Endangered
	Mammal	Peninsular bighorn sheep	*Ovis canadensis nelsoni*	Endangered
	Mammal	San Bernardino Merriam's kangaroo rat	*Dipodomys merriami parvus*	Endangered
	Reptile	Desert tortoise	*Gopherus agassizii*	Threatened
8	Bird	Hawaiian (=koloa) Duck	*Anas wyvilliana*	Endangered
	Bird	Laysan duck	*Anas laysanensis*	Endangered
	Bird	Laysan finch (honeycreeper)	*Telespyza cantans*	Endangered
	Bird	Nihoa finch (honeycreeper)	*Telespyza ultima*	Endangered
	Bird	Nihoa millerbird (old world warbler)	*Acrocephalus familiaris kingi*	Endangered
	Bird	Hawaiian common gallinule	*Gallinula galeata sandvicensis*	Endangered
	Bird	Short-tailed albatross	*Phoebastria (=Diomedea) albatrus*	Endangered
	Bird	Hawaii akepa	*Loxops coccineus*	Endangered
	Bird	Oahu creeper	*Paroreomyza maculata*	Endangered
	Bird	Hawaiian stilt	*Himantopus mexicanus knudseni*	Endangered
	Bird	Hawaiian coot	*Fulica americana alai*	Endangered
	Bird	Newell's Townsend's shearwater	*Puffinus auricularis newelli*	Threatened
	Bird	Oahu elepaio	*Chasiempis ibidis*	Endangered
	Fern and Ally	Pendant kihi fern	*Adenophorus periens*	Endangered
	Fern and Ally	Asplenium-leaved diellia	*Asplenium dielerectum*	Endangered
	Fern and Ally	No common name	*Asplenium dielfalcatum*	Endangered
	Fern and Ally	No common name	*Diplazium molokaiense*	Endangered
	Fern and Ally	Ihi`ihi	*Marsilea villosa*	Endangered
	Fern and Ally	No common name	*Pteris lidgatei*	Endangered
	Fern and Ally	Pauoa	*Ctenitis squamigera*	Endangered
	Fern and Ally	Wawae`iole	*Huperzia nutans*	Endangered
	Fern and Ally	No common name	*Asplenium unisorum*	Endangered
	Fern and Ally	No common name	*Doryopteris takeuchii*	Endangered
	Fern and Ally	No common name	*Microlepia strigosa var. mauiensis*	Endangered
	Fern and Ally	Kupukupu makalii	*Cyclosorus boydiae*	Endangered

Flowering Plant	No common name	*Amaranthus brownii*	Endangered
Flowering Plant	Uhi uhi	*Mezoneuron kavaiense*	Endangered
Flowering Plant	Haha	*Cyanea humboldtiana*	Endangered
Flowering Plant	Haha	*Cyanea lanceolata*	Endangered
Flowering Plant	Ha`iwale	*Cyrtandra dentata*	Endangered
Flowering Plant	Ha`iwale	*Cyrtandra kaulantha*	Endangered
Flowering Plant	Haiwale	*Cyrtandra waiolani*	Endangered
Flowering Plant	Fosberg's love grass	*Eragrostis fosbergii*	Endangered
Flowering Plant	`Akoko	*Euphorbia haeleeleana*	Endangered
Flowering Plant	Aupaka	*Isodendrion laurifolium*	Endangered
Flowering Plant	Aupaka	*Isodendrion longifolium*	Threatened
Flowering Plant	`Anaunau	*Lepidium arbuscula*	Endangered
Flowering Plant	No common name	*Lobelia koolauensis*	Endangered
Flowering Plant	Alani	*Melicope saint-johnii*	Endangered
Flowering Plant	`Aiea	*Nothocestrum latifolium*	Endangered
Flowering Plant	No common name	*Phyllostegia hirsuta*	Endangered
Flowering Plant	No common name	*Phyllostegia parviflora*	Endangered
Flowering Plant	Lo`ulu	*Pritchardia remota*	Endangered
Flowering Plant	Kaulu	*Pteralyxia macrocarpa*	Endangered
Flowering Plant	Haha	*Cyanea calycina*	Endangered
Flowering Plant	Haha	*Cyanea purpurellifolia*	Endangered
Flowering Plant	No common name	*Sanicula purpurea*	Endangered
Flowering Plant	No common name	*Schiedea hookeri*	Endangered
Flowering Plant	Ma`oli`oli	*Schiedea kealiae*	Endangered
Flowering Plant	No common name	*Stenogyne kaalae ssp. sherffii*	Endangered
Flowering Plant	Ko`oloa`ula	*Abutilon menziesii*	Endangered
Flowering Plant	No common name	*Abutilon sandwicense*	Endangered
Flowering Plant	Mahoe	*Alectryon macrococcus*	Endangered
Flowering Plant	No common name	*Schiedea obovata*	Endangered

Flowering Plant	No common name	*Schiedea trinervis*	Endangered
Flowering Plant	No common name	*Bonamia menziesii*	Endangered
Flowering Plant	Kamanomano	*Cenchrus agrimonioides*	Endangered
Flowering Plant	`Akoko	*Euphorbia celastroides var. kaenana*	Endangered
Flowering Plant	`Akoko	*Euphorbia deppeana*	Endangered
Flowering Plant	Ewa Plains `akoko	*Euphorbia skottsbergii var. skottsbergii*	Endangered
Flowering Plant	Kauila	*Colubrina oppositifolia*	Endangered
Flowering Plant	haha	*Cyanea crispa*	Endangered
Flowering Plant	Haha	*Cyanea grimesiana ssp. grimesiana*	Endangered
Flowering Plant	Haha	*Cyanea st.-johnii*	Endangered
Flowering Plant	Haha	*Cyanea superba*	Endangered
Flowering Plant	Ha`iwale	*Cyrtandra polyantha*	Endangered
Flowering Plant	Ha`iwale	*Cyrtandra subumbellata*	Endangered
Flowering Plant	Oha	*Delissea subcordata*	Endangered
Flowering Plant	Hawaiian gardenia (=Na`u)	*Gardenia brighamii*	Endangered
Flowering Plant	No common name	*Gouania meyenii*	Endangered
Flowering Plant	No common name	*Gouania vitifolia*	Endangered
Flowering Plant	Kio`ele	*Kadua coriacea*	Endangered
Flowering Plant	No common name	*Kadua degeneri*	Endangered
Flowering Plant	No common name	*Kadua parvula*	Endangered
Flowering Plant	No common name	*Hesperomannia arborescens*	Endangered
Flowering Plant	No common name	*Hesperomannia arbuscula*	Endangered
Flowering Plant	(=Native yellow hibiscus) ma`o hau hele	*Hibiscus brackenridgei*	Endangered
Flowering Plant	Kula wahine noho	*Isodendrion pyrifolium*	Endangered
Flowering Plant	`Ohe	*Joinvillea ascendens ascendens*	Endangered
Flowering Plant	Nehe	*Lipochaeta lobata var. leptophylla*	Endangered
Flowering Plant	No common name	*Lobelia niihauensis*	Endangered
Flowering Plant	No common name	*Lobelia oahuensis*	Endangered
Flowering Plant	Alani	*Melicope christophersenii*	Endangered

Flowering Plant	Alani	*Melicope lydgatei*	Endangered
Flowering Plant	Alani	*Melicope makahae*	Endangered
Flowering Plant	Alani	*Melicope pallida*	Endangered
Flowering Plant	No common name	*Neraudia angulata*	Endangered
Flowering Plant	Kulu`i	*Nototrichium humile*	Endangered
Flowering Plant	Makou	*Peucedanum sandwicense*	Threatened
Flowering Plant	Kuahiwi laukahi	*Plantago princeps*	Endangered
Flowering Plant	Dwarf naupaka	*Scaevola coriacea*	Endangered
Flowering Plant	Diamond Head schiedea	*Schiedea adamantis*	Endangered
Flowering Plant	No common name	*Schiedea kaalae*	Endangered
Flowering Plant	No common name	*Silene lanceolata*	Endangered
Flowering Plant	Popolo	*Solanum nelsonii*	Endangered
Flowering Plant	`Aiakeakua, popolo	*Solanum sandwicense*	Endangered
Flowering Plant	No common name	*Stenogyne kanehoana*	Endangered
Flowering Plant	No common name	*Tetramolopium filiforme*	Endangered
Flowering Plant	No common name	*Tetramolopium lepidotum ssp. lepidotum*	Endangered
Flowering Plant	`Ohe`ohe	*Polyscias gymnocarpa*	Endangered
Flowering Plant	Opuhe	*Urera kaalae*	Endangered
Flowering Plant	No common name	*Vigna o-wahuensis*	Endangered
Flowering Plant	Pamakani	*Viola chamissoniana ssp. chamissoniana*	Endangered
Flowering Plant	No common name	*Viola oahuensis*	Endangered
Flowering Plant	Round-leaved chaff-flower	*Achyranthes splendens var. rotundata*	Endangered
Flowering Plant	Haha	*Cyanea pinnatifida*	Endangered
Flowering Plant	Ha`iwale	*Cyrtandra crenata*	Endangered
Flowering Plant	Haiwale	*Cyrtandra gracilis*	Endangered
Flowering Plant	Hilo ischaemum	*Ischaemum byrone*	Endangered
Flowering Plant	Kamakahala	*Labordia cyrtandrae*	Endangered
Flowering Plant	Nehe	*Melanthera tenuifolia*	Endangered
Flowering Plant	Nehe	*Lipochaeta waimeaensis*	Endangered

Flowering Plant	No common name	*Lobelia monostachya*	Endangered
Flowering Plant	No common name	*Lysimachia filifolia*	Endangered
Flowering Plant	Alani	*Melicope hiiakae*	Endangered
Flowering Plant	No common name	*Phyllostegia mollis*	Endangered
Flowering Plant	No common name	*Platanthera holochila*	Endangered
Flowering Plant	Hala pepe	*Pleomele forbesii*	Endangered
Flowering Plant	Ohai	*Sesbania tomentosa*	Endangered
Flowering Plant	No common name	*Cyperus pennatiformis*	Endangered
Flowering Plant	Ko`oko`olau	*Bidens campylotheca ssp. pentamera*	Endangered
Flowering Plant	Haha	*Cyanea grimesiana ssp. obatae*	Endangered
Flowering Plant	Na`ena`e	*Dubautia herbstobatae*	Endangered
Flowering Plant	Kamapua`a	*Kadua fluviatilis*	Endangered
Flowering Plant	Lo`ulu	*Pritchardia kaalae*	Endangered
Flowering Plant	Makou	*Ranunculus mauiensis*	Endangered
Flowering Plant	Awiwi	*Schenkia sebaeoides*	Endangered
Flowering Plant	`Akoko	*Euphorbia kuwaleana*	Endangered
Flowering Plant	Haha	*Cyanea truncata*	Endangered
Flowering Plant	Pu`uka`a	*Cyperus trachysanthos*	Endangered
Flowering Plant	Ha`iwale	*Cyrtandra sessilis*	Endangered
Flowering Plant	Ha`iwale	*Cyrtandra viridiflora*	Endangered
Flowering Plant	Nioi	*Eugenia koolauensis*	Endangered
Flowering Plant	Mehamehame	*Flueggea neowawraea*	Endangered
Flowering Plant	Hulumoa	*Korthalsella degeneri*	Endangered
Flowering Plant	Kolea	*Myrsine juddii*	Endangered
Flowering Plant	No common name	*Platydesma cornuta var. cornuta*	Endangered
Flowering Plant	No common name	*Platydesma cornuta var. decurrens*	Endangered
Flowering Plant	Ihi	*Portulaca villosa*	Endangered
Flowering Plant	No common name	*Sanicula mariversa*	Endangered
Flowering Plant	No common name	*Schiedea nuttallii*	Endangered

Flowering Plant	No common name	*Silene perlmanii*	Endangered
Flowering Plant	No common name	*Spermolepis hawaiiensis*	Endangered
Flowering Plant	No common name	*Trematolobelia singularis*	Endangered
Flowering Plant	Haha	*Cyanea acuminata*	Endangered
Flowering Plant	`Akoko	*Euphorbia herbstii*	Endangered
Flowering Plant	`Akoko	*Euphorbia rockii*	Endangered
Flowering Plant	Haha	*Cyanea koolauensis*	Endangered
Flowering Plant	Haha	*Cyanea longiflora*	Endangered
Flowering Plant	Nanu	*Gardenia mannii*	Endangered
Flowering Plant	No common name	*Phyllostegia kaalaensis*	Endangered
Flowering Plant	Kopiko	*Psychotria hexandra ssp. oahuensis*	Endangered
Flowering Plant	A`e	*Zanthoxylum oahuense*	Endangered
Flowering Plant	Ko`oko`olau	*Bidens amplectens*	Endangered
Flowering Plant	Kolea	*Myrsine fosbergii*	Endangered
Flowering Plant	Baker"s Loulu	*Pritchardia bakeri*	Endangered
Flowering Plant	No common name	*Sicyos lanceoloideus*	Endangered
Insect	Blackline Hawaiian damselfly	*Megalagrion nigrohamatum nigrolineatum*	Endangered
Insect	Crimson Hawaiian damselfly	*Megalagrion leptodemas*	Endangered
Insect	Oceanic Hawaiian damselfly	*Megalagrion oceanicum*	Endangered
Insect	Orangeblack Hawaiian damselfly	*Megalagrion xanthomelas*	Endangered
Insect	Pacific Hawaiian damselfly	*Megalagrion pacificum*	Endangered
Insect	Anthricinan yellow-faced bee	*Hylaeus anthracinus*	Endangered
Insect	Easy yellow-faced bee	*Hylaeus facilis*	Endangered
Insect	Hawaiian yellow-faced bee	*Hylaeus longiceps*	Endangered
Insect	Hawaiian picture-wing fly	*Drosophila aglaia*	Endangered
Insect	Hawaiian picture-wing fly	*Drosophila sharpi*	Endangered
Insect	Hawaiian picture-wing fly	*Drosophila montgomeryi*	Endangered
Insect	Hawaiian picture-wing fly	*Drosophila obatai*	Endangered
Insect	Hawaiian picture-wing fly	*Drosophila substenoptera*	Endangered

	Insect	Hawaiian picture-wing fly	*Drosophila tarphytrichia*	Endangered
	Insect	Hawaiian picture-wing fly	*Drosophila hemipeza*	Endangered
	Insect	Hawaiian yellow-faced bee	*Hylaeus mana*	Endangered
	Insect	Hawaiian yellow-faced bee	*Hylaeus kuakea*	Endangered
	Mammal	Hawaiian hoary bat	*Lasiurus cinereus semotus*	Endangered
	Snail	Oahu tree snails	*Achatinella spp.*	Endangered
9	Clam	Round Ebonyshell	*Fusconaia rotulata*	Endangered
	Clam	Southern kidneyshell	*Ptychobranchus jonesi*	Endangered
	Mammal	Perdido Key beach mouse	*Peromyscus polionotus trissyllepsis*	Endangered
10	Bird	Cape Sable seaside sparrow	*Ammodramus maritimus mirabilis*	Endangered
	Bird	Bachman's warbler (=wood)	*Vermivora bachmanii*	Endangered
	Fern and Ally	Florida bristle fern	*Trichomanes punctatum ssp. floridanum*	Endangered
	Flowering Plant	Blodgett's silverbush	*Argythamnia blodgettii*	Threatened
	Flowering Plant	Florida brickell-bush	*Brickellia mosieri*	Endangered
	Flowering Plant	Sand flax	*Linum arenicola*	Endangered
	Flowering Plant	Carter's small-flowered flax	*Linum carteri carteri*	Endangered
	Flowering Plant	Garber's spurge	*Chamaesyce garberi*	Threatened
	Flowering Plant	Florida pineland crabgrass	*Digitaria pauciflora*	Threatened
	Flowering Plant	Deltoid spurge	*Chamaesyce deltoidea ssp. deltoidea*	Endangered
	Flowering Plant	Okeechobee gourd	*Cucurbita okeechobeensis ssp. okeechobeensis*	Endangered
	Flowering Plant	Beach jacquemontia	*Jacquemontia reclinata*	Endangered
	Flowering Plant	Tiny polygala	*Polygala smallii*	Endangered
	Flowering Plant	Crenulate lead-plant	*Amorpha crenulata*	Endangered
	Flowering Plant	Small's milkpea	*Galactia smallii*	Endangered
	Flowering Plant	Pineland sandmat	*Chamaesyce deltoidea pinetorum*	Threatened
	Flowering Plant	Cape Sable Thoroughwort	*Chromolaena frustrata*	Endangered
	Flowering Plant	Florida prairie-clover	*Dalea carthagenensis floridana*	Endangered
	Flowering Plant	Florida semaphore Cactus	*Consolea corallicola*	Endangered
	Flowering Plant	Everglades bully	*Sideroxylon reclinatum ssp. austrofloridense*	Threatened

	Insect	Schaus swallowtail butterfly	*Heraclides aristodemus ponceanus*	Endangered
	Insect	Miami Blue Butterfly	*Cyclargus (=Hemiargus) thomasi bethunebakeri*	Endangered
	Insect	Bartram's hairstreak Butterfly	*Strymon acis bartrami*	Endangered
	Insect	Florida leafwing Butterfly	*Anaea troglodyta floridalis*	Endangered
	Reptile	American crocodile	*Crocodylus acutus*	Threatened
	Snail	Stock Island tree snail	*Orthalicus reses (not incl. nesodryas)*	Threatened
11	Fish	Okaloosa darter	*Etheostoma okaloosae*	Threatened
	Mammal	Choctawhatchee beach mouse	*Peromyscus polionotus allophrys*	Endangered
12	Flowering Plant	Short-leaved rosemary	*Conradina brevifolia*	Endangered
	Flowering Plant	Scrub mint	*Dicerandra frutescens*	Endangered
	Flowering Plant	Highlands scrub hypericum	*Hypericum cumulicola*	Endangered
	Flowering Plant	Scrub blazingstar	*Liatris ohlingerae*	Endangered
	Flowering Plant	Papery whitlow-wort	*Paronychia chartacea*	Threatened
	Flowering Plant	Lewton's polygala	*Polygala lewtonii*	Endangered
	Flowering Plant	Wireweed	*Polygonella basiramia*	Endangered
	Flowering Plant	Sandlace	*Polygonella myriophylla*	Endangered
	Flowering Plant	Scrub plum	*Prunus geniculata*	Endangered
	Flowering Plant	Florida bonamia	*Bonamia grandiflora*	Threatened
	Flowering Plant	Pygmy fringe-tree	*Chionanthus pygmaeus*	Endangered
	Flowering Plant	Pigeon wings	*Clitoria fragrans*	Threatened
	Flowering Plant	Scrub buckwheat	*Eriogonum longifolium var. gnaphalifolium*	Threatened
	Flowering Plant	Snakeroot	*Eryngium cuneifolium*	Endangered
	Flowering Plant	Britton's beargrass	*Nolina brittoniana*	Endangered
	Flowering Plant	Garrett's mint	*Dicerandra christmanii*	Endangered
	Flowering Plant	Florida ziziphus	*Ziziphus celata*	Endangered
	Flowering Plant	Avon Park harebells	*Crotalaria avonensis*	Endangered
	Reptile	Bluetail mole skink	*Eumeces egregius lividus*	Threatened
	Reptile	Sand skink	*Neoseps reynoldsi*	Threatened

13	-	-	-	-
14	Amphibian	Oregon spotted frog	*Rana pretiosa*	Threatened
	Flowering Plant	Marsh Sandwort	*Arenaria paludicola*	Endangered
	Flowering Plant	Water howellia	*Howellia aquatilis*	Threatened
	Mammal	Gray wolf	*Canis lupus*	Endangered
	Mammal	Canada Lynx	*Lynx canadensis*	Threatened
	Mammal	Roy Prairie pocket gopher	*Thomomys mazama glacialis*	Threatened
	Mammal	Olympia pocket gopher	*Thomomys mazama pugetensis*	Threatened
	Mammal	Tenino pocket gopher	*Thomomys mazama tumuli*	Threatened
	Mammal	Yelm pocket gopher	*Thomomys mazama yelmensis*	Threatened
Shared:				
9, 11	Amphibian	Reticulated flatwoods salamander	*Ambystoma bishopi*	Endangered
1, 4, 9, 10, 11	Bird	Piping Plover	*Charadrius melodus*	Threatened
1, 4	Bird	Roseate tern	*Sterna dougallii dougallii*	Endangered
1, 3, 4, 6, 9, 10, 11	Bird	Red knot	*Calidris canutus rufa*	Threatened
2, 10, 11, 12	Bird	Red-cockaded woodpecker	*Picoides borealis*	Endangered
2, 7	Bird	Least tern	*Sterna antillarum*	Endangered
3, 10	Bird	Kirtland's Warbler	*Setophaga kirtlandii (= Dendroica kirtlandii)*	Endangered
9, 10, 11, 12	Bird	Wood stork	*Mycteria americana*	Threatened
10, 12	Bird	Everglade snail kite	*Rostrhamus sociabilis plumbeus*	Endangered
10, 12	Bird	Audubon's crested caracara	*Polyborus plancus audubonii*	Threatened
10, 12	Bird	Florida grasshopper sparrow	*Ammodramus savannarum floridanus*	Endangered
10, 12	Bird	Florida scrub-jay	*Aphelocoma coerulescens*	Threatened
13, 14	Bird	Yellow-billed Cuckoo	*Coccyzus americanus*	Threatened
13, 14	Bird	Northern spotted owl	*Strix occidentalis caurina*	Threatened
13, 14	Bird	Marbled murrelet	*Brachyramphus marmoratus*	Threatened
13, 14	Bird	Streaked Horned lark	*Eremophila alpestris strigata*	Threatened
9, 11	Clam	Choctaw bean	*Villosa choctawensis*	Endangered
9, 11	Clam	Narrow pigtoe	*Fusconaia escambia*	Threatened

9, 11	Clam	Southern sandshell	*Hamiota australis*	Threatened
9, 11	Clam	Fuzzy pigtoe	*Pleurobema strodeanum*	Threatened
9, 11	Fish	Atlantic sturgeon (Gulf subspecies)	*Acipenser oxyrinchus (=oxyrhynchus) desotoi*	Threatened
13, 14	Fish	Bull Trout	*Salvelinus confluentus*	Threatened
10, 12	Flowering Plant	Carter's mustard	*Warea carteri*	Endangered
13, 14	Flowering Plant	Golden paintbrush	*Castilleja levisecta*	Threatened
13, 14	Insect	Taylor's (=whulge) Checkerspot	*Euphydryas editha taylori*	Endangered
11, 12	Lichen	Florida perforate cladonia	*Cladonia perforata*	Endangered
1, 2, 3, 4, 6	Mammal	Northern Long-Eared Bat	*Myotis septentrionalis*	Threatened
9, 10, 11, 12	Mammal	West Indian Manatee	*Trichechus manatus*	Threatened
10, 12	Mammal	Florida panther	*Puma (=Felis) concolor coryi*	Endangered
10, 12	Mammal	Florida bonneted bat	*Eumops floridanus*	Endangered
1, 4, 8, 9, 10, 11	Reptile	Hawksbill sea turtle	*Eretmochelys imbricata*	Endangered
1, 4, 7, 8, 9, 10, 11, 13	Reptile	Leatherback sea turtle	*Dermochelys coriacea*	Endangered
4, 9, 11	Reptile	Kemp's ridley sea turtle	*Lepidochelys kempii*	Endangered
4, 9 ,11	Reptile	Green sea turtle	*Chelonia mydas*	Threatened
4, 9, 10, 11	Reptile	Loggerhead sea turtle	*Caretta caretta*	Threatened
7, 8	Reptile	Olive ridley sea turtle	*Lepidochelys olivacea*	Threatened
9, 10, 11, 12	Reptile	Eastern indigo snake	*Drymarchon corais couperi*	Threatened

Appendix III. PFAS bioaccumulation model equations.

Activity-Concentration Relationship:

$$a_B = \frac{C_B}{S_B} \quad (1)$$

Time dependent mass of chemical in the organism

$$\frac{dM_B}{dt} = [W_B(k_{R1}C_R + k_D C_D)] - (k_{R2}+k_F+k_U + k_{BILE} + k_L + k_M + k_{GROWTH})M_B \quad (2)$$

Time dependent concentration in the organism

$$\frac{dC_B}{dt} = k_{R1}C_R + k_D C_D - (k_{R2}+k_F+k_U + k_{BILE} + k_L + k_M + k_{GROWTH})M_B \quad (3)$$

Steady-state concentration in the organism

$$C_B = k_{R1}C_R + k_D C_D - (k_{R2}+k_F+k_U + k_{BILE} + k_L + k_M + k_{GROWTH})M_B) \quad (4)$$

Solubilities (S, mol/m^3) and distribution coefficients (D, unitless) used to derive rate constant values:

$$D_{BA} = \frac{S_B}{S_A} = \frac{C_B}{C_A} = \nu_{NL,B}\, D_{OA} + \nu_{PL,B}\, D_{MA} + \nu_{TP,B}\, D_{TP,A} + \nu_{SP,B}\, D_{SP,A} + \nu_{C,B}\, D_{CA} + \frac{\nu_{WB}}{D_{AW}} \quad (5)$$

$$D_{BW} = \frac{S_B}{S_W} = \frac{C_B}{C_{WD}} = \nu_{NL,B}\, D_{OW} + \nu_{PL,B}\, D_{MW} + \nu_{TP,B}\, D_{TP,W} + \nu_{SP,B}\, D_{SP,W} + \nu_{C,B}\, D_{CW} + \nu_{WB} \quad (6)$$

$$D_{BG} = \frac{S_B}{S_G} = \frac{C_B}{C_G} = \frac{\left(\nu_{NL,B}\, D_{OW} + \nu_{PL,B}\, D_{MW} + \nu_{TP,B}\, D_{TP,W} + \nu_{SP,B}\, D_{SP,W} + \nu_{C,B}\, D_{CW} + \nu_{WB}\right)}{\left(\nu_{NL,G}\, D_{OW} + \nu_{PL,G}\, D_{MW} + \nu_{TP,G} D_{TP,W} + \nu_{SP,G} D_{SP,W} + \nu_{C,G}\, D_{CW} + \nu_{WB}\right)} \quad (7)$$

and

$$D_{DG} = \frac{S_D}{S_G} = \frac{C_D}{C_G} = \frac{\left(\nu_{NL,D}\, D_{OW} + \nu_{PL,D}\, D_{MW} + \nu_{TP,BD} D_{TP,W} + \nu_{SP,D}\, D_{SP,W} + \nu_{C,D}\, D_{CW} + \nu_{WD}\right)}{\left(\nu_{NL,G}\, D_{OW} + \nu_{PL,G}\, D_{MW} + \nu_{TP,G} D_{TP,W} + \nu_{SP,G} D_{SP,W} + \nu_{C,G}\, D_{CW} + \nu_{WB}\right)} \quad (8)$$

where

$$\nu_{NL,G} = \frac{(1-\varepsilon_{NL}) \times \nu_{NL,D}}{\left[(1-\varepsilon_{NL}) \times \nu_{NL,D}\right] + \left[(1-\varepsilon_{PL}) \times \nu_{PL,D}\right] + \left[(1-\varepsilon_{TP}) \times \nu_{TP,D}\right] + \left[(1-\varepsilon_{SP}) \times \nu_{SP,D}\right] + \left[(1-\varepsilon_{C}) \times \nu_{CD}\right] + \left[(1-\varepsilon_{W}) \times \nu_{WD}\right]} \quad (9)$$

$$\nu_{PL,G} = \frac{(1-\varepsilon_{PL}) \times \nu_{PL,D}}{\left[(1-\varepsilon_{NL}) \times \nu_{NL,D}\right] + \left[(1-\varepsilon_{PL}) \times \nu_{PL,D}\right] + \left[(1-\varepsilon_{TP}) \times \nu_{TP,D}\right] + \left[(1-\varepsilon_{SP}) \times \nu_{SP,D}\right] + \left[(1-\varepsilon_{C}) \times \nu_{CD}\right] + \left[(1-\varepsilon_{W}) \times \nu_{WD}\right]} \quad (10)$$

$$v_{TP,G} = \frac{(1-\varepsilon_{TP}) \times v_{TP,D}}{\left[(1-\varepsilon_{NL}) \times v_{NL,D}\right] + \left[(1-\varepsilon_{PL}) \times v_{PL,D}\right] + \left[(1-\varepsilon_{TP}) \times v_{TP,D}\right] + \left[(1-\varepsilon_{SP}) \times v_{SP,D}\right] + \left[(1-\varepsilon_{C}) \times v_{CD}\right] + \left[(1-\varepsilon_{W}) \times v_{WD}\right]} \quad (11)$$

$$v_{SP,G} = \frac{(1-\varepsilon_{SP}) \times v_{SP,D}}{\left[(1-\varepsilon_{NL}) \times v_{NL,D}\right] + \left[(1-\varepsilon_{PL}) \times v_{PL,D}\right] + \left[(1-\varepsilon_{TP}) \times v_{TP,D}\right] + \left[(1-\varepsilon_{SP}) \times v_{SP,D}\right] + \left[(1-\varepsilon_{C}) \times v_{CD}\right] + \left[(1-\varepsilon_{W}) \times v_{WD}\right]} \quad (12)$$

$$v_{CG} = \frac{(1-\varepsilon_{C}) \times v_{CD}}{\left[(1-\varepsilon_{NL}) \times v_{NL,D}\right] + \left[(1-\varepsilon_{PL}) \times v_{PL,D}\right] + \left[(1-\varepsilon_{TP}) \times v_{TP,D}\right] + \left[(1-\varepsilon_{SP}) \times v_{SP,D}\right] + \left[(1-\varepsilon_{C}) \times v_{CD}\right] + \left[(1-\varepsilon_{W}) \times v_{WD}\right]} \quad (13)$$

$$v_{WG} = \frac{(1-\varepsilon_{W}) \times v_{WD}}{\left[(1-\varepsilon_{NL}) \times v_{NL,D}\right] + \left[(1-\varepsilon_{PL}) \times v_{PL,D}\right] + \left[(1-\varepsilon_{TP}) \times v_{TP,D}\right] + \left[(1-\varepsilon_{SP}) \times v_{SP,D}\right] + \left[(1-\varepsilon_{C}) \times v_{CD}\right] + \left[(1-\varepsilon_{W}) \times v_{WD}\right]} \quad (14)$$

Uptake and elimination rate constants (*k*, d^{-1}):

$k_{R1} = \frac{G_W E_W}{V_B}$ (water-respiring organisms) *(15)*

$k_{R2} = \frac{k_{R1}}{D_{BW}}$ (water-respiring organisms) *(16)*

$k_{R1} = \frac{G_A E_A}{V_B}$ (air-breathing organisms *(17)*

$k_{R2} = \frac{k_{R1}}{D_{BA}}$ (air-breathing organisms) *(18)*

$k_D = \frac{G_F}{D_{BF} V_B}$ (all organisms) *(19)*

$k_U = \frac{G_U}{D_{BW} V_B}$ (select organisms) *(20)*

$k_{BILE} = \frac{G_{BILE}}{V_B D_{BW} / \beta}$ (select organisms) *(21)*

$k_L = \frac{G_L}{D_{BW} V_B}$ (lactating female mammals only) *(22)*

$k_M = \frac{\ln(2)}{t_{1/2}}$ (all organisms) *(23)*

$k_{GROWTH} = \frac{dW_B / d_t}{W_B}$ (all organisms) *(24)*

Appendix IV. Definitions and units of various parameters used in the PFAS food web bioaccumulation model.

Symbol	Parameter Definition	Units
a_B	Chemical activity in organism	unitless
W_B	Organism Weight	kg
C_B	Organism Chemical Concentration	mol/m^3
S_B	Chemical Solubility in Organism	mol/m^3
C_D	Organism Diet Chemical Concentration	mol/m^3
C_R	Dissolved Concentration measured in respiratory medium	mol/m^3
D_{OW}	Octanol-Water Distribution Coefficient	unitless
D_{OA}	Octanol-Air Distribution Coefficient	unitless
D_{AW}	Air-Water Distribution Coefficient	unitless
$D_{TP,W}$	Transporter Protein-Water Distribution Coefficient	unitless
$D_{TP,A}$	Transporter Protein-Air Distribution Coefficient	unitless
$D_{SP,W}$	Structural Protein-Water Distribution Coefficient	unitless
$D_{SP,A}$	Structural-Protein-Air Distribution Coefficient	unitless
$D_{NL,W}$	Neutral Lipid-Water Distribution Coefficient	unitless
$D_{NL,A}$	Neutral Lipid-Air Distribution Coefficient	unitless
$D_{PL,W}$	Phospholipid-Water Distribution Coefficient	unitless
$D_{PL,A}$	Phospholipid-Air Distribution Coefficient	unitless
D_{CW}	Carbohydrate-Water Distribution Coefficient	unitless
D_{CA}	Carbohydrate-Air Distribution Coefficient	unitless
D_{BW}	Biota-Water Distribution Coefficient	unitless
D_{BA}	Biota-Air Distribution Coefficient	unitless
D_{BG}	Biota-Gut Distribution Coefficient	unitless
D_{DG}	Diet-Gut Distribution Coefficient	unitless
$\nu_{NL,B}$	Neutral Lipid Composition in Biota	kg lipid/kg wet wt. organism
$\nu_{PL,B}$	Phospholipid Composition in Biota	kg lipid/kg wet wt. organism
$\nu_{TP,B}$	Transporter Protein Composition in Biota	kg protein/kg wet wt. organism
$\nu_{SP,B}$	Structural Protein Composition in Biota	kg protein/kg wet wt. organism
$\nu_{C,B}$	Carbohydrate Composition in Biota	kg protein/kg wet wt. organism
ν_{WB}	Water Composition in Biota	kg water/kg wet wt. organism

$\nu_{NL,D}$	Neutral Lipid Composition in Diet	kg lipid/kg wet wt. organism
$\nu_{PL,D}$	Phospholipid Composition in Diet	kg lipid/kg wet wt. organism
$\nu_{TP,D}$	Transporter Protein Composition in Diet	kg protein/kg wet wt. organism
$\nu_{SP,D}$	Structural Protein Composition in Diet	kg protein/kg wet wt. organism
$\nu_{C,D}$	Carbohydrate Composition in Diet	kg protein/kg wet wt. organism
ν_{WD}	Water Composition in Diet	kg water/kg wet wt. organism
$\nu_{NL,G}$	Neutral Lipid Composition in Gut Digesta	kg lipid/kg wet wt. organism
$\nu_{PL,G}$	Phospholipid Composition in Gut Digesta	kg lipid/kg wet wt. organism
$\nu_{TP,G}$	Transporter Protein Composition in Gut Digesta	kg protein/kg wet wt. organism
$\nu_{SP,G}$	Structural Protein Composition in Gut Digesta	kg protein/kg wet wt. organism
$\nu_{C,G}$	Carbohydrate Composition in Gut Digesta	kg protein/kg wet wt. organism
ν_{WG}	Water Composition in Gut Digesta	kg water/kg wet wt. organism
ε_{NL}	Neutral Lipid Extraction Efficiency	-
ε_{PL}	Phospholipid Extraction Efficiency	-
ε_{TP}	Transporter Protein Extraction Efficiency	-
ε_{SP}	Structural Protein Extraction Efficiency	-
ε_{W}	Carbohydrate Extraction Efficiency	-
ε_{W}	Water Extraction Efficiency	-
k_D	Dietary Uptake Rate Constant	d^{-1}
k_{R1}	Respiratory Uptake Rate Constant	d^{-1}
k_{R2}	Respiratory Elimination Rate Constant	d^{-1}
k_U	Urinary Excretion Rate Constant	d^{-1}
k_F	Faecal Egestion Rate Constant	d^{-1}
k_L	Lactation Rate Constant	d^{-1}
k_B	Bile Elimination Rate Constant	d^{-1}
k_M	Metabolism Rate Constant	d^{-1}
k_{GROWTH}	Growth Rate Constant	d^{-1}
B	Constant representing the degree to which bile fluids exceed the solubility of contaminants over that in water	unitless

Appendix V. Model parameter values used for simulation of PFOS bioaccumulation in aquatic and terrestrial food webs at DoD sites.

	Body Weight (kg) W_B	Body Volume (m^3) V_B	Body Composition (ν, unitless) ν_{NL}	ν_{PL}	ν_{TP}	ν_{SP}	ν_C	ν_W	Uptake and Elimination Rates (G, m^3/d) G_D	G_A, G_W	G_F	G_U	G_{BILE}	G_L
Terrestrial Food Web														
Plants	-	-	0.002	0.001	0.00	0.01	0.03	0.96	0	0	0	0	0	0
Herbivorous Insects	0.0025	2.50E-06	0.005	0.005	0.05	0.10	0	0.84	1.75E-07	5.43E-03	5.17E-08	0	0	0
Terrestrial Reptiles	5	5.00E-03	0.05	0.01	0.06	0.14	0	0.74	3.50E-04	1.62E+00	8.31E-05	0	0	0
Terrestrial Insectivorous Bird	0.025	2.50E-05	0.04	0.01	0.05	0.12	0	0.78	1.75E-06	3.05E-02	4.64E-07	0	0	0
Aquatic Insectivorous Bird	0.025	2.50E-05	0.04	0.01	0.05	0.12	0	0.78	1.75E-06	3.05E-02	4.53E-07	0	0	0
Piscivorous Bird	5	5.00E-03	0.1	0.01	0.06	0.12	0	0.71	3.50E-04	1.62E+00	8.75E-05	1.00E-06	2.80E-08	0
Terrestrial Insectivorous Mammal	1	1.00E-03	0.04	0.01	0.06	0.14	0	0.75	7.00E-05	4.86E-01	1.82E-05	1.00E-06	3.14E-06	0
Herbivorous Mammal	100	1.00E-01	0.04	0.01	0.06	0.14	0	0.75	7.00E-03	1.54E+01	2.04E-03	1.00E-03	1.54E-06	0
Terrestrial Carnivorous Mammal	60	6.00E-02	0.1	0.01	0.06	0.14	0	0.69	4.20E-03	1.05E+01	9.98E-04	1.00E-03	3.00E-06	0
Human	70	7.00E-02	0.1	0.01	0.06	0.14	0	0.69	4.90E-03	1.18E+01	1.16E-03	1.00E-03	0	0
Aquatic Food Web														
Phytoplankton	-	-	0.005	0.005	0.05	0.10	0.03	0.81	0	0	0	0	0	0
Benthic Invertebrate	5.7E-06	5.7E-09	0.005	0.005	0.05	0.10	0	0.84	1.39E-08	5.30E-05	4.16E-09	0	0	0
Zooplankton	5.7E-06	5.7E-09	0.005	0.005	0.05	0.10	0	0.84	1.39E-08	5.30E-05	3.90E-09	0	0	0
Bivalve	0.15	1.50E-04	0.005	0.005	0.05	0.10	0.02	0.82	1.04E-05	3.96E-02	2.91E-06	0	0	0
Benthic Fish	0.25	2.50E-04	0.04	0.01	0.06	0.14	0	0.75	1.44E-05	5.52E-02	4.06E-06	2.92E-04	0	0
Forage Fish	0.1	1.00E-04	0.04	0.01	0.06	0.14	0	0.75	7.96E-06	3.04E-02	2.26E-06	7.30E-04	0	0
Predatory Fish	1	1.00E-03	0.04	0.01	0.06	0.14	0	0.75	3.56E-05	1.36E-01	8.44E-06	7.30E-05	0	0
Aquatic Reptile	3	3.00E-03	0.04	0.01	0.06	0.14	0	0.78	7.26E-05	2.78E-01	1.95E-05	5.00E-06	0	0
Amphibian	1	1.00E-03	0.04	0.01	0.06	0.14	0	0.75	3.56E-05	1.36E-01	9.53E-06	1.00E-06	0	0
Amphibious Mammals	20	2.00E-02	0.04	0.01	0.06	0.14	0	0.75	2.49E-04	9.53E-01	9.97E-05	1.00E-04	1.54E-07	0

Gastrointestinal Extraction Efficiency (ε)

	ε_{NL}	ε_{PL}	ε_{TP}	ε_{SP}	ε_{C}	ε_{W}
Terrestrial Food Web						
Plants	0	0	0	0		0
Herbivorous Insects	0.8	0.8	0.8	0.8	0.8	0.7
Terrestrial Reptiles	0.95	0.95	0.95	0.95	0.95	0.7
Terrestrial Insectivorous Bird	0.95	0.95	0.95	0.9	0.9	0.7
Aquatic Insectivorous Bird	0.95	0.95	0.95	0.95	0.95	0.7
Piscivorous Bird	0.9	0.9	0.9	0.9	0.9	0.7
Terrestrial Insectivorous Mammal	0.95	0.95	0.95	0.95	0.95	0.7
Herbivorous Mammal	0.9	0.9	0.9	0.9	0.9	0.7
Terrestrial Carnivorous Mammal	0.95	0.95	0.95	0.95	0.95	0.7
Human	0.95	0.95	0.95	0.95	0.95	0.7
Aquatic Food Web						
Phytoplankton	-	-	-	-	-	-
Benthic Invertebrate	0.7	0.7	0.7	0.7	0.7	0.7
Zooplankton	0.8	0.8	0.8	0.8	0.8	0.7
Bivalve	0.8	0.8	0.8	0.8	0.8	0.7
Benthic Fish	0.8	0.8	0.8	0.8	0.8	0.7
Forage Fish	0.8	0.8	0.8	0.8	0.8	0.7
Predatory Fish	0.95	0.95	0.95	0.95	0.95	0.7
Aquatic Reptile	0.90	0.90	0.90	0.90	0.90	0.7
Amphibian	0.90	0.90	0.90	0.90	0.90	0.7
Amphibious Mammals	0.90	0.90	0.90	0.90	0.90	0.5

Appendix VI. Model input values used for simulation of food web bioaccumulation in aquatic and terrestrial organisms at select DoD sites.

	Former Wurtsmith AFB	Barksdale AFB	Former Pease AFB	Joint Base McGuire-Dix-Lakehurst	Peterson AFB
Surface Water Concentration (C_W, ng/L)	1,710 (140-20,954)	368 (83.1-1,630)	1,105 (273-4,462)	52.2 (17.2-159)	16.6 (2.1-133)
Sediment Concentration ($C_{SEDIMENT}$, ng/g dry weight)	4,280	1.4 (0.33-6.4)	2,779	8.5 (2.7-26.9)	0.5
Soil Concentration (C_{SOIL} ng/g dry weight)	0.24 (0.08-0.74)	0.24 (0.08-0.74)	0.24 (0.08-0.74)	0.24 (0.08-0.74)	0.24 (0.08-0.74)
Organic carbon content in Sediment ($f_{OC, SEDIMENT}$)	0.03	0.03	0.03	0.03	0.03
Organic carbon content in Soil ($f_{OC, SOIL}$)	0.05	0.05	0.05	0.05	0.05

Appendix VII. Model predicted PFOS activities in aquatic and terrestrial organisms (a_B, unitless) at select DoD sites.

	Former Wurtsmith AFB	Barksdale AFB	Former Pease AFB	Joint Base McGuire-Dix-Lakehurst	Peterson AFB
	Model Predicted PFOS activity in biota (*a*, unitless)				
Terrestrial Food Web					
Plants	2.3E-08 (7.6E-09-7.0E-08)	2.3E-08 (7.6E-09-7.0E-08)	2.3E-08 (7.6E-09-7.0E-08)	2.3E-08 (7.6E-09-7.0E-08)	1.1E-07 (3.8E-08-3.5E-07)
Herbivorous Insects	9.0E-08 (3.0E-08-2.8E-07)	9.0E-08 (3.0E-08-2.8E-07)	9.0E-08 (3.0E-08-2.8E-07)	9.0E-08 (3.0E-08-2.8E-07)	4.5E-07 (1.5E-07-1.4E-06)
Insectivorous Birds	1.7E-06 (5.5E-07-5.1E-06)	1.7E-06 (5.5E-07-5.1E-06)	1.7E-06 (5.5E-07-5.1E-06)	1.7E-06 (5.5E-07-5.1E-06)	8.3E-06 (2.8E-06-2.6E-05)
Insectivorous Mammals	1.7E-06 (5.6E-07-5.2E-06)	1.7E-06 (5.6E-07-5.2E-06)	1.7E-06 (5.6E-07-5.2E-06)	1.7E-06 (5.6E-07-5.2E-06)	8.5E-06 (2.8E-06-2.6E-05)
Terrestrial Reptiles	8.3E-06 (2.8E-06-2.6E-05)	8.3E-06 (2.8E-06-2.6E-05)	8.3E-06 (2.8E-06-2.6E-05)	8.3E-06 (2.8E-06-2.6E-05)	4.1E-05 (1.4E-05-1.3E-04)
Herbivorous Mammals	5.9E-09 (2.0E-09-1.8E-08)	5.9E-09 (2.0E-09-1.8E-08)	5.9E-09 (2.0E-09-1.8E-08)	5.9E-09 (2.0E-09-1.8E-08)	9.4E-07 (3.1E-07-2.9E-06)
Terrestrial Carnivorous Mammals	1.0E-07 (3.5E-08-3.2E-07)	1.0E-07 (3.5E-08-3.2E-07)	1.0E-07 (3.5E-08-3.2E-07)	1.0E-07 (3.5E-08-3.2E-07)	1.7E-05 (5.6E-06-5.2E-05)
Human	2.7E-05 (2.3E-06-3.4E-04)	5.9E-06 (1.3E-06-2.6E-05)	1.8E-05 (4.4E-06-7.2E-05)	8.9E-07 (2.9E-07-2.7E-06)	3.2E-07 (5.2E-08-2.3E-06)
Aquatic Food Web					
Phytoplankton	2.5E-06 (2.1E-07-3.1E-05)	5.4E-07 (1.2E-07-2.4E-06)	1.6E-06 (4.0E-07-6.6E-06)	7.7E-08 (2.5E-08-2.3E-07)	2.4E-08 (3.1E-09-2.0E-07)
Benthic Invertebrates	2.0E-03 (-)	6.6E-07 (1.6E-07-3.0E-06)	1.3E-03 (-)	4.0E-06 (1.3E-06-1.3E-05)	2.4E-07 (-)

Zooplankton	2.8E-06 (2.3E-07-3.5E-05)	6.1E-07 (1.4E-07-2.7E-06)	1.8E-06 (4.5E-07-7.4E-06)	8.7E-08 (2.9E-08-2.6E-07)	2.8E-08 (3.5E-09-2.2E-07)
Bivalves	2.8E-06 (2.3E-07-3.4E-05)	6.0E-07 (1.4E-07-2.6E-06)	1.8E-06 (4.4E-07-7.2E-06)	8.5E-08 (2.8E-08-2.6E-07)	2.7E-08 (3.4E-09)
Benthic Fish	1.7E-04 (1.6E-04-1.9E-04)	5.8E-07 (1.3E-07-2.6E-06)	1.1E-04 (-)	4.0E-07 (1.3E-07-1.3E-06)	4.3E-08 (2.2E-08-2.1E-07)
Mid Trophic Fish	2.4E-06 (2.0E-07-3.0E-05)	5.3E-07 (1.2E-07-2.4E-06)	1.6E-06 (4.0E-07-6.5E-06)	7.6E-08 (2.5E-08-2.3E-07)	2.4E-08 (3.0E-09-1.9E-07)
Upper Trophic Fish	2.9E-06 (2.4E-07-3.6E-05)	6.3E-07 (1.4E-07-2.8E-06)	1.9E-06 (4.6E-07-7.6E-06)	8.9E-08 (2.9E-08-2.7E-07)	2.8E-08 (3.6E-09-2.3E-07)
Amphibians	2.5E-05 (2.1E-06-3.1E-04)	5.5E-06 (1.2E-06-2.4E-05)	1.6E-05 (4.1E-06-6.6E-05)	7.8E-07 (2.6E-07-2.4E-06)	2.5E-07 (3.1E-08-2.0E-06)
Aquatic Reptiles	2.5E-05 (2.1E-06-3.1E-04)	5.5E-06 (1.2E-06-2.4E-05)	1.6E-05 (4.1E-06-6.6E-05)	7.8E-07 (2.6E-07-2.4E-06)	2.5E-07 (3.1E-08-2.0E-06)
Aquatic Insectivorous Birds	9.5E-03 (9.5E-03-9.7E-03)	7.7E-06 (1.6E-06-3.7E-05)	6.2E-03 (6.2E-03-6.2E-03)	2.1E-05 (6.3E-06-7.1E-05)	3.0E-06 (1.3E-06-1.3E-05)
Piscivorous Birds	4.6E-05 (3.8E-06-5.8E-04)	1.0E-05 (2.3E-06-4.5E-05)	3.0E-05 (7.5E-06-1.2E-04)	1.4E-06 (4.7E-07-4.4E-06)	4.6E-07 (5.8E-08-3.7E-06)
Amphibious Mammals	3.5E-05 (3.1E-06-4.0E-04)	1.3E-05 (2.1E-06-6.9E-05)	2.6E-05 (5.4E-06-1.2E-04)	7.8E-06 (9.9E-07-4.4E-05)	7.2E-06 (7.3E-07-4.4E-05)

Appendix VIII. Model predicted PFOS concentrations (C_B, µg/g wet wt.) in aquatic and terrestrial organisms at select DoD sites. Concentrations represent predicted PFOS concentrations on a whole organism basis. Concentrations in different tissues/compartments (C_i, mol/m^3) can be calculated using the predicted activity in the organism (a_B) and the corresponding solubility for a given tissue/compartment i (i.e., $C_i = a_B \times S_i$).

	Former Wurtsmith AFB (µg/g wet wt.)	Barksdale AFB (µg/g wet wt.)	Former Pease AFB (µg/g wet wt.)	Joint Base McGuire-Dix-Lakehurst (µg/g wet wt.)	Peterson AFB (µg/g wet wt.)
Terrestrial Food Web					
Plants	6×10^{-4} (2×10^{-4} - 1.9×10^{-3})	6×10^{-4} (2×10^{-4} - 1.9×10^{-3})	6×10^{-4} (2×10^{-4} - 1.9×10^{-3})	6×10^{-4} (2×10^{-4} - 1.9×10^{-3})	6×10^{-4} (2×10^{-4} - 1.9×10^{-3})
Herbivorous Insects	0.04 (0.01-0.13)	0.04 (0.01-0.13)	0.04 (0.01-0.13)	0.04 (0.01-0.13)	0.04 (0.01-0.13)
Insectivorous Birds	0.91 (0.30-2.81)	0.91 (0.30-2.81)	0.91 (0.30-2.81)	0.91 (0.30-2.81)	0.91 (0.30-2.81)
Insectivorous Mammals	1.08 (0.36-3.33)	1.08 (0.36-3.33)	1.08 (0.36-3.33)	1.08 (0.36-3.33)	1.08 (0.36-3.33)
Terrestrial Reptiles	5.32 (1.77-16.4)	5.32 (1.77-16.4)	5.32 (1.77-16.4)	5.32 (1.77-16.4)	5.32 (1.77-16.4)
Herbivorous Mammals	3.8×10^{-3} (1.3×10^{-3} - 1.1×10^{-2})	3.8×10^{-3} (1.3×10^{-3} - 1.1×10^{-2})	3.8×10^{-3} (1.3×10^{-3} - 1.1×10^{-2})	3.8×10^{-3} (1.3×10^{-3} - 1.1×10^{-2})	3.8×10^{-3} (1.3×10^{-3} - 1.1×10^{-2})
Terrestrial Carnivorous Mammals	0.07 (0.02-0.21)	0.07 (0.02-0.21)	0.07 (0.02-0.21)	0.07 (0.02-0.21)	0.07 (0.02-0.21)
Human	18.2 (1.50-220)	3.90 (0.9-17.4)	11.8 (2.9-48))	0.6 (0.2-1.8)	0.21 (0.03-1.5)
Aquatic Food Web					
Phytoplankton	1.25 (0.10-15.35)	0.27 (0.06-1.19)	0.81 (0.20-3.27)	0.04 (0.01-0.12)	0.012 (0.0015-0.097)
Benthic Invertebrates	980	0.32	637	1.95	0.11

	(-)	(0.08-1.47)		(0.62-6.17)	(-)
	1.38	0.30	0.89	0.04	0.013
Zooplankton	(0.11-16.87)	(0.07-1.31)	(0.22-3.59)	(0.01-0.13)	(0.002-0.1)
	1.37	0.30	0.89	0.04	0.013
Bivalves	(0.11-16.82)	(0.07-1.31)	(0.22-3.58)	(0.01-0.13)	(0.002-0.1)
	111.2	0.37	69.05	0.26	0.03
Benthic Fish	(104.90-124.03)	(0.08-1.65)	(68.28-72.13)	(0.08-0.80)	(0.01-0.13)
	1.54	0.34	1.03	0.05	0.02
Mid Trophic Fish	(0.13-19.4)	(0.08-1.51)	(0.25-4.14)	(0.02-0.15)	(0.002-0.12)
	1.86	0.40	1.20	0.06	0.02
Upper Trophic Fish	(0.15-22.7)	(0.09-1.77)	(0.30-4.84)	(0.02-0.17)	(0.002-0.14)
	16.3	3.50	10.50	0.50	0.16
Amphibians	(1.33-199)	(0.79-15.49)	(2.60-42.41)	(0.16-1.51)	(0.02-1.26)
	16.3	3.50	10.50	0.50	0.16
Aquatic Reptiles	(1.33-199)	(0.79-15.49)	(2.59-42.39)	(0.16-1.51	(0.02-1.26)
Aquatic Insectivorous	5,230	4.24	3396	11.56	1.65
Birds	(5,220-5,320)	(0.85-20.56)	(3392-3416)	(3.46-39.29)	(0.72-6.96)
	29.9	6.61	19.85	0.94	0.30
Piscivorous Birds	(2.51-376.41)	(1.49-29.28)	(4.90-80.15)	(0.31-2.85)	(0.04-2.39)
	22.6	8.44	16.50	4.99	4.60
Amphibious Mammals	(1.97-258.14)	(1.35-44.34)	(3.43-75.40)	(0.63-28.25)	(0.46-27.96)

Appendix IX. Details of species-sensitivity distribution (SSD) relationships used to determine HC5 values.

Number	Fit Model	Equation	Adjusted R-Square
1	Exponential	y =exp(-108.65792+45.1074*x+-4.65975*x^2)	0.94
2	Parabola	y = -0.16458 + -0.46857*x + 0.11846*x^2	0.96
3	Gompertz	y = 1.02918*exp(-exp(-2.68127*(x-4.84405)))	0.99
4	Exponential	y = 0.01579 + 2.87315E-4*exp(1.87702*x)	0.99
5	Dose Response	y = 0.04726 + (1.02214-0.04726)/(1 + 10^((4.33-x)*1.30866))	0.99
6	Linear	y = -0.04407 + 0.18389*x	0.98
7	Exponential	y =exp(-6.61433+0.88043*x+0.07097*x^2)	0.96
8	Cubic	y = 1.26551 + -0.8911*x + 0.15833*x^2 + 2.25313E-4*x^3	0.95
9	Cubic	y = -4.70569 + 3.66149*x + -0.94495*x^2 + 0.08593*x^3	0.97
10	Dose Response	y = -0.00644 + (1.31272--0.00644)/(1 + 10^((4.4162-x)*0.99871))	0.99
11	Dose Response	y = 0.04774 + (0.95221-0.04774)/(1 + 10^((4.15582-x)*3.52455))	0.99
12	Dose Response	y = -0.08189 + (1.17934--0.08189)/(1 + 10^((4.16839-x)*1.11473))	0.99
13	Linear	y = 0.23731 + 0.14539*x	0.94
14	Boltzmann	y = 0.96896 + (0.02559-0.96896)/(1 + exp((x-4.1631)/0.17811))	0.99
15	Dose Response	y = 0.0412 + (0.9929-0.0412)/(1 + 10^((4.16053-x)*2.11576))	0.99
16	Dose Response	y = 0.01846 + (0.62166-0.01846)/(1 + 10^((4.3565-x)*3.61885))	1.00
17	Gompertz	y = 0.95482*exp(-exp(-1.16215*(x-1.81529)))	0.99

Appendix X. 5% hazardous concentration effect levels (HC5s) and corresponding 95% lower confidence limits of HC5s for individual PFASs derived from various species-sensitivity distributions (SSDs).

Animal Group	Exposure Type	Exposure Medium	PFAS	Metric	Unit	HC5	Lower 95%	Upper 95%
1. Aquatic Biota	External	Aqueous	PFNA	EC50	µg/L	23,400	-	-
2. Aquatic Biota	External	Aqueous	PFOA	EC50	µg/L	27,000	25,800	28,300
3. Aquatic Biota	External	Aqueous	PFOS	EC50	µg/L	241	-	-
4. Aquatic Biota	External	Aqueous	PFOS	NOEL	µg/L	3.2	1.1	9.8
5. Terrestrial Invertebrates	External	Soil	PFOS	LOEL	µg/kg	1,800	-	-
6. Birds and Mammals	External	Dietary	PFOS	LOEL	µg/kg/d	1,790	-	-
7. Birds and Mammals	External	Dietary	PFOS	NOEL	µg/kg/d	738.0	-	-
8. Birds and Mammals	Internal	In vitro assay	N-Et-PFOSA	AC50	µg/L	1,160	880	1,400
9. Birds and Mammals	Internal	In vitro assay	PFDA	AC50	µg/L	2,620	-	-
10. Birds and Mammals	Internal	In vitro assay	PFHpA	AC50	µg/L	2,150	-	-
11. Birds and Mammals	Internal	In vitro assay	PFNA	AC50	µg/L	3,290	2,490	3,790
12. Birds and Mammals	Internal	In vitro assay	PFOS	AC50	µg/L	1,590	740	2,030
13. Birds and Mammals	Internal	In vitro assay, implantation, dietary	PFOS	NOEL	µg/L	10,200	9,540	10,700
14. Birds and Mammals	Internal	In vitro assay, air sac injection, dietary	PFOS	NOEL	µg/kg	7.7	6.5	9.0

Appendix XI. Lowest reported toxicity values for individual PFASs in different species and exposure conditions.

Chemical/ Organism	Exposure Type	Exposure Media	Metric	Unit	Lowest	Reference
PFHxS						
Danio rerio	External	Aqueous	AC50	µg/L	3,970	ToxCast
Eisenia fetida	External	Soil	LOEL	µg/kg	100,000	Karnjanapiboonwong et al., (2018)
Eisenia fetida	External	Soil	NOEL	µg/kg	1,000	Karnjanapiboonwong et al., (2018)
Homo sapiens	Internal	In vitro assay	AC50	µg/L	32.9	ToxCast
Gallus sp.	Internal	In vitro assay	NOEL	µg/L	20,000	Hickey et al., (2009)
Gallus gallus ssp. domesticus	Internal	Air sac injection	LOEL	µg/kg	890	Cassone et al., (2012)
Gallus gallus ssp. domesticus	Internal	Air sac injection	NOEL	µg/kg	890	Cassone et al., (2012)
PFOS						
Chironomus tentans	External	Aqueous	EC50	µg/L	87.2	MacDonald et al., (2004)
Rana pipiens	External	Aqueous	LC50	µg/L	6,210	Ankley et al., (2004)
Danio rerio	External	Aqueous	LOEL	µg/L	0.6	Keiter et al., (2012)
Chironomus tentans	External	Aqueous	NOEL	µg/L	2.3	MacDonald et al., (2004)
Danio rerio	External	Aqueous	AC50	µg/L	273	ToxCast
Brassica chinensis	External	Soil	EC50	µg/kg	95,000	Zhao et al., (2011)
Eisenia fetida	External	Soil	LOEL	µg/kg	250	Xu et al., (2013)
Aporrectodea caliginosa	External	Soil	NOEL	µg/kg	1,000	Zareitalabad et al., (2013)
Colinus virginianus	External	Dietary	LD50	µg/kg/d	61,000	Newsted et al., (2006)
Colinus virginianus	External	Dietary	LOEL	µg/kg/d	770	Newsted et al., (2005)
Colinus virginianus	External	Dietary	NOEL	µg/kg/d	580	Newsted et al., (2005)

Colinus virginianus	Internal	Serum	LOEL	µg/mL	8,700	Newsted et al., (2005)
Colinus virginianus	Internal	Liver	NOEL	µg/g	1.3	Newsted et al., (2005)
Homo sapiens	Internal	In vitro assay	AC50	µg/L	6.5	ToxCast
Tursiops truncatus	Internal	In vitro assay	LOEL	µg/L	5,000	Wirth et al., (2014)
Gallus sp.	Internal	In vitro assay	NOEL	µg/L	500	Hickey et al., (2009)
Gallus domesticus	Internal	Air sac injection	LOEL	µg/kg	10.0	Molina et al., (2006)
Gallus domesticus	Internal	Air sac injection	NOEL	µg/kg	1.0	Molina et al., (2006)
PFOA						
Psetta maxima	External	Aqueous	EC50	µg/L	11,900	Mhadhbi et al., (2012)
Neocaridina denticulate	External	Aqueous	LC50	µg/L	454,000	Li, (2009)
Pseudokirchneriella subcapitata	External	Aqueous	NOEL	µg/L	96.9	González-Naranjo and Boltes, (2014)
Sorghum bicolor	External	Soil	EC50	µg/kg	66,000	Gonzalez-Naranjo et al., (2015)
Aporrectodea caliginosa	External	Soil	LOEL	µg/kg	1,000	Zareitalabad et al., (2013)
Aporrectodea caliginosa	External	Soil	NOEL	µg/kg	1,000	Zareitalabad et al., (2013)
Rattus norvegicus	External	Dietary	LOEL	µg/kg/d	100,000	Dupont Haskell Laboratory, (2000)
Homo sapiens	Internal	In vitro assay	AC50	µg/L	83.6	ToxCast
Pusa sibirica	Internal	In vitro assay	LOEL	µg/L	25,900	Ishibashi et al., (2008)
Gallus sp.	Internal	In vitro assay	NOEL	µg/L	20,700	Hickey et al., (2009)
Gallus sp.	Internal	Air sac injection	LOEL	µg/kg	20	Stromqvist et al., (2012)
Gallus gallus ssp. domesticus	Internal	Air sac injection	NOEL	µg/kg	5.0	Norden et al., (2016)
PFOSA						
Danio rerio	External	Aqueous	AC50	µg/L	200	ToxCast
Homo sapiens	Internal	In vitro assay	AC50	µg/L	0.02	ToxCast

Appendix XII. Maps showing locations and satellite images of the studied DoD sites, including (a) Former Wurtsmith Air Force Base, (b) former Pease Air Force Base, (c) Joint Base McGuire-Dix-Lakehurst, (d) Barksdale Air Force Base and (e) Peterson Air Force Base.