



Extraordinary levels of per- and polyfluoroalkyl substances (PFAS) in vertebrate animals at a New Mexico desert oasis: Multiple pathways for wildlife and human exposure

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) in the environment pose persistent and complex threats to human and wildlife health. Around the world, PFAS point sources such as military bases expose thousands of populations of wildlife and game species, with potentially far-reaching implications for population and ecosystem health. But few studies shed light on the extent to which PFAS permeate food webs, particularly ecologically and taxonomically diverse communities of primary and secondary consumers. Here we conducted >2000 assays to measure tissue-concentrations of 17 PFAS in 23 species of mammals and migratory birds at Holloman Air Force Base (AFB), New Mexico, USA, where wastewater catchment lakes form biodiverse oases. PFAS concentrations were among the highest reported in animal tissues, and high levels have persisted for at least three decades. Twenty of 23 species sampled at Holloman AFB were heavily contaminated, representing middle trophic levels and wetland to desert microhabitats, implicating pathways for PFAS uptake: ingestion of surface water, sediments, and soil; foraging on aquatic invertebrates and plants; and preying upon birds or mammals. The hazardous long carbon-chain form, perfluorooctanesulfonic acid (PFOS), was most abundant, with liver concentrations averaging >10,000 ng/g wet weight (ww) in birds and mammals, respectively, and reaching as high 97,000 ng/g ww in a 1994 specimen. Perfluorohexanesulfonic acid (PFHxS) averaged thousands of ng/g ww in the livers of aquatic birds and littoral-zone house mice, but one order of magnitude lower in the livers of upland desert rodent species. Piscivores and upland desert songbirds were relatively uncontaminated. At control sites, PFAS levels were strikingly lower on average and different in composition. In sum, legacy PFAS at this desert oasis have permeated local aquatic and terrestrial food webs across decades, severely contaminating populations of resident and migrant animals, and exposing people via game meat consumption and outdoor recreation.

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a pernicious threat to global wildlife and human health because of their long-term stability, nonbiodegradability in the environment, biopersistence in tissues, and documented serious health effects (Giesy and Kannan, 2001; Lau et al., 2007; Evich et al., 2022). These human-made chemicals have been in widespread use since the 1940s for applications ranging from non-stick and stain-resistant coatings to fire-fighting foams and waterproofing materials (US EPA, 2016; US Food and Drug Administration, 2022). Globally, military bases tend to be among the most PFAS-contaminated sites (Anderson et al., 2016). Surface and ground water on military bases are centers of accumulation and movement of PFAS that are known to be harmful to wildlife and human health, particularly ‘legacy’, long carbon-chain compounds that were phased out of manufacturing due to toxicity, including perfluorooctanosulfonic acid (PFOS), perfluorohexanesulfonic acid (PFHxS), and perfluorooctanoic acid (PFOA) (Buck et al., 2011).

Even low-level exposure has been implicated in a wide range of health and ecological impacts (Fenton et al., 2021; Grandjean and Clapp, 2015; Sebastiano et al., 2023). In the human body, modest tissue concentrations of PFAS have been linked to cancer, developmental problems, reproductive problems such as pre-term birth, autoimmune disease, and endocrine disruptions, among other serious health problems (DeWitt, 2015; Fenton et al., 2021; Taibl et al., 2023). However, movements of PFAS through food webs that include humans and domesticated and wild animals are poorly understood, in part because of the lack of strategic and comprehensive biodiversity sampling infrastructure (Malaney and Cook, 2018) and the challenges associated with assessing exposure, sampling diverse species, and conducting assays (De Silva et al., 2021).

In wildlife, a growing list of studies have reported specific detrimental effects and declines in overall condition due to PFAS (D’Holander et al., 2014; Costantini et al., 2019; Banyoi et al., 2022; Guillette et al., 2022; Jouanneau et al., 2022; Sebastiano et al., 2023). Toxicity estimates based on lab experiments are similar for rodents and birds; for both vertebrate classes, perfluoroalkane sulfonic acids (PFASs) such as PFOS tend to bioaccumulate faster and be more toxic than perfluoroalkyl carboxylic acids (PFCAs) such as PFOA, and longer carbon compounds have higher tendencies for bioaccumulation and toxicity than shorter molecules (Conder et al., 2008; Ankley et al., 2021). In birds, experimentally estimated toxicity reference values (TRV) for PFOS in blood serum and liver tissues ranged from hundreds to tens of thousands of parts per billion (Newsted et al., 2005). However, chronic toxicity occurs at tissue concentrations 2–4 orders of magnitude lower (Dennis et al., 2021), at levels that have been linked to endocrine disruption, immune dysfunction, and other maladies (Guillette et al., 2020; Sebastiano et al., 2023).

Understanding how variable rates of exposure and elimination cause differential bioaccumulation across species is a frontier for PFAS research. The toxicokinetics of various PFAS are known to be variable among species of birds and mammals and among classes of PFAS. Comparisons of PFAS levels in different tissues and among wild species at focal study sites over time can help to reveal rates and mechanisms of movement through individual organisms and food webs, as well as specific pathways of PFAS transport that pose potential threats to animal and human health (Pizzurro et al., 2019). PFAS are proteinophilic and move easily into protein-rich tissues, such as blood and liver, where rates of bioaccumulation and trophic magnification vary profoundly among species and among classes of PFAS (Munoz et al., 2022; Ren et al., 2022; Sun et al., 2022).

Metabolic conversion of precursor compounds is one source of exposure that may affect species differently (Butt et al., 2014). Metabolic elimination of PFAS, however, is generally negligible due to strong C–F bonds. Elimination occurs by excretion, but excretion rates are highly variable among species as indicated by tissue half-life variation.

Blood serum or plasma half-lives of PFAS in varied rodent and bird species have been found to range from hours to over one year but tended to be on a scale of weeks to months, longer for PFOS than PFOA, and shorter for females that shed PFAS through egg or placental tissue (Death et al., 2021). Elimination half-lives of long carbon-chain PFAS tend to be much longer for humans than for other species that have been tested, ranging as high as 5.4 years for PFOS, 8.5 years for PFOA, and 15.5 years for PFHxS (Pizzurro et al., 2019). Another example that shows species-specific biokinetics is provided by a recent comparative study between co-occurring great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) across a gradient of exposure, showing that even closely related species can differ in how they sequester long carbon-chain versus short carbon-chain perfluoroalkyl carboxylic acids (PFCAs) (Lasters et al., 2021). At higher-level taxonomic scales, terrestrial plants and herbivorous invertebrates tended to sequester more short-chain PFCAs, whereas vertebrate animals and higher trophic level invertebrates tended to sequester more long-chain PFCAs (Groffen et al., 2022).

Holloman Air Force Base and its immediate vicinity, in the Chihuahuan Desert of south-central New Mexico, contain artificial wetlands contaminated with PFOS, PFHxS, PFOA, and other PFAS (Jarvis et al., 2021). Holloman Lake was created in 1965 by installing an earthen dam on a playa lake (ephemeral desert wetland). The lake receives storm-water runoff and treated sewage from Holloman Air Force Base. Many contaminants accumulated over decades of wastewater deposition, among which were components of aqueous film-forming foams (AFFF) that had been used extensively in fire-fighting training since the 1970s (Moody and Field, 2000). While some components of the various AFFF formulations degrade to PFOA and other perfluoroalkyl carboxylic acids (PFCAs), others degrade to PFOS and other perfluoroalkyl sulfonic acids (PFASs); however, the ingredients of proprietary AFFF formulations and their pathways of degradation in the environment are not completely known (Anderson et al., 2016). A recent analysis of publicly available data for surface waters across the United States showed that Holloman Lake is one of the most polluted with PFOS, with measured concentrations as high as 5.9 ng/mL and a median concentration of 4.5 ng/mL; other wetlands on and around Holloman Airforce base are also heavily contaminated (Jarvis et al., 2021).

The wetlands, riparian, and littoral habitat surrounding Holloman Lake are largely managed by the U. S. Department of Defense, the U. S. Department of Interior (Bureau of Land Management), and the New Mexico State Lands Office. This biodiverse ecosystem includes a woodland area at the head of the lake, tall emergent vegetation, mudflats, permanent open water, muddy shorelines, and extensive desert shrubland surrounding the lake. Holloman Lake is the largest and most ecologically significant water source in the Tularosa Basin (~16,800 km²). The lake, surrounding vegetation, and the inflow of nutrient rich treated sewage support a diverse vertebrate fauna, including birds, mammals, reptiles, and amphibians. At least 252 species of birds occur at Holloman Lake (ebird.org), 113 of which are aquatic species, and 41 of which are game species that can be legally hunted. The mammal community is also diverse and typical of the northern Chihuahuan Desert scrub community, with more than 35 non-volant mammal species occurring locally (Frey and Yates, 1996; Malaney et al., 2022) and at least 19 species of bats occurring within 100 km, some of which are long-distance migrant species (Cryan, 2003; Russell et al., 2005). Free-ranging beef cattle (*Bos taurus*) and oryx (*Oryx gazella*) graze in the vicinity and drink directly from the lake, the latter species having been introduced in the late 1960s and 1970s for sport hunting.

Hunting for sport or subsistence provides a pathway for PFAS movement from contaminated wildlife into human tissues (Haug et al., 2010), although concerns about PFAS-contaminated fish consumption have received substantially more attention (Guillette et al., 2020). In the few cases worldwide where assays have been conducted on game species, such as wild boars and wild ducks, their tissues have been shown to harbor potentially dangerous concentrations of PFAS (typically PFOS, PFHxS, PFOA et al.) (Death et al., 2021; Rupp et al., 2023). A few

previous studies have published data on PFAS in waterfowl meat, and these have shown that contaminated waterfowl can travel far from point sources. In Japan (two species), Canada (two species), and Australia (four species), duck meat averaged in the single digits or tens of ng/g ww, and ranged as high as the hundreds of ng/g ww, high enough to trigger consumption warnings (Taniyasu et al., 2003; Kelly et al., 2009; Senversa, 2018; Environmental Protection Authority Victoria, 2019; Sharp et al., 2021).

In this study, we explored the extent and pathways of PFAS contamination in wild animal populations in the area of Holloman AFB (Fig. 1). Specifically, we asked: (1) To what extent do PFAS accumulate in diverse aquatic and terrestrial vertebrate species at a point source, relative to control sites? (2) Have PFAS been present since at least 1994, when the first museum-archived tissues samples from the area were collected? (3) To what extent does the meat of migratory game bird species pose an ongoing hazard to human health? (4) To what extent do PFAS from the point source permeate upland desert rodent communities that have no direct contact with lake water or sediments? (5) How do PFAS profiles and tissue distributions vary across species with varying phylogenetic affinities, habitats, and diets? (6) What does this indicate about pathways for PFAS uptake, bioaccumulation, trophic magnification, and human exposure?

2. Materials and methods

2.1. Sampling and museum archiving

We screened 99 samples (n = 63 liver, 24 muscle, 10 blood, 2 leaves and stems) for 17 PFAS and six constituent isomeric forms. The samples represented 34 individual birds, 40 mammals, and 2 composite plant samples (Table S1). The bird and mammal species were selected in part because of their varied phylogenetic affinities, diets, and habitat preferences. The sampled bird community included game and non-game bird species, aquatic and terrestrial species, as well as winter and breeding season residents; in total a representative set of 11 aquatic game bird species, 3 songbird species, and 1 shorebird species, all of which occur in or at the margins of contaminated wetlands at Holloman AFB. The 11 aquatic game species that we screened included 10 duck species (Anseriformes: Anatidae) and the American coot (Gruiformes: Rallidae), all of which vary in foraging depths, and, with the possible exception of the piscivorous common merganser (*Mergus merganser*), all of which are commonly hunted and eaten. The mammals comprised nine rodent species that are resident throughout their lives in the immediate littoral zone and/or surrounding desert habitats.

Four mammal specimens had been collected in 1994 in the vicinity of a golf course on Holloman AFB, near a contaminated wetland, Lagoon G. Livers from these specimens were cryogenically preserved in liquid nitrogen and archived at the Museum of Southwestern Biology (MSB), University of New Mexico. We screened these four samples, collected

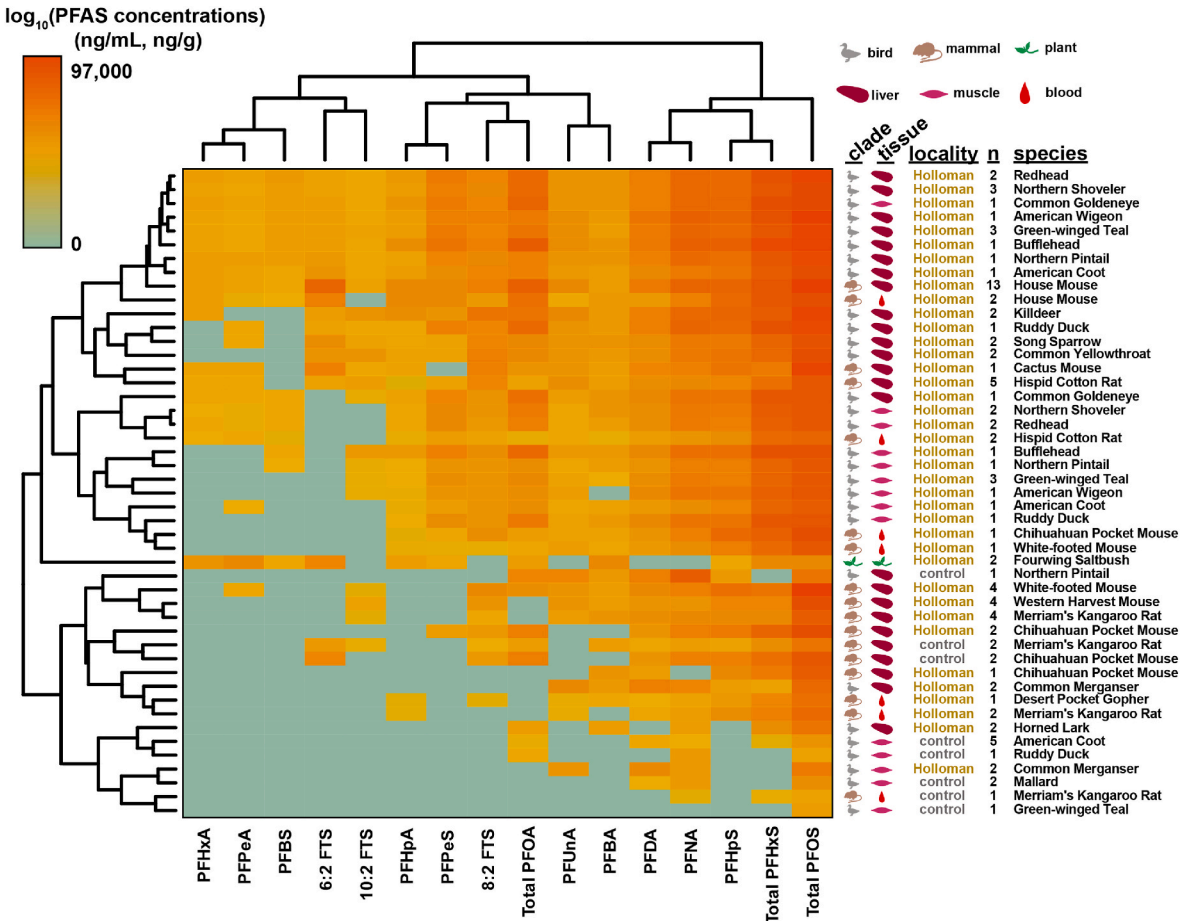


Fig. 1. Heat map showing similarity of PFAS concentration-profiles in 47 unique species-tissue combinations from Holloman Air Force base (mustard font) versus control sites (gray font). Tissues include bird muscle (ng/g), bird and small mammal liver (ng/g), small mammal blood (ng/mL), and plant leaf and stem. Sample sizes are indicated under 'n'. Dendrograms depict hierarchical relationships among species-tissue combinations (left) based on similarity of 16 PFAS concentrations, and among 16 PFAS (top) based on similarity of their concentrations across species-tissue categories. Samples clustered in accordance with ecology and phylogeny, as described in Results.

within 2 km of our recent sampling, in order to gain insights into historical contamination. We were not granted access to resample sites on Holloman AFB during our 2021–23 sampling efforts.

The remainder of samples in this study were collected during 2021–2023 adjacent to Holloman AFB. All historical and contemporary samples were taken within a ~2 km radius, in or near Holloman Lake (32.81 N, 106.12 W ± 2 km). To provide ‘control’ samples, additional specimens were collected from uncontaminated sites ~10–150 km from Holloman AFB (see Table S1).

Birds were collected by licensed hunters using shotguns with non-toxic shot, operating under federal and state scientific collecting permits and, for game species, in compliance with federal and state hunting regulations. Small mammals were trapped overnight using live-capture Sherman traps, following standard protocols for museum collection (Yates et al., 1996) and under state scientific collecting permits. Field research protocols were approved by the University of New Mexico Institutional Animal Care and Use Committee (Protocols 21-201225-MC and 19-200908-MC).

After collection, animals were prepared as museum specimens with an associated suite of tissues permanently frozen under ultra-cold conditions (−80 °C freezers or −196 °C vapor-phase liquid nitrogen storage) in the Division of Genomic Resources of the MSB. Specimen records included spatial, temporal and natural history data (e.g., georeferenced locality, collection date, and reproductive and mensural data). All specimens were screened for ecto- and/or endoparasites that were also preserved and linked to voucher specimens (Galbreath et al., 2019). All specimen info is available online through the Arctos collection management system (Cicero et al., 2023) and links to specimen records are included in Table S1. Contemporary tissues were sampled following strict protocols to avoid PFAS contamination during handling. We stored samples in PFAS-free, non-leaching polypropylene tubes (Greiner Bio-One®). Dissecting equipment was free of PFAS-containing materials and was cleaned thoroughly between specimens in HDPE (high density polyethylene) containers with Liquinox® and rinsed with deionized PFAS-free (ASTM Type II) water to avoid cross-sample contamination. Historical samples (1994) were flash frozen in liquid N₂ and archived in NUNC® polypropylene cryovials at −80 °C for ~30 years, although specific field protocols for tissue collection were not recorded. Bird specimens were frozen for a period at −20 °C before being thawed for specimen preparation, at which time tissues were dissected out and re-frozen.

As a preliminary test of plant PFAS content, we collected two plant samples, each comprised of a composite of stems and leaves from multiple individuals of an abundant shrub, four-winged saltbush, along the Holloman Lake shoreline. We screened these samples and description of plant PFAS profiles to gain insights into PFAS movement from ground-water to plants to terrestrial herbivores.

2.2. Tissue selection for screening

We chose liver because it is a target tissue for PFAS accumulation in multiple vertebrate classes (D’Hollander et al., 2014; Groffen et al., 2017), likely because PFAS readily bind to fatty acid binding proteins that are abundant in liver (Cara et al., 2022). We additionally screened pectoral muscle for a set of game bird species because it is preferred for human consumption, although many hunters also prepare and eat liver. For a subset of the small mammals, we supplemented liver screening with whole blood screening. The median serum or plasma to whole blood ratio of PFOS, PFOA, PFHxS, PFNA and PFUnDA tends to be approximately 2:1; however, the ratio can vary in other PFAS, indicating that it is preferable to screen whole blood rather than serum or plasma to assay these pollutants (EFSA CONTAM Panel et al., 2020).

2.3. PFAS extraction and cleanup

Targeted screening for PFAS (Table 1) was conducted by Eurofins

Table 1

List of 17 PFAS compounds and 6 alternative isomers assayed in this study. PFSA’s with 6 or more C atoms and PFCA’s with 7 or more C atoms are considered ‘long-chain’ (Buck et al., 2011).

Abbreviation	PFAS compound	Chemical formula
Fluorotelomer sulfonic acids		
10:2 FTS	10:2 Fluorotelomer sulfonic acid	C ₁₀ F ₂₁ CH ₂ CH ₂ SO ₃ H
4:2 FTS	4:2 Fluorotelomer sulfonic acid	C ₆ H ₅ F ₉ O ₃ S
6:2 FTS	6:2 Fluorotelomer sulfonic acid	C ₆ F ₁₃ CH ₂ CH ₂ SO ₃ K
8:2 FTS	8:2 Fluorotelomer sulfonic acid	C ₈ F ₁₇ CH ₂ CH ₂ SO ₃ H
Perfluoroalkane sulfonic acids (PFSA’s)		
PFBS	Perfluorobutanesulfonic acid	C ₄ F ₉ SO ₃ [−]
PFHpS	Perfluoroheptanesulfonic acid	C ₇ F ₁₅ SO ₃ H
PFPeS	Perfluoropentanesulfonic acid	C ₅ HF ₁₁ O ₃ S
Total PFHxS	Total Perfluorohexanesulfonic acid	C ₆ F ₁₃ SO ₃ [−]
Br-PFHxS	“ “	Branched isomer, perfluorohexanesulfonic acid
L-PFHxS	“ “	Linear isomer, perfluorohexanesulfonic acid
Total PFOS	Perfluorooctanesulfonic acid	C ₈ F ₁₇ SO ₃ H
Br-PFOS	“ “	Branched isomer, perfluorooctanesulfonic acid
L-PFOS	“ “	Linear isomer, perfluorooctanesulfonic acid
Perfluoroalkyl carboxylic acids (PFCA’s)		
PFBA	Perfluorobutanoic acid	C ₃ F ₇ CO ₂ H
PFDA	Perfluorodecanoic acid	C ₉ F ₁₉ COO [−]
PFHpA	Perfluoroheptanoic acid	C ₆ F ₁₃ COO [−]
PFHxA	Perfluorohexanoic acid	C ₅ F ₁₁ COO [−]
PFNA	Perfluorononanoic acid	C ₈ F ₁₇ COO [−]
Total PFOA	Perfluorooctanoic acid	C ₇ F ₁₅ COO [−]
Br-PFOA	“ “	Branched isomer, perfluorooctanoic acid
L-PFOA	“ “	Linear isomer, perfluorooctanoic acid
PFPeA	Perfluoropentanoic acid	C ₄ F ₉ COO [−]
PFUnA	Perfluoroundecanoic acid	C ₁₀ F ₂₁ COO [−]

(Sacramento, California, USA), with details provided in Supplementary Materials, Appendix 1.

2.4. Data analysis

Assayed concentrations for each PFAS and isomer were summarized, compared, and visualized in R. To assess differences among contamination profiles of species, tissues, localities, and substances, we created a heat map with log₁₀-transformed PFAS concentrations and applied marginal dendrograms to visualize similarity among species-tissue-locality combinations and substances, respectively. We conducted classical non-metric multidimensional scaling (NMDS) to assess variation among individual tissues with respect to their PFAS profiles and in relation to site (Holloman AFB vs. control). Because sample sizes for species-tissue combinations from control localities were low (n ≤ 9), we assessed differences between localities with non-parametric Kruskal-Wallis tests (Altman et al., 1983).

We compared concentrations among tissues within individuals to quantify tissue-specific exposure and examine toxic potency and bio-accumulation (Gomis et al., 2018). We assessed equal variance between bird tissues from contaminated sites using paired t-tests and F-tests (sampling was adequate for parametric approaches). Due to unequal variances, we used paired-sample Mann-Whitney Wilcoxon signed-rank tests with a normal approximation to compare bird muscle to liver and mammal blood to liver. We note that tissue comparisons should be interpreted with caution because of potential matrix effects, such as ion enhancement or suppression, that can add systematic error (Berger and

Haukås, 2005).

3. Results

PFAS were strikingly abundant and widespread among animal tissues sampled from Holloman AFB. We obtained measurements above reporting limits for 16 of 17 PFAS (Table S2), and the most common PFAS were detected above reporting limits in the overwhelming majority of Holloman AFB vertebrate samples (Table 2, S2). For Holloman liver samples, mammals had higher mean concentrations of total PFAS (Σ PFAS; $\bar{x}$ = 15,589 ng/g, SD = 24,623) than birds ($\bar{x}$ = 11,508 ng/g, SD = 11,284), although this difference was driven largely by high Σ PFAS concentrations in house mice (*Mus musculus*) ($\bar{x}$ = 28,276 ng/g, SD = 25,644); mammals excluding house mice averaged lower ($\bar{x}$ = 8,173 ng/g, SD = 21,062).

Vertebrate sample types, when grouped by species, tissue type, and locality, fell into two large clusters with respect to PFAS composition and concentrations (Fig. 1). The first group comprised only Holloman AFB samples that were highly contaminated with a broad range of PFAS, including all of the aquatic-habitat birds except mergansers, as well as house mice and four other rodent species (Fig. 1). The second group comprised the remaining rodent species and an upland desert songbird species from Holloman AFB, as well as control-site samples (Fig. 1). We found multiple PFAS in the tissues of all eleven game bird species that were tested at Holloman AFB, and ten of eleven species contained high levels. Only the common mergansers (n = 2) collected at Holloman Lake were not highly contaminated and more closely resembled control samples in their PFAS profiles (Fig. 1).

PFAS in animal tissues was strikingly higher at Holloman AFB than at control sites (Table 2; Fig. 1; Fig. 2; Fig. S1; Fig. S2). Among all taxa and tissues, samples from Holloman AFB were more than 30-fold higher in Σ PFAS ($\bar{x}$ = 10,527 ng/g or ng/mL, SD = 17,641) than samples from control sites ($\bar{x}$ = 324 ng/g or ng/mL, SD = 965). Among bird muscle samples, 13 of 17 targeted PFAS substances (plus six constituent isomers) were detected at significantly higher levels in Holloman AFB samples relative to control sites: PFBA, PFHpA, L-PFOA, Br-PFOA, Total PFOA, PFNA, PFDA, PFUnA, PFBS, PFPeS, Br-PFHxS, L-PFHxS, Total PFHxS, PFHpS, L-PFOS, Br-PFOS, Total PFOS, 8:2 FTS, and 10:2 FTS (Fig. 2; Fig. S1; full names of each compound listed in Table 1). Among mammal liver samples, four PFAS and two constituent isomers were measured at significantly higher levels among Holloman AFB samples relative to control sites (despite limited statistical power due to the small number of control samples assayed): PFDA, PFUnA, PFHpS, L-PFOS, Br-PFOS, and Total PFOS (Fig. S2).

PFOS was the most abundant PFAS in animal tissues, both at Holloman AFB and control sites, followed by PFHxS (Table 2; Table 3; Fig. 1; Fig. 3). A white-footed mouse (*Peromyscus leucopus*) collected in 1994 had the highest PFAS level among all samples (liver [PFOS] = 97,000 ng/g ww); however, the four samples from 1994 exhibited a wide range of PFOS levels (as low as 24 ng/g ww) and could not be distinguished from modern samples based on their PFAS profiles. An American wigeon (*Mareca americana*) collected in 2022 had the highest PFAS levels among sampled birds (liver [PFOS] = 38,000 ng/g ww). 4:2 fluorotelomer sulfonic acid (FTS) was the only tested substance that we never detected above reporting limits (Table 2).

Overall, there were strong similarities of the PFAS contamination profiles among bird and mammal samples (Fig 1; Fig. 3). Most bird and mammal tissues from Holloman AFB sites clustered along the first two NMDS dimensions (Fig. 3A). Control samples including bird muscle samples and a single mammal blood sample clustered separately (Fig. 3A). PFAS composition was dominated by PFOS (Fig. 3B), averaging ~10,000 ng/g ww in liver for both birds and mammals from Holloman AFB sites. One prominent exception was a northern pintail liver sample from a control site that contained the highest levels of PFNA of any sample in our study (850 ng/g ww) but very little PFOS. PFHxS was the second most abundant PFAS detected, averaging in the 1000s of

ng/g ww among bird and mammal tissues from Holloman AFB. Together PFOS and PFHxS comprised >75% of Σ PFAS. Additional abundant PFAS in vertebrate tissues at Holloman AFB included PFOA, PFNA, PFHpS, 6:2 FTS, PFDA, 8:2 FTS, and PFPeS (Fig. 3C). Isomer ratios were somewhat different between birds and mammals (Fig. S5) and are described in Supplementary Materials, Appendix 2.

Two key differences between birds and mammals emerged from our data. First, PFHxS and PFOA were far more abundant in avian liver than in mammal liver, with the exception that the house mice tended to resemble the birds (Fig. 1). Second, 6:2 FTS was found at high levels in some mammal livers ($\bar{x}$ = 160.3 ng/g ww), but not avian livers ($\bar{x}$ = 1.1 ng/g ww) (Table 2). Samples of the plant species (four-wing salt-bush) from Holloman Lake clustered apart from the animals, with lower overall levels of PFAS and starkly contrasting composition (6:2 FTS was the most abundant compound; Fig. 3).

For both birds and mammals, liver showed evidence of higher bioaccumulation than other tissues, as expected (Fig. 4; Fig. S3; Fig. S4). Eleven out of 17 PFAS were significantly higher in bird liver than muscle (Fig. 4; Fig. S3). Mammals showed higher bioaccumulation in liver than blood; seven out of 17 PFAS were significantly more concentrated in mammal liver tissue than blood from the same individuals (Fig. S4).

4. Discussion

4.1. A contaminated desert oasis

This study shows that diverse species in the vicinity of Holloman AFB have contained high PFAS tissue-concentrations from at least 1994 to present. PFAS matching those typically found at AFFF-contaminated sites (Anderson et al., 2016) have permeated the wetland and upland desert food webs, consistent with multiple exposure pathways, and with likely consequences for wildlife, livestock, and human health. Our data provide the first direct evidence that PFAS originating at a military base actively contaminate the entire community of migratory birds — thousands of individuals annually — that use its constructed wetlands for migratory stopover, wintering, and breeding.

4.2. Extraordinary PFAS concentrations

The birds and rodents at Holloman AFB had PFAS tissue concentrations far higher than those measured in nearly all previous surveys of wildlife, with liver concentrations of PFOS and Σ PFAS, respectively, averaging in the 10,000s of ng/g ww. For perspective, at Cannon AFB in eastern New Mexico, ground water contamination resulted in destruction of ~3000 dairy cattle whose milk contained PFOS at concentrations 3–4 orders of magnitude lower than we measured in Holloman wildlife (Jha et al., 2021).

The highest previous measurements in wild populations were from a fluorochemical plant in Belgium. In 2002, wood mouse (*Apodemus sylvaticus*) livers near the plant contained a median of ~5000 ng/g ww PFOS, with one sample containing an astounding 179,000 ng/g ww, and two others measuring 98,000 ng/g ww (Hoff et al., 2004)—similar to the highest level of contamination that we observed at Holloman AFB. By 2004, the highest measurement from a wood mouse liver at the site was ~22,000 ng/g ww (D'Hollander et al., 2014). Great tit (*Parus major*; a songbird) livers at the same site measured as high as 11,359 ng/g ww (Dauwe et al., 2007). A scops owl (*Otus* sp.) from South Korea had similar liver Σ PFAS (11,283 ng/g ww), suggesting exposure at an unknown point source (Barghi et al., 2018).

The vast majority of PFOS (or Σ PFAS) tissue concentrations in wildlife have tended to be in the 1s–100s of ng/g ww (liver) or ng/ml (serum). However, a small proportion of published measurements have ranged to the 1000s of ng/g ww, with top predator species and piscivorous mammals and birds generally over-represented among these outliers: bald eagle (*Haliaeetus leucocephalus*), two hawk species (*Buteo buteo*, *Accipiter gentilis*), a vulture (*Aegypius monachus*) a gull (*Larus*

Table 2

Detection levels of 17 PFAS compounds and six isomeric constituents in bird liver (ng/g), bird pectoral muscle (ng/g), mammal blood (ng/mL), mammal liver (ng/g), and plant stem & leaf tissue (ng/g), respectively, for Holloman AFB and control sites. Bold values represent the highest mean values for each compound across the nine tissue sample types. BDL indicates samples for which detection levels fell below detection limits.

PFAS	bird							mammal							plant		
	liver			muscle				blood			liver				stem/leaves		
	control		Holloman	control		Holloman		control		Holloman		control		Holloman		Holloman	
	n = 1	n = 24		n = 9		n = 15		n = 1	n = 9		n = 4		n = 34		n = 2		
	value	mean	range	mean	range	mean	range	value	mean	range	mean	range	mean	range	mean	range	
10:2 FTS	BDL	0.66	BDL–4.5	BDL	–	0.13	BDL–1.2	BDL	BDL	–	0.04	BDL–0.15	0.40	BDL–1.8	BDL	–	
4:2 FTS	BDL	BDL	–	BDL	–	BDL	–	BDL	BDL	–	BDL	–	BDL	–	BDL	–	
6:2 FTS	BDL	1.12	BDL–8.6	BDL	–	0.05	BDL–0.71	BDL	6.53	BDL–54	6.38	BDL–23	160.28	BDL–2200	10.15	4.3–16	
8:2 FTS	BDL	16.35	BDL–70	BDL	–	2.65	BDL–12	BDL	1.04	BDL–5.8	0.85	BDL–3	15.50	BDL–77	BDL	–	
PFBS	BDL	0.40	BDL–1.4	BDL	–	0.14	BDL–0.7	BDL	0.04	BDL–0.3	BDL	–	0.08	BDL–1.6	0.32	0.23–0.4	
PFBA	1.60	1.57	BDL–3.3	BDL	–	0.90	BDL–2.6	BDL	0.48	BDL–2	0.48	BDL–1.9	1.00	BDL–5.3	8.50	3–14	
PFDA	31.00	22.13	BDL–57	0.16	BDL–0.4	6.24	0.94–24	BDL	0.96	0.18–2.4	1.18	0.25–3.7	18.41	BDL–120	BDL	–	
PFHpS	1.20	150.99	BDL–480	BDL	–	50.00	BDL–230	BDL	64.56	1.7–310	10	BDL–39	151.31	BDL–1700	0.21	0.2–0.22	
PFHpA	BDL	1.02	BDL–7.4	BDL	–	0.37	BDL–1.9	BDL	1.38	0.05–11	BDL	–	2.90	BDL–34	0.70	0.39–1	
PFHxA	BDL	0.39	BDL–1.9	BDL	–	0.12	BDL–1	BDL	0.21	BDL–1.5	BDL	–	0.38	BDL–3.6	2.70	1.9–3.5	
PFNA	850.00	202.82	0.5–630	0.49	BDL–1.7	52.54	0.78–270	0.07	9.24	0.53–34	9.23	BDL–35	173.19	BDL–2000	BDL	–	
PFPeS	BDL	17.18	BDL–77	BDL	–	7.21	BDL–37	BDL	1.61	BDL–11	BDL	–	3.61	BDL–48	0.40	0.33–0.47	
PFPeA	BDL	0.23	BDL–0.7	BDL	–	0.09	BDL–0.6	BDL	0.10	BDL–0.3	BDL	–	0.34	BDL–2.5	9.00	8.8–9.2	
PFUnA	13.00	3.49	BDL–16	BDL	–	0.87	BDL–3.9	BDL	0.28	0.13–0.7	BDL	–	4.26	BDL–23	BDL	–	
Br–PFHxS	BDL	104.92	BDL–450	BDL	–	43.53	BDL–200	–	–	–	6.14	BDL–24	139.42	BDL–2600	0.98	0.76–1.2	
L–PFHxS	BDL	1738.43	0.38–8600	0.03	BDL–0.3	946.67	BDL–4500	–	–	–	54.69	BDL–210	755.68	BDL–8000	5.75	5.2–6.3	
Total PFHxS	BDL	1832.18	0.38–9000	0.03	BDL–0.3	992.00	BDL–4700	0.08	499.69	5.2–2700	59.81	BDL–230	905.09	BDL–11000	6.70	5.9–7.5	
Br–PFOA	BDL	2.43	BDL–37	BDL	–	0.29	BDL–1.2	BDL	0.75	BDL–6.4	1.3	BDL–5.2	16.90	BDL–170	BDL	–	
L–PFOA	11.00	101.06	BDL–940	0.1	BDL–0.3	52.81	BDL–380	BDL	20.60	BDL–170	15.15	BDL–59	243.97	BDL–3500	0.67	0.54–0.8	
Total PFOA	11.00	103.52	BDL–980	0.1	BDL–0.3	53.09	BDL–380	BDL	20.71	BDL–170	16.4	BDL–64	256.94	BDL–3600	0.67	0.54–0.8	
Br–PFOS	6.500	2209.34	2.4–9500	0.3	BDL–0.9	437.71	2.5–2200	0.06	125.44	10–400	286.28	4.9–1100	4375.00	6.1–25000	3.20	2.8–3.6	
L–PFOS	52.00	7020.88	12–29000	2.37	0.4–5.0	1454.20	15–6600	0.36	1795.44	79–6200	575.25	12–2200	9708.38	18–74000	3.80	3.1–4.5	
Total PFOS	59.00	9154.04	14–38000	2.68	0.6–5.3	1903.20	17–8800	0.42	1917.67	89–6600	861	16–3300	14165.74	24–97000	7.00	6–8	

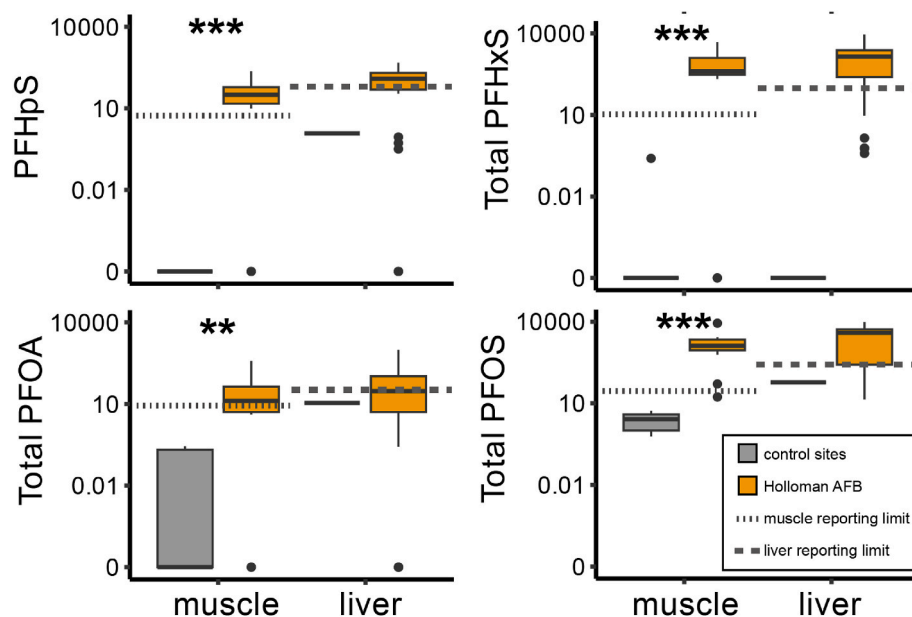


Fig. 2. Comparisons of select PFAS compound concentrations (ng/g) in bird muscle and liver samples at Holloman AFB (orange) versus offsite control localities (gray). Thick and thin gray dashed lines (see legend) indicate mean reporting limit thresholds per compound for muscle and liver, respectively. Asterisks indicate significant differences, calculated from Kruskal-Wallis tests (for muscle samples only).

* $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$). Because we had only a single liver sample from a control site, we did not conduct significance tests for liver. See Table S1 for additional PFAS compounds.

crassirostris), two owl species (*Otus* sp., *Ninox scutulata*), a cormorant (*Phalacrocorax penicillatus*), a dove (*Streptopelia orientalis*), a crow (*Corvus corone*), a seal (*Arctocephalus forsteri*), polar bear (*Ursus maritimus*), various mustelid species (mink, *Neogale vison*; Eurasian otter, *Lutra*; river otter, *Lontra canadensis*), and, in at least one case, a human (*Homo sapiens*) (Giesy and Kannan, 2001; Kannan et al., 2001, 2002; Taniyasu et al., 2003; Verreault et al., 2005; Lau et al., 2007; Yoo et al., 2008; Greaves et al., 2012; Persson and Magnusson, 2015; Barghi et al., 2018; Lopez-Antia et al., 2021; Park et al., 2021; Androulakis et al., 2022; Badry et al., 2022; Herzke et al., 2023). Taken together, published PFAS concentrations from wildlife around the world show that the level of community-wide contamination of primary and secondary consumer species at Holloman AFB is unprecedented, with 20 species — 87% of those sampled — showing tissue concentrations of PFOS >1000 ng/g ww.

4.3. Effects on wildlife health

Although there is a shortage of PFAS toxicity studies for wild populations, published benchmarks suggest that the liver PFOS concentrations observed in this study would be more than sufficient to damage health and diminish ecological performance. Avian thresholds reported by Newsted et al. (2005) and Dennis et al. (2021) are most relevant for chronically exposed wild birds at Holloman AFB. Newsted et al. estimated 'predicted no effect concentrations' (PNEC; 350 ng/g ww) and a toxicity reference value for upper-trophic avian predators (TRV; 600 ng/g ww) (Newsted et al., 2005). Dennis et al. reported chronic toxicity reference values (CRVs) of liver PFOS as 226 and 50.4 ng/g ww for adult and juvenile northern bobwhites (*Colinus virginianus*), respectively (Dennis et al., 2021). Forty-two out of 47 liver or muscle samples of aquatic or wetland-habitat bird species from Holloman AFB exceeded all three of these thresholds (Table 3). Dennis et al. further observed that PFOS was absorbed and distributed differently when combined with PFHxS, as it is at Holloman AFB. Previous work has demonstrated striking health consequences for bird populations even at far lower PFAS tissue concentrations than we recorded in this study. Low doses of PFOS reduced body weight and reproduction of northern

bobwhites (Ankley et al., 2021). In four species of gulls (*Charadriiformes*), declines in condition, antioxidant capacity, or thyroid function were linked with several PFAS at concentrations in the 1s to low 10s of ng/g ww (Costantini et al., 2019; Sebastiano et al., 2023).

For mammals, a 'no-observed-adverse-effect-concentration' (NOAEC) for rodent serum of 50,000 ng/mL has been estimated by repeated dose toxicity (Colnot and Dekant, 2022); but as in birds, adverse effects have been reported at far lower concentrations. In domestic cats, PFAS increased body weight and risk of liver, thyroid, and kidney disease at blood concentrations averaging 6.9 ng/mL PFHxS (maximum 235 ng/mL), 8.9 ng/mL PFOS (maximum 121 ng/mL) (Bost et al., 2016) and 9.5 ng/mL ΣPFAS (Wang et al., 2018), respectively. Dogs exposed to firefighting foams or dietary PFAS showed dose-dependent alterations in amylase, cholesterol, and several indicators of blood chemistry at ΣPFAS levels averaging 3.6 ng/mL (maximum 16.6 ng/mL) (You et al., 2022). Immunotoxicity resulted from low daily doses of 6:2 FTS in white-footed mice (Bohannon et al., 2023). Aquatic secondary consumer species across a variety of vertebrate and invertebrate taxa were adversely affected by serum PFAS as low as 13.5 ng/mL (Banyoi et al., 2022). Mouse livers that approximated Holloman AFB levels of PFOS, ~50,000 ng/g ww, under experimental dosing were linked to perturbed placental gene expression and corticosterone, and reduced body weight (Wan et al., 2020). Humans, mice, and rats showed altered development after early exposure to any of a suite of PFAS at tissue concentrations in the single digits ng/g ww (Blake and Fenton, 2020). Serum PFHxS and PFOA in pregnant women were linked to poor birth outcomes at only 1.09 (2.30) ng/mL PFHxS and 0.57 (2.31) ng/mL PFOA, respectively (geometric means and standard deviations) (Taibl et al., 2023).

The above evidence indicates that low-level chronic exposure leads to diminished health for diverse animal species at tissue concentrations far lower than those needed to produce effects in laboratory acute toxicity studies, and 3–4 orders of magnitude lower than we observed in the Holloman AFB fauna.

Table 3

Total PFOS, PFOA, and PFHxS, respectively, by species and tissue type, for Holloman AFB and control samples. Liver and muscle units are ng/g; blood units are in ng/mL. BDL indicates samples for which detection levels fell below detection limits.

Locality	Class	English name	Tissue type	n	Mean total PFOS	PFOS range	Mean total PFOA	PFOA range	mean total PFHxS	PFHxS range
Holloman	bird	American Coot	liver	1	5000.0	–	17.0	–	1200.0	–
control	bird	American Coot	muscle	5	3.0	0.77–5.3	0.1	BDL–0.28	0.1	BDL–0.25
Holloman	bird	American Coot	muscle	1	960.0	–	4.1	–	330.0	–
Holloman	bird	American Wigeon	liver	1	38000.0	–	40.0	–	3500.0	–
Holloman	bird	American Wigeon	muscle	1	2100.0	–	5.2	–	500.0	–
Holloman	bird	Bufflehead	liver	1	20000.0	–	980.0	–	9000.0	–
Holloman	bird	Bufflehead	muscle	1	2700.0	–	200.0	–	3000.0	–
Holloman	bird	Common Goldeneye	liver	1	2200.0	–	100.0	–	1700.0	–
Holloman	bird	Common Goldeneye	muscle	1	8800.0	–	380.0	–	4700.0	–
Holloman	bird	Common Merganser	liver	2	265.0	220–310	BDL	–	0.5	0.38–0.57
Holloman	bird	Common Merganser	muscle	2	34.0	17–51	BDL	–	–	–
Holloman	bird	Common Yellowthroat	liver	2	5750.0	4300–7200	9.2	7.3–11	285.0	280–290
Holloman	bird	Green-winged Teal	liver	3	14333.3	11000–20000	66.3	47–97	2266.7	1600–3000
control	bird	Green-winged Teal	muscle	1	1.0	–	BDL	–	–	–
Holloman	bird	Green-winged Teal	muscle	3	1536.7	810–2600	9.4	5.6–13	413.3	280–640
Holloman	bird	Horned Lark	liver	2	76.5	23–130	0.9	BDL–1.7	5.0	0.6–9.4
Holloman	bird	Killdeer	liver	2	14200.0	5400–23000	77.5	15–140	1095.0	390–1800
control	bird	Mallard	muscle	2	3.9	2.6–5.1	BDL	–	–	–
control	bird	Northern Pintail	liver	1	59.0	–	11.0	–	–	–
Holloman	bird	Northern Pintail	liver	1	10000.0	–	22.0	–	1800.0	–
Holloman	bird	Northern Pintail	muscle	1	1300.0	–	4.9	–	400.0	–
Holloman	bird	Northern Shoveler	liver	3	7371.3	14–17000	147.8	0.27–360	2500.5	1.4–5900
Holloman	bird	Northern Shoveler	muscle	2	1600.0	1100–2100	33.5	15–52	900.0	400–1400
Holloman	bird	Redhead	liver	2	11850.0	9700–14000	160.0	120–200	2650.0	1000–4300
Holloman	bird	Redhead	muscle	2	1255.0	610–1900	30.5	20–41	655.0	210–1100
Holloman	bird	Ruddy Duck	liver	1	9400.0	–	170.0	–	3800.0	–
control	bird	Ruddy Duck	muscle	1	0.6	–	0.2	–	–	–
Holloman	bird	Ruddy Duck	muscle	1	2300.0	–	46.0	–	1600.0	–
Holloman	bird	Song Sparrow	liver	2	2850.0	1800–3900	9.1	1.2–17	300.0	170–430
Holloman	dicot	Four-wing saltbush	leaf & stem	2	7.0	6–8	0.7	0.54–0.8	6.7	5.9–7.5
Holloman	mammal	Cactus mouse	liver	1	22000.0	–	3.3	–	38.0	–
control	mammal	Chihuahuan pocket mouse	liver	2	1664.0	28–3300	32.0	BDL–64	115.3	0.64–230
Holloman	mammal	Chihuahuan pocket mouse	liver	2	4420.0	440–8400	25.6	4.2–47	357.0	34–680
Holloman	mammal	Desert pocket gopher	blood	1	89.0	–	BDL	–	12.0	–
Holloman	mammal	Hispid cotton rat	blood	2	370.0	310–430	0.1	BDL–0.27	170.0	110–230
Holloman	mammal	Hispid cotton rat	liver	5	2000.0	1000–3900	0.4	BDL–0.65	88.4	36–160
Holloman	mammal	House mouse	blood	2	4250.0	1900–6600	92.5	15–170	1620.0	540–2700
Holloman	mammal	House mouse	liver	13	24081.6	61–65000	666.5	3.1–3600	2244.8	2.2–11000
Holloman	mammal	Chihuah. grasshopper mouse	blood	1	5900.0	–	0.8	–	720.0	–
Holloman	mammal	Chihuah. grasshopper mouse	liver	1	850.0	–	BDL	–	37.0	–
control	mammal	Merriam's kangaroo rat	blood	1	0.4	–	BDL	–	0.1	–
Holloman	mammal	Merriam's kangaroo rat	blood	2	215.0	160–270	BDL	–	17.6	5.2–30
control	mammal	Merriam's kangaroo rat	liver	2	58.0	16–100	0.8	BDL–1.6	4.3	BDL–8.6
Holloman	mammal	Merriam's kangaroo rat	liver	4	1165.0	700–1700	BDL	–	8.5	BDL–27
Holloman	mammal	Western Harvest Mouse	liver	4	4475.0	1600–7500	BDL	–	14.8	BDL–33
Holloman	mammal	White-footed mouse	blood	1	1600.0	–	0.3	–	150.0	–
Holloman	mammal	White-footed mouse	liver	4	26081.0	24–97000	3.9	BDL–15	66.7	0.86–160

4.4. Implications for rare species

Numerous migratory bird species of conservation concern use Holloman Lake regularly, including raptors (Accipitriformes and Falconiformes) and shorebirds (Charadriiformes). The Western Snowy Plover (*Charadrius alexandrinus nivosus*) of the interior U.S., is considered “Greatest Conservation Concern” by the U.S. Shorebird Conservation Partnership (shorebirdplan.org) and is a breeding resident around the margins of Holloman Lake. We sampled a related species that forages in the same habitat, the Killdeer (*Charadrius vociferus*), and we found it to be highly contaminated (Table 3, Fig. 1), as expected for species that forage on the sediment at lake margins (Larson et al., 2018). Reproductive and developmental toxicity of several PFAS compounds for both birds and mammals suggests that reproductive functions may be impaired. In birds, of which at least 43 species breed in the vicinity, mother to egg transfer of PFAS occurs predictably, and most heavily for

PFCAs with longer carbon chains (Jouanneau et al., 2022). Additionally, bird and mammal species from various parts of the Tularosa Basin may depend on the lake's resources for breeding; for example, the peregrine falcon (*Falco peregrinus*) makes foraging sorties to the lake from nesting sites in nearby mountains, ≥ 20 km away.

4.5. Implications for human exposure

Hunting is a popular activity at Holloman Lake and environs. Oryx, mule deer, pronghorn, javelina, wild boar, jackrabbits, and cottontails are all potentially hunted mammal species that should be considered susceptible to PFAS ingestion in the vicinity. Oryx were present in upland scrub areas around Holloman Lake during our sampling efforts, and 826 individuals were hunted on adjacent lands during the 2021–2022 season (NMDGF, 2022). Ear-tagged, free-ranging beef cattle also frequent the wetlands.

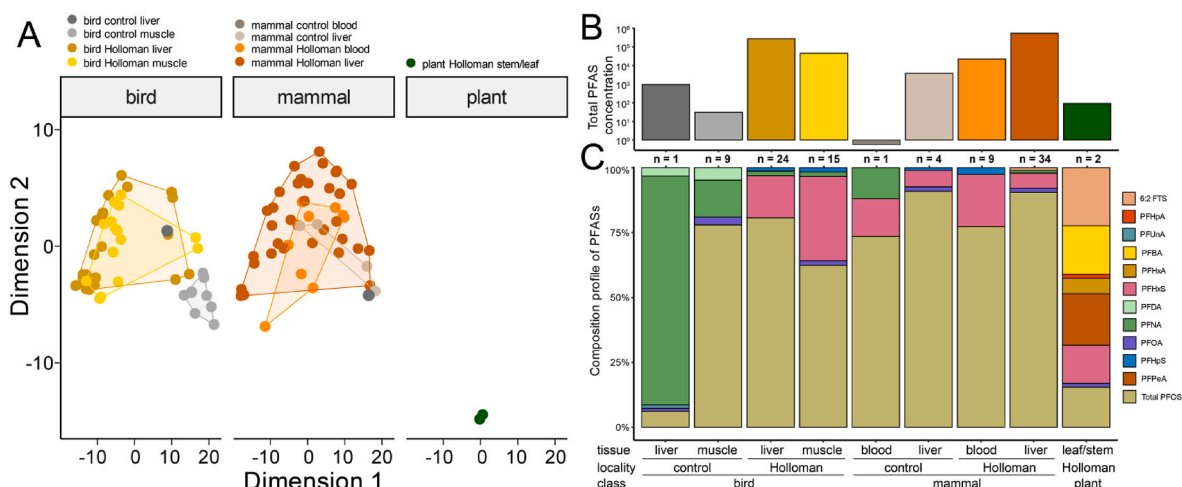


Fig. 3. (A) Multidimensional scaling of PFAS concentrations by locality-tissue combinations, plotted separately by clade. Clusters are bounded by convex hulls. (B) Σ PFAS concentrations (ng/g, or ng/ml for blood) colored by clade-locality-tissue combination (see key in panel A). (C) Composition of compounds that comprised $\geq 1\%$ of total PFAS, averaged by clade, tissue type, and locality. X-axis labels are shared between panels B and C.

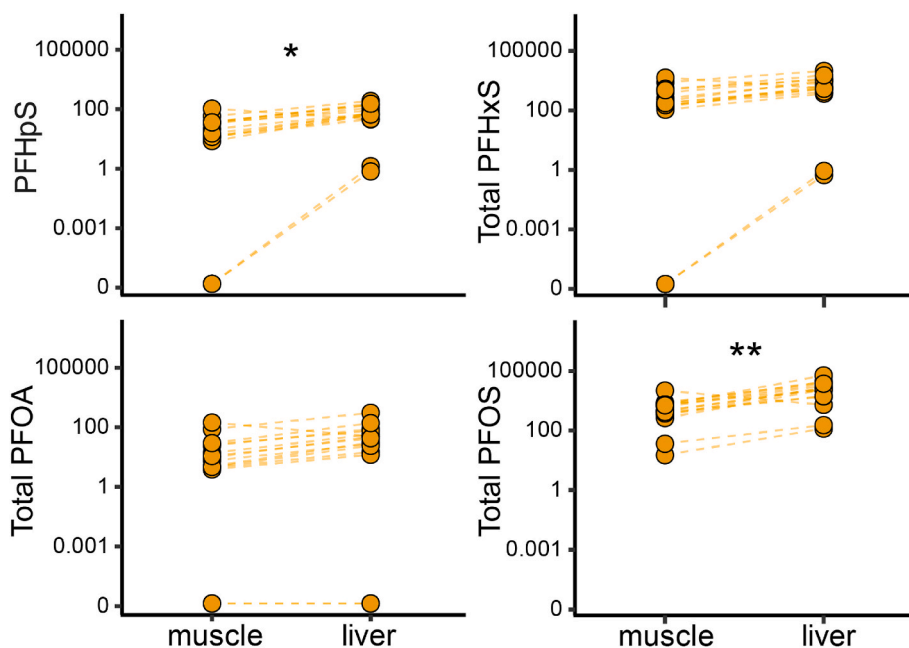


Fig. 4. Between-tissue comparison of select PFAS compound concentrations for muscle and liver of birds. Points indicate PFAS concentrations for muscle or liver, measured in ng/g. Lines connect points from the same animal. Steeper lines connecting tissues within individuals represent larger between tissue differences in PFAS concentrations. Asterisks indicate significant differences in PFAS concentrations between tissues based on Wilcoxon signed-rank tests using normal approximation. * $p < 0.05$; ** $p < 0.005$. See Table S3 for additional PFAS compounds.

At least 41 game bird species have been recorded at Holloman Lake (Table S3). Hunting of both aquatic (waterfowl and coots) and non-aquatic game species (quail and doves) frequently occurs around the edges of Holloman Lake; waterfowl hunters were present on six of nine days that we visited the lake during 2021–2022 and 2022–2023 seasons. Hunters invariably consume the meat of their quarry after harvest, as required by New Mexico hunting regulations.

As with upland desert rodents, human exposure during outdoor activities is plausible via incidental ingestion of water, soil, or airborne particulates. The sample exhibiting the highest PFOS level encountered in this study (97,000 ng/g) was collected in 1994 at Lagoon G, a wastewater discharge area adjacent to a heavily used golf course. The samples with the second and third highest contamination levels in 2021–2023 were collected along the Lagoon G outfall canal where it

drains into Holloman Lake. Dispersed camping, recreation, bird watching, and hunting have occurred regularly in this area over most of the nearly three-decade period represented by our sampling.

4.6. Implications for human health

Eating contaminated game provides a pathway for PFAS contamination of human tissues. The European Food Safety Authority (EFSA) recommended a tolerable weekly intake (TWI) not exceeding 4.4 ng per kg of body weight for the sum of PFOA, PFNA, PFHxS, and PFOS (EFSA CONTAM Panel et al., 2020). The total of Holloman Lake bird meat that could be consumed within this TWI, for a 70-kg adult, and based on the means of our measurements in game birds (excluding the fishy-tasting common merganser), would be 89 mg of muscle or 21 mg of liver.

Under the guidelines of the US EPA (2016), 25 ng/kg bw/day PFOS would be considered tolerable (Nolen et al., 2022), corresponding to 799 mg muscle or 141 mg liver per day at the mean PFOS concentrations that we observed at Holloman Lake. Thus, our findings suggest it would never be safe to eat more than 1 g of game meat per day from Holloman Lake. In Australia, consumption advisories were issued for duck meat at PFOS concentrations more than two orders of magnitude lower (Environmental Protection Authority Victoria, 2019; Sharp et al., 2021).

4.7. Dispersal of PFAS-contaminated animals

Migrating and wintering waterbirds routinely fly between Holloman Lake and other suitable habitats in the region, providing potential for PFAS-contaminated individuals to be hunted and consumed from uncontaminated sites. On a smaller spatial scale, the same is true for wide-ranging mammalian game species. Among the 10 gamebirds that were screened from control sites, we did not detect individuals heavily contaminated with PFOS, PFOA, or PFHxS. However, one northern pintail from the Middle Rio Grande Valley, central New Mexico, ~150 km NNW of Holloman Lake, contained among the highest levels that we recorded (850 ng/g ww) of PFNA, a highly toxic, long carbon-chain PFCA (Table 2, Fig. 1). The PFAS profile of this bird (Fig. 3, C) was unique among our samples, suggesting that it had been exposed to PFAS at a site other than Holloman AFB. More PFAS screening will be needed from broadly dispersed localities to test the extent to which PFAS pose a health risk to consumers of gamebird meat that was hunted away from point sources.

4.8. Pathways of PFAS movement into aquatic animals

Aquatic animals like ducks and coots accumulate PFAS by feeding on aquatic invertebrates and plants, and by incidental ingestion of sediment and soil (Larson et al., 2018). The highest concentrations of PFAS are generally found in soil and sediment samples, with levels of PFAS in surface water samples averaging lower (De Silva et al., 2021). Soil mineral contents, soil PFAS concentrations, and PFAS chain length, strongly influence the level of PFAS uptake by aquatic plants (Pi et al., 2017; Zhang et al., 2020). Zhang et al. experimentally demonstrated that long-chain PFAS were integrated into root tissues, while short-chain PFAS were translocated to shoot tissues (Zhang et al., 2020).

At the level of primary consumer, benthic macroinvertebrates accumulate PFAS from ingestion of aquatic plants or exposure to sediment (Brase et al., 2022) and their PFOS concentrations have been measured as high as 61 ng/g ww (St. Lawrence River) (Munoz et al., 2022). Predatory macroinvertebrates generally accumulate higher PFAS in tissues relative to herbivores, though lifespan and physiological differences also affect accumulation (Brase et al., 2022). Terrestrial invertebrates of various trophic positions near the fluorochemical plant in Belgium measured 28–9000 ng/g ww PFOS (D'Hollander et al., 2014). An American wigeon sampled from Holloman Lake had the highest levels of PFAS among any bird in our dataset. This species is primarily herbivorous, feeding mainly on aquatic and terrestrial plants, suggesting that high PFAS exposure in gamebirds at Holloman AFB extends to the lowest trophic levels. Stomach contents of several game bird species in our sampling showed that they were eating large quantities of omnivorous aquatic corixids (Insecta: Hemiptera), a likely major pathway for trophic transfer of PFAS (see Arctos.org specimen data linked from Table S1).

4.9. Pathways of PFAS movement into terrestrial animals

Ingestion of food and incidental ingestion of soil are the most likely routes of exposure for most terrestrial rodents at Holloman AFB; a subset of species that occur at the lake edge likely also ingest PFAS with surface water. The suite of rodent species that we screened span the breadth of foraging niches and environments around Holloman AFB: desert

granivores (Merriam's kangaroo rat, *Dipodomys merriami*; Chihuahuan pocket mouse, *Chaetodipus eremicus*; western harvest mouse, *Reithrodontomys megalotis*), omnivores (white-footed mouse, *Peromyscus leucopus*; house mouse), a carnivore (Chihuahuan grasshopper mouse, *Onychomys arenicola*), and a relatively mesic adapted herbivore (hispid cotton rat, *Sigmodon hispidus*). In general, Chihuahuan desert rodents consume both plant material and arthropods, in proportions that vary by species and season (Hope and Parmenter, 2007). Soil ingestion while foraging or grooming, or dust inhalation, likely overshadows exposure through ingestion of water at Holloman Lake species other than white-footed mouse and house mouse. The upland desert rodents in our dataset may not directly ingest water, but rather obtain water from vegetation and invertebrates that they consume. A single white-footed mouse (MSB:Mamm:92667) and several house mice (MSB:Mamm:340078, 340121), both species with relatively low tolerance for dehydration (Haines and Schmidt-Nielsen, 1967; MacMillen, 1983) had the highest levels of PFAS among all sampled species (Fig. 1, Table 3). The white-footed mouse was collected from Lagoon G in 1994 (prior to construction of a wastewater treatment plant in 1996). Approximately 1.2 million gallons of domestic and industrial wastewater were discharged to the sewage lagoon area daily, with overflow draining through an outflow canal into Holloman Lake (Amec Foster Wheeler Programs, Inc., 2018). Although resampling at this exact location (Lagoon G) was not permitted, we were able to collect samples about 1.8 km from Lagoon G in the area of the outfall canal into Holloman Lake. The house mice exhibiting the highest PFAS levels in our contemporary sampling were collected from this location.

Upland desert rodent species that would not be expected to contact or ingest surface water directly generally exhibited similar PFOS levels as found in aquatic zone birds and rodents, with two key differences: PFHxS was lower, and the number of detected PFAS was reduced (Fig. 1). The plant, four-wing saltbush, was relatively high in 6:2 FTS, though ΣPFAS was four orders of magnitude lower than in bird or mammal tissues (Fig. 1; Fig. 3; Table 2). Soil PFAS are taken up by roots (Stahl et al., 2009), and short-chain PFAS differentially accumulate in plant tissues and invertebrates that feed on them (Ghisi et al., 2019; Groffen et al., 2022). The strikingly different PFAS profiles of rodents versus four-winged saltbush suggest that pathways other than herbivory are the most important for rodent PFAS uptake. We did observe an excess of 6:2 FTS in some rodents relative to aquatic birds, although levels were highly variable. This preliminary finding suggests at least some PFAS transfer via herbivory. The overall similarity of rodents PFAS profiles with those of avian aquatic primary and secondary consumer species (Fig. 1; Fig. 3) suggests that they share key exposure pathways. These shared pathways may include incidental ingestion of soil, consumption of arthropods that have aquatic life stages, and inhalation of dust or aerosolized foam. However, additional sampling of plants and invertebrates is needed to evaluate the relative importance of PFAS transport via insectivory and herbivory, respectively.

4.10. Trophic magnification

Carnivorous mammals and raptors routinely forage on primary and secondary consumer species that we studied here and are expected to be at high risk of long carbon-chain PFAS bioaccumulation and trophic magnification (Jouanneau et al., 2020). Bioaccumulation processes tend to be more complex for terrestrial than aquatic food chains (EFSA CONTAM Panel et al., 2020). Highlighting the dangers for upper trophic predators at Holloman AFB, nearly all tissues that we screened from potential prey species exceeded a benchmark tissue concentration for PFOS of 33 ng/g that was established to protect upper trophic level wildlife species from secondary poisoning (European Union (EU), 2014).

Trophic magnification of PFOS tends to be most severe in piscivorous tetrapods, with contaminants from sediments and water being translocated into macroinvertebrates, then fish, then piscivores (Lau et al., 2007; Larson et al., 2018; Ankley et al., 2021). Trophic magnification

factors for various PFAS have been estimated to be between ~1 and 20 in aquatic systems, ~2–6 in fish, ~0.7–7.2 in terrestrial food webs containing birds, and ~1.1–2.7 in terrestrial food webs with mammals (Kelly et al., 2009; De Silva et al., 2021; Fremlin et al., 2023). PFAS is enriched in longer food chains and, as a result, herbivores tend to have the lowest tissue concentrations (Guckert et al., 2023; Miranda et al., 2022). PFAS levels in piscivorous birds are highly variable (Kannan et al., 2001) and affected by local PFAS concentrations where foraging occurs. Samples from the piscivorous common merganser collected at Holloman Lake had relatively low PFAS levels, similar to those of control sites. Interestingly, Holloman Lake seems to contain only one species of fish, the mosquitofish (*Gambusia affinis*), an introduced species with very small body size. The stomach of one merganser specimen contained a sunfish (*Leptomis* sp.), which is not known to occur in the lake; thus, we suspect that common mergansers had been using the lake as a rest site between foraging sites in adjacent watersheds >100 km away.

4.11. Museum collections and biorepositories for environmental monitoring

This study demonstrates how museum collections of biodiversity research specimens, or biorepositories, are well suited for measuring PFAS contamination in wild populations over space and time. Museum collections have proven essential for ecotoxicological challenges in the past, as exemplified by the discovery that eggshell thinning was caused by the pesticide, DDT (Hickey and Anderson, 1968). Other PFAS studies have also used collections to assess PFAS in ways that would have been impossible without these resources. For example, a study using herbarium specimens showed that pine needles have tracked temporal changes since the 1960s in airborne concentrations of over 70 PFAS (Kirkwood et al., 2022). In South Korea, birds from a museum collection revealed high levels of contamination in various diurnal and nocturnal birds of prey (Barghi et al., 2018). In the present study, we screened four rodent samples that had been collected during the mid-1990s for other purposes. One specimen proved to be contaminated with PFAS at exceptionally high levels, establishing a nearly three-decade timeline for persistent exposure to these contaminants. Furthermore, the voucher specimens collected for this study in 2021–2023 are accompanied by frozen tissue samples, ecto- and endoparasites, and online-open data, all of which will provide a temporal baseline of the Holloman Lake faunal community for future studies of environmental change. Biorepositories broaden the range of questions we can ask about PFAS or other aspects of environmental change; for example, species-specific patterns of PFAS exposure, biokinematics, and tolerance will require taxonomically diverse samples that archival specimens can provide. Combining these contaminant analyses with genomic and epigenetic studies of the specimens or other aspects of their biology provides a powerful framework for assessing the impact of these perturbations on individuals, biotic communities, and ecosystems.

5. Conclusions

The biotic communities of Holloman AFB wetlands and surrounding terrestrial habitats are extraordinarily contaminated with PFAS, especially legacy, long carbon-chain forms. Our results show that diverse bird and mammal species at middle trophic levels accumulate PFAS in their tissues at levels that far exceed those known to be harmful. Permeation of the primary and secondary consumer community at such high tissue concentrations is essentially unprecedented among previous PFAS surveys.

Expanded monitoring of this desert oasis site is needed, including at higher and lower trophic levels. This self-contained, isolated wetland provides an unusually tractable opportunity to understand PFAS movement through a food web, as well as to assess effects on animal health and condition. Few PFAS-exposed wild populations or communities have been adequately screened to date, but such data are urgently

needed to establish baselines, parameterize models, and estimate risks to wildlife, livestock, and human health. Biorepositories such as the frozen tissue collections of natural history museums, could bolster such efforts by providing spatiotemporally and taxonomically broad sampling with robust data (Schindel and Cook, 2018). The health of humans who use the Holloman AFB area for hunting or recreation should also be monitored closely, and this population could be considered a sentinel for the overall health of this contaminated environment (Andrews et al., 2023).

A key concern arising from our results is the potential effects of PFAS on migratory animal populations that are attracted to these wetlands at all times of year. Numerous migratory populations — including game species and declining species — stopover at Holloman AFB wetlands while traveling between widely dispersed localities in North, Central, and South America. Dispersal of these contaminated migratory animals potentially poses risks to predators and hunters across a broad area. To understand these risks, we need to study species-specific dynamics of PFAS bioaccumulation in conjunction with full annual cycles of migration and reproduction. More specifically, it should be considered urgent for both conservation and public health to understand the temporal dynamics of PFAS tissue accumulation during stopover or wintering periods.

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CRediT authorship contribution statement

Christopher C. Witt: Funding acquisition, Supervision, Writing – review & editing, Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision. **Chauncey R. Gadek:** Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. **Jean-Luc E. Carttron:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Michael J. Andersen:** Funding acquisition, Supervision, Writing – review & editing. **Mariel L. Campbell:** Investigation, Supervision, Writing – review & editing. **Marialejandra Castro-Farías:** Investigation, Writing – review & editing. **Ethan F. Gyllenhaal:** Investigation, Writing – review & editing. **Andrew B. Johnson:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. **Jason L. Malaney:** Investigation, Writing – review & editing. **Kyana N. Montoya:** Investigation, Writing – review & editing. **Andrew Patterson:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Nicholas T. Vinciguerra:** Investigation, Writing – review & editing. **Jessie L. Williamson:** Investigation, Visualization, Writing – review & editing. **Joseph A. Cook:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft. **Jonathan L. Dunnun:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the data is shared in supplementary files that I uploaded for review.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2024.118229>.

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