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Per- and Polyfluoroalkyl Substances (PFAS) in Terrestrial Mammals from a Typical Chinese Agricultural Region: Non-Target Screening, Biomagnification, and Risk Assessment

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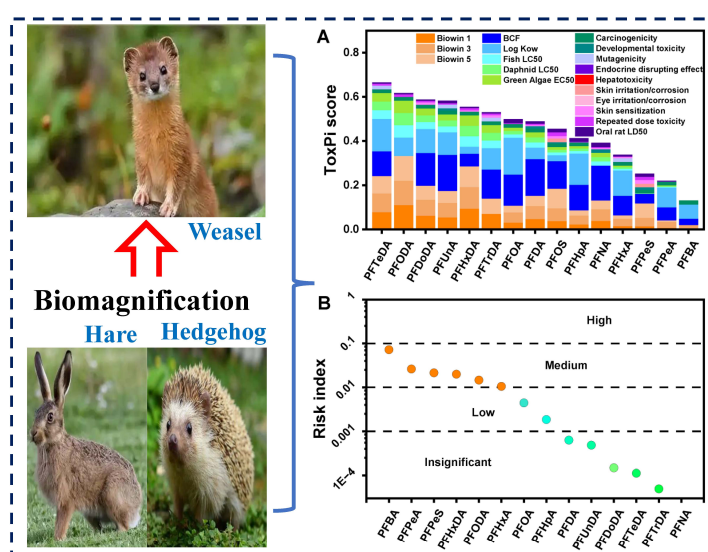
Keywords

per- and polyfluoroalkyl substances;
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Highlights

- Using non-target analysis, 11 PFAS were identified with confidence level 2.
- Weasels obviously exhibited significant higher PFAS concentrations among the three terrestrial mammals.
- For 15 PFAS examined except PFHxDA and PFODA, biomagnification factors were found to be greater than 1 in the food chains.
- Four PFAS, including PFOA, PFPeA, PFPeS, and PFOS were flagged with high priority (risk index > 0.1).

Abstract: With perfluoroalkyl and polyfluoroalkyl substances (PFAS) widely distributed across environmental media and prone to bioaccumulation, terrestrial mammals face ecological health risks, yet related research remains limited. This study addresses the gap by investigating the occurrence and biomagnification of PFAS in terrestrial mammals in a typical agricultural region in China. Using non-target analysis, fifteen PFAS from twelve classes were identified with confidence level 2. Total PFAS concentrations ranged from 0.121 to 0.386 ng/g wet weight (ww) in hares, 0.086 to 0.434 ng/g ww in hedgehogs, and 0.306 to 0.973 ng/g ww in weasels. For 15 PFAS examined except PFHxDA and PFODA, biomagnification factors were found to be greater than 1 in both the hare-weasel and hedgehog-weasel food chains, indicating significant biomagnification of PFAS in hare-weasel and hedgehog-weasel food chains. A risk-based prioritization approach was developed to rank the identified PFAS, flagging four as high priority (risk index > 0.1). These findings enhance understanding of PFAS behavior in terrestrial ecosystems and provide valuable insights to mitigate their environmental and ecological impacts.



1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) have become growing environmental pollutants because of their extensive use, environmental persistence, and ability to bioaccumulate in ecosystems [1,2]. These synthetic compounds, often referred to as “forever chemicals” are widely utilized in industrial applications and consumer products, including nonstick cookware, waterproof textiles, and fire-fighting foams [3]. Their unique physicochemical properties, such as high thermal stability and resistance to degradation, make them beneficial for industrial purposes but also lead to their accumulation in the environment. Over the past decades, concerns have escalated regarding the environmental and health risks posed by PFAS exposure, particularly through food chains in terrestrial ecosystems.

In agricultural areas, PFAS contamination has increasingly been recognized as a significant issue [4]. These regions often experience contamination through multiple pathways, including the application of PFAS-containing biosolids as fertilizers, irrigation with polluted water, and atmospheric deposition from industrial emissions [5]. Once in the environment, PFAS can persist in soils and groundwater and can be taken up from soil by plants [6], potentially entering terrestrial food webs. Consequently, PFAS have been detected in plants [7] and in several wildlife species, with evidence of biomagnification in the terrestrial environment [8–10]. Possible exposure routes for terrestrial animals are food, drinking water and atmospheric exposure. Roe deer are particularly suitable indicators for a large number of bioaccumulative xenobiotics as summarized [11]. Terrestrial mammals such as hares, hedgehogs and weasels are ecologically significant in agricultural landscapes [12]. These species collectively offer a broad perspective on PFAS exposure pathways and trophic interactions within agricultural ecosystems.

Biomagnification, the process by which PFAS concentrations increase along the food chain, is well-documented in aquatic systems but less understood in terrestrial ecosystems [13–15]. Studying PFAS biomagnification in these mammals can shed light on contamination propagation in agricultural environments. Additionally, while traditional targeted analyses focus on a limited set of PFAS, non-targeted screening methods enable the detection of a broader range of compounds, providing a comprehensive understanding of PFAS diversity and exposure.

The toxicological risks associated with PFAS exposure in terrestrial mammals are of particular concern, given their potential to impact both wildlife and human populations. PFAS have been linked to immunotoxicity [16], reproductive issues [17], and metabolic disruptions [18] in various species [19]. In agricultural regions, these mammals may serve as vectors

of PFAS transfer to humans, either through direct consumption or secondary exposure via contaminated crops or water. Evaluating the risks of PFAS posed to hares, hedgehogs, and weasels is essential not only for understanding wildlife health but also assessing potential implications for ecosystem integrity and indirect human exposure on account of these species may act as vectors for PFAS transfer within the food web.

This study investigates PFAS occurrence, biomagnification, and risk assessment in hares, hedgehogs, and weasels from a typical Chinese agricultural region. Specifically, it aims to (1) identify PFAS compounds in terrestrial mammals using non-targeted screening, (2) evaluate biomagnification potential in the terrestrial food web (from hedgehogs and hares to weasels), and (3) assess ecological health risks of PFAS exposure on terrestrial mammals. The findings will contribute to understanding PFAS behavior in terrestrial ecosystems and guide strategies to mitigate their impacts.

2. Materials and Methods

2.1. Chemicals and Reagents

Eighteen commercially available PFAS standards and nine internal standards (ISs) were purchased from three suppliers: ANPEL Laboratory Technologies (Shanghai, China), Wellington Laboratories (Guelph, Ontario, Canada), and Tokyo Chemical Industry (Tokyo, Japan) (Supplementary Material, Table S1). Acetonitrile, cyclohexane, ethyl acetate, and methanol of pesticide grade were all obtained from ANPEL Laboratory Technologies. Ammonium acetate (NH_4OAc , purity $\geq 99.9\%$) was purchased from Sigma-Aldrich (Stockholm, Sweden), while Milli-Q water was produced using an ultrapure water filtration system (Millipore, Billerica, MA, USA).

2.2. Study Area and Sample Collection

In this study, extensive agricultural areas in central Henan Province, China, including the cities of Xuchang, Luohe, and Zhoukou, were selected as the study region (Figure S1). The agricultural areas are primarily located within the Huai River Basin, one of the major river systems in China, whose tributaries provide essential water resources for irrigation and agriculture in these regions. The agricultural areas cultivate a diverse range of crops. The primary crops include wheat and corn, often grown in rotation, with wheat in the winter and corn in the summer. Peanuts and soybeans are also widely cultivated as important cash crops. Cotton is grown to support the textile industry, while rapeseed is planted in the winter for oil production. Additionally, various vegetables like cabbage, carrots, peppers, and eggplants are cultivated to meet local market demands. Fruits such as pears, apples, grapes, and watermelons are also commonly grown in these areas.

A total of 59 terrestrial mammals including hare (herbivore, $N = 20$), hedgehog (omnivore, $N = 20$), and weasel (carnivore, $N = 19$) as representatives of different trophic levels in the agricultural ecosystem, were collected from September 2020 to August 2023. All of the animals were found either dead or severely injured due to harvesting machinery activities or attacks by dogs kept by farmers. The samples were placed in sealed bags as soon as they were found and stored in $-20\text{ }^{\circ}\text{C}$ freezers at the farmers' homes. They were then periodically sent to the laboratory at the Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, where they were dissected. As liver or blood samples of sufficient quantity for comprehensive analysis could not be reliably obtained from deceased or critically injured individuals, muscle tissue samples were selected for collection and analysis, it is a widely used indicator of persistent organic pollutant body burden in wildlife studies. Consequently, the analytical scope of this study was determined by the availability of this specific tissue type. Before dissection, thaw the sample and measure the body weight. Detailed information of these samples was documented in Table S2. Then, separated the muscle tissue. Freeze-dried the separated muscle tissue and ground it into a powder, while recording the wet weight and dry weight data of the muscle tissue. The ground muscle tissue sample was stored in a $-20\text{ }^{\circ}\text{C}$ freezer until further chemical analysis. This study was approved by the Research Ethics Committee of Guangdong University of Technology (Approval No. GDUTXS2023093, approved on March 13, 2023).

2.3. Sample Preparation and Instrumental Analysis

2.3.1. Sample Preparation

The sample preparation method was adapted from a previous study [20] with some modifications. A ground muscle sample (1 g dry weight) was weighed in a 15 mL polypropylene (PP) centrifuge tube. Then, the ISs were spiked into the muscle sample. After a 20 min equilibration, added 10 mL of 0.01 mol/L NaOH-methanol, and then shake the tube vigorously for 3 min, followed by ultrasonic extraction for 10 min, and then centrifuged at 4000 rpm (5 min, $4\text{ }^{\circ}\text{C}$). Transferred the supernatant to a 50 mL bottle, and repeated the extraction process three times. The supernatant was concentrated to 1 mL and purified by solid-phase extraction (SPE) using a 200 mg weak anion exchange (WAX) cartridge (J&K Scientific, Beijing, China), which was activated with 0.1% ammonia methanol, methanol, and ultrapure water, respectively. The sample was loaded onto the SPE cartridge at a slow flow rate (1 mL/min), rinsed with ultrapure water, and eluted with 8 mL of 1% ammonia methanol. The eluate was concentrated to near dryness under a gentle stream of purified nitrogen gas and reconstituted to 0.5 mL with methanol and filtered through a PTFE membrane ($0.22\text{ }\mu\text{m}$) prior to instrument analysis.

2.3.2. Instrument Analysis

The non-target analysis of PFAS was performed using a Dionex UltiMate 3000 HPLC system coupled with a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA), equipped with a negative electrospray ionization (ESI) source and operated in negative ion mode. The negative ion electrospray voltage was set to 2500 V. Gas flows were configured at 40, 10, and 0 arb. for shear gas, aux gas, and sweep gas, respectively. The vaporizer and ion transfer tube temperatures were maintained at $300\text{ }^{\circ}\text{C}$ and $320\text{ }^{\circ}\text{C}$, respectively. MS/MS analysis was performed with normalized collision energy (CE) settings of 10%, 20%, and 40%, and in data-dependent acquisition mode. Chromatographic separation was achieved using a Hypersil Gold C18 ($100 \times 2.1\text{ mm}$, $1.9\text{ }\mu\text{m}$; Thermo Fisher Scientific, Waltham, MA, USA). The mobile phase consisted of water with 5 mM ammonium acetate (solvent A) and methanol (solvent B), and the flow rate was maintained at $300\text{ }\mu\text{L/min}$, with the column oven and autosampler temperatures set to $35\text{ }^{\circ}\text{C}$ and $10\text{ }^{\circ}\text{C}$, respectively. The solvent elution gradient was optimized as follows: 10% B for 1 min, 10–70% B over 6 min, 70–95% B over 14 min, followed by a hold at 95% B for 7 min. The column should be equilibration at 10% B for 4 min between injections.

The process of suspect and non-target screening for PFAS followed a previous study with some modification [21]. In brief, raw data were preprocessed using Compound Discoverer 3.2 for peak picking. Then performed the suspect screening using a list of PFAS from our in-house PFAS database, compiled from the NORMAN Suspect List Exchange and the US EPA CompTox Chemistry Dashboard. As for non-target screening, extracted peaks with mass defects higher than 0.85 or less than 0.15 were retained. Potential PFAS homologues were identified on the basis of mass differences corresponding to CF_2 , C_2F_4 , CF_2O , $\text{C}_2\text{H}_2\text{F}_2$, and $\text{C}_2\text{F}_4\text{O}$ units among the exact masses of peaks, using the non-target package [22]. Additionally, the retention time (RT) of each peak was required to increase with the mass for homologues within each series. Identified the remaining peaks by comparing the accurate MS/MS spectra and masses in the in-house PFAS database, as well as the RTs of commercially available standard compounds. PFAS identification confidence levels were assigned according to the criteria established in a previous study [23].

The target analysis of PFAS was conducted using an ExionLC AC ultrafast liquid chromatograph (LC) coupled to a Triple Quad 5500 mass spectrometer (MS/MS; AB Sciex, Framingham, MA, USA), with chromatographic separation performed on an XBridge BEH C18 XP Column ($10 \times 2.1\text{ mm}$, $2.5\text{ }\mu\text{m}$; Waters, Milford, MA, USA) and temperature maintained at $40\text{ }^{\circ}\text{C}$. The mobile phase consisted of water with 5 mM ammonium acetate (solvent A) and methanol (solvent B). The gradient was set as follows: 0–14 min,

20–90% B; 14–16 min, 90% B; 16–16.01 min, 90–20% B; 16.01–20 min, 20% B. The flow rate and the sample injection volume were set to 300 $\mu\text{L}/\text{min}$ and 5 μL , respectively. Capillary temperature and vaporizer temperature were configured at 320 $^{\circ}\text{C}$ and 350 $^{\circ}\text{C}$. Sheath gas and auxiliary gas were set to 35 (arb.) and 10 (arb.). The samples were analyzed in ESI mode using multiple reaction monitoring (MRM). Table S3 provided detailed information on monitoring transitions of the target PFASs and ISs. The quality assurance and quality control procedures for instrument analysis were described in Text S1 and Table S4.

2.4. Quality Assurance and Quality Control

Rigorous quality assurance and control protocols were implemented throughout the study. To prevent cross-contamination, all glassware was combusted at 450 $^{\circ}\text{C}$ and then sequentially rinsed with acetone, dichloromethane, and n-hexane prior to use. For every batch of twelve samples, triplicate procedural blanks were analyzed to monitor background interference and contamination. Instrumental sensitivity was verified after every twenty sample injections using a 20 ng/mL quality control standard. Reported sample concentrations were blank-corrected. For the LC-MS/MS analysis, matrix recoveries for target PFAS ranged from 87% to 109% in the spiked samples (Table S4).

2.5. Stable Isotope Analysis and Biomagnification Calculation

Stable isotope analysis was the main method employed in this study to investigate the diet and trophic levels of three terrestrial mammal species. Carbon isotope ($\delta^{13}\text{C}$) and nitrogen isotope ($\delta^{15}\text{N}$) measurements were conducted using a Flash HT element analyzer (Thermo Fisher Scientific, Waltham, MA, USA) connected to a Thermo Scientific MAT 253 isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Further details on the stable isotope analysis can be found elsewhere [24].

The biomagnification factor (BMF) was calculated using the Equation (1).

$$\text{BMF} = \frac{C_{\text{predator}}}{C_{\text{prey}}} \quad (1)$$

C_{predator} and C_{prey} represent the concentrations of PFASs in terrestrial mammal muscle tissues for the predator and prey, respectively.

2.6. Risk Modeling

To prioritize the identified PFASs, we applied a risk-based approach developed by Hu et al. (2023) [21] that takes into account both their potential hazard effects and environmental exposure. This methodology consists three key steps: (1) calculating a PBT-based score for each PFAS; (2) assessing exposure through concentrations and detection frequency; (3) ranking PFASs according to their

hazard and exposure levels. Firstly, the persistence, bioaccumulation, and toxicity (PBT) of each PFAS were estimated (encompassing 18 different attributes). We utilized quantitative structure activity relationship (QSAR) models, including EPI Suite v4.11, ECOSAR v2.0, T.E.S.T. v5.1.1, and VEGA v1.2.0 (Text S2 and Table S5). These models were applied following the criteria outlined in REACH Annex XIII and III, as well as GHS classification (Table S6). To evaluate the hazards levels of PFAS, we utilized the normalized PBT values to calculate the ToxPi score for each compound, using the software of Toxicological Priority Index (ToxPi) [25,26]. Secondly, the detection frequency (DF) was defined as the regularity and prevalence of occurrence, while magnitude reflects the intensity of its concentration [Equation (2)] [27]. Exposure was estimated by multiplying DF by PFAS concentration magnitude [Equation (3)], and then normalizing the result to a range of 0–1 [Equation (4)]. Thirdly, the risk index (RI) for each compound was calculated by multiplying its ToxPi score with its normalized exposure value [Equation (5)] [26]. Based on RI values, PFAS were classified into four priority levels: high ($\text{RI} > 0.1$), medium ($0.01 < \text{RI} \leq 0.1$), low ($0.001 < \text{RI} \leq 0.01$), and insignificant priority ($\text{RI} \leq 0.001$).

$$\text{Magnitude} = \frac{C_i - C_{\min}}{C_{\max} - C_{\min}} \quad (2)$$

$$\text{Exposure} = \text{DF} \times \text{Magnitude} \quad (3)$$

$$\text{Exposure}_{\text{normalized}} = \frac{\text{Exposure}_i - \text{Exposure}_{\min}}{\text{Exposure}_{\max} - \text{Exposure}_{\min}} \quad (4)$$

$$\text{RI} = \text{ToxPi Score} \times \text{Exposure}_{\text{normalized}} \quad (5)$$

where C_i refers to the maximum concentration of PFAS i ; $C_{\max}$ represents the highest maximum concentration observed among all PFAS; $C_{\min}$ indicates the lowest maximum concentration found across all PFAS; DF is the detection frequency of PFAS; Magnitude denotes the concentration magnitude of PFAS; Exposure_i is the exposure value for PFAS i ; $\text{Exposure}_{\min}$ is the lowest exposure value observed for all PFAS; $\text{Exposure}_{\max}$ is the highest exposure value among all PFAS.

2.7. Statistical Analysis

Statistical analysis for this study was conducted using IBM SPSS Statistics 25. The normality of the data was assessed using Shapiro-Wilk test. To evaluate statistical significance and examine differences, such as species- and gender- related variations in the PFASs presence among mammals, nonparametric tests and one-way analysis of variance (ANOVA) were used. A significant level of 0.05 was set. The method detection limit (MDL), defined by a signal-to-noise ratio of 3, concentrations below the MDL were imputed as $\text{MDL}/\sqrt{2}$ for statistical analysis (Table S4). Additionally, Spearman's rank correlation test was applied

to examine correlations in the occurrence of PFAS. Principal component analysis was conducted on individual compound concentrations to explore the patterns of PFAS among different terrestrial animals.

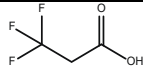
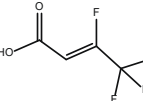
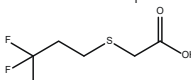
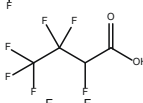
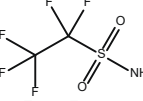
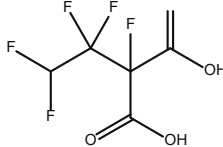
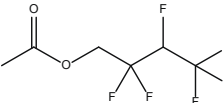
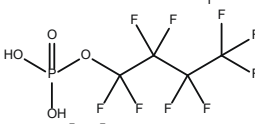
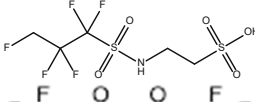
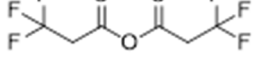
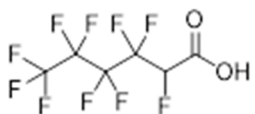
3. Results and Discussion

3.1. Non-Target and Suspect Screening of PFAS

Due to the limited availability of data regarding pollution characteristics such as PFAS species and concentrations in wild animals, a non-target workflow (Figure S2) was devised to identify potential PFAS compounds existing within the terrestrial animal food chain, specifically focusing on hares, hedgehogs, and weasels. Utilizing a non-targeted HPLC-Orbitrap/MS analysis protocol and subsequent procedures, a total of

1922, 1924, and 2019 peaks were initially annotated by comparing accurate masses in the in-house database with a 5 ppm variance in hare, hedgehog, and weasel samples, respectively. Following mass defect filtering and homologue detection based on structural units of C₂F₄, CF₂, CF₂O, C₂H₂F₂, and C₂F₄O, 45465, 43956, and 47104 peaks were retained in the mammal muscle samples of hares, hedgehogs, and weasels, respectively. Subsequently, after suspect and non-target screening processes, 1913, 1823, and 1822 peaks were identified as potential PFAS candidates for weasels, hedgehogs, and hares. Among these candidates, 11 PFAS compounds were successfully identified at a confidence level of 2, as detailed in (Table 1, Figures S3–S13). The 18 PFAS with available reference standards were not identified using non-targeted analysis.

Table 1. Summary of PFAS with level 2 identified by using suspect and non-target analysis. R, H, and W represent samples of hare, hedgehog, and weasel, respectively.

Class	Structure	Formula	Theoretical Mass	Observed Mass	RT/min	Groups	Level
1		C ₃ H ₃ F ₃ O ₂	127.0012	127.0013	2.12	R H	2
2		C ₄ H ₂ F ₄ O ₂	156.9918	156.9909	2.62	H W	2
3		C ₅ H ₇ F ₃ O ₂ S	187.0046	187.0099	3.12	W	2
4		C ₄ H ₂ F ₆ O ₂	194.9886	194.9888	3.25	R W	2
5		C ₂ H ₂ F ₅ NO ₂ S	197.9654	197.9633	3.30	H W	2
6		C ₅ H ₃ F ₅ O ₄	220.9879	220.9960	3.68	H W	2
7		C ₆ H ₆ F ₆ O ₂	223.0199	223.0202	3.72	R H W	2
8		C ₄ H ₂ F ₉ O ₄ P	314.9474	314.9461	5.25	H	2
9		C ₅ H ₈ F ₅ NO ₅ S ₂	319.9691	319.9792	5.33	H W	2
10		C ₆ H ₄ F ₆ O ₃	236.9925	236.9736	8.0601	H	2
11		C ₆ H ₂ F ₁₀ O ₂	294.9822	294.9825	8.8715	W	2

3, 8 and 8 PFAS were detected in the muscle tissues of hares, hedgehogs, and weasels, respectively. Among them, 5

PFAS were co-existed in hedgehogs and weasels, while only 1 PFAS was the same in hares and the other two animals,

respectively. In the food chains involving weasel muscles was found to harbor the highest number of PFAS species compared to hares, and hedgehogs, indicating a significant accumulation of PFAS in weasels. The accumulation and potential transfer of PFAS along food chains raise critical concerns regarding their bioaccumulation and biomagnification in ecosystems [28]. The diversity of PFAS compounds detected in hares, hedgehogs, and weasels—including perfluoroalkyl acids, sulfonic acids, and related derivatives—differs notably from the profiles typically observed in environmental media [29–31].

By situating these findings within the existing literature, this study provides valuable insights into the complexity of PFAS contamination in terrestrial animals and elucidates potential accumulation sources. Furthermore, the utilization of non-targeted screening approaches enhances our understanding of PFAS contamination in wildlife and highlights the need for ongoing monitoring and research to address the environmental and health implications of these persistent compounds [32]. In conclusion, the meticulous analysis of PFAS in wild animals conducted in this study provides essential information on evaluating the extent of pollutants in terrestrial ecosystems and emphasizes the imperative need for further investigations to mitigate the risks linked with PFAS exposure in wildlife and their habitats.

3.2. Concentrations and Distribution Profiles of PFAS

We conducted quantitative analysis of PFAS in the samples using 18 PFAS standards, among which 15 PFAS were detected (Table S7). For subsequent studies on PFAS distribution, biomagnification, and risk assessment in terrestrial mammals, we based the analysis on 15 compounds quantified through target analysis. A total of 15 PFASs were quantified, with at least 14 detected in each of the terrestrial species. PFASs were consistently present in all hare, hedgehog, and weasel samples (Figure 1 and Table S7), indicating their widespread contamination among terrestrial mammal in the agricultural region. One-way ANOVA revealed no significant differences ($p > 0.05$) in $\Sigma_{15}\text{PFAS}$ among terrestrial species at similar trophic level across the sampling areas. Therefore, samples from the same mammal species collected in Xuchang, Luohe, and Zhoukou were pooled for further analysis. The detection rates of individual PFAS compounds varied. With the exception of PFHpA, detection rates of PFAS in the three mammal samples were as follows: PFPeA (100%), followed by PFOA (93.2%), PFOS (89.8%), PFODA (83.1%), PFPeS (81.4%), PFHxDA (79.7%), PFBA (78.0%), PFUnDA (69.5%), PFDA (62.7%), PFHxA (61.0%), PFNA (39.0%), PFDoNA (27.1%), PFTrDA (27.1%), PFHpA, (15.3%), and PFTeDA (8.47%) (Table S8).

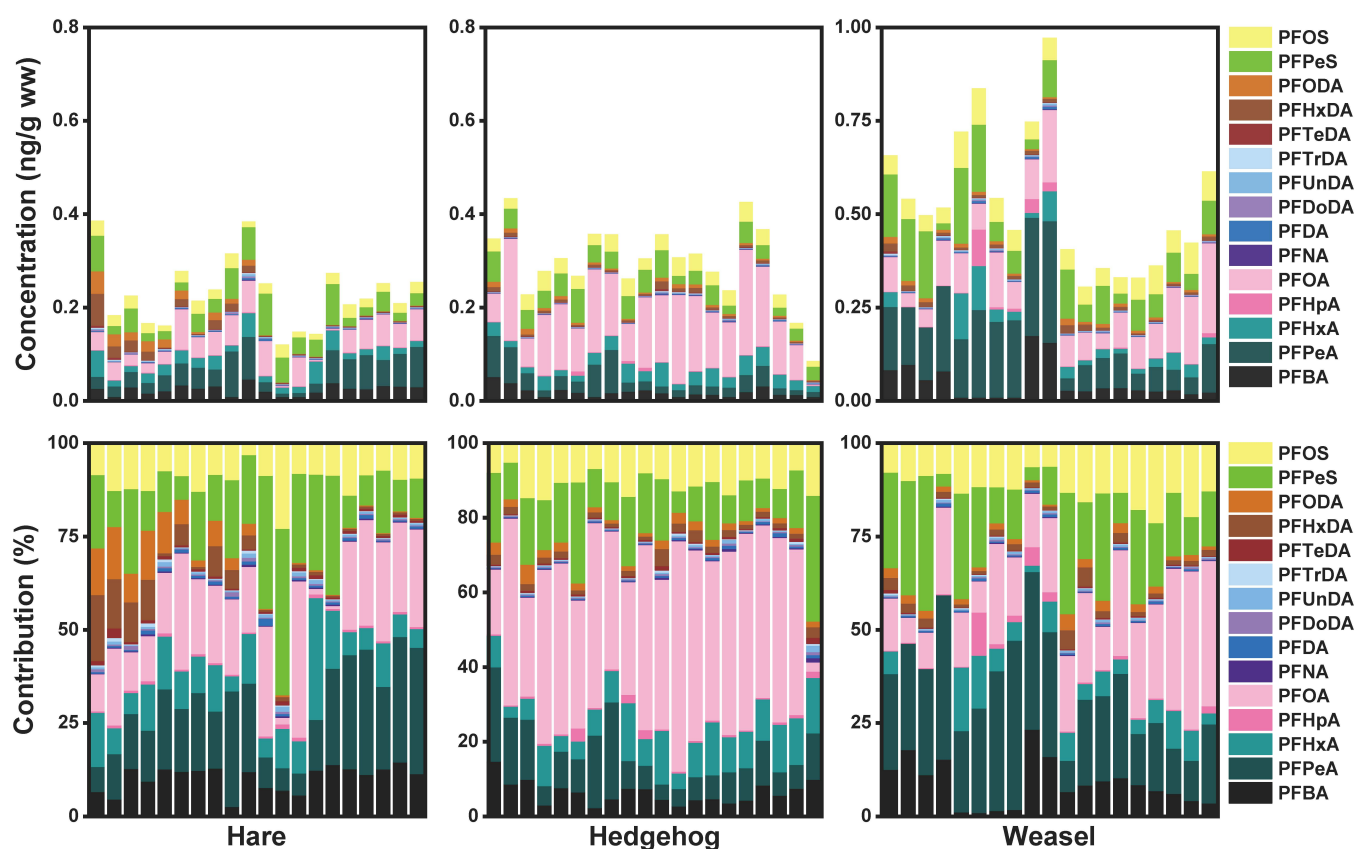


Figure 1. Concentration and accumulation of 15 target PFAS in three biota species collected from the agricultural growing area.

The concentrations and distribution profiles of $\Sigma_{15}\text{PFAS}$ in terrestrial mammals are summarized in Figure

1, with detailed data for individual PFASs in each terrestrial species provided in Table S8. $\Sigma_{15}\text{PFAS}$ concentrations

ranged from 0.121 to 0.386 ng/g wet weight (ww) in hares, 0.086 to 0.434 ng/g ww in hedgehog, and 0.306 to 0.973 ng/g ww in weasels. Among the three terrestrial species, weasels exhibited the highest average PFAS levels ($p < 0.001$, Figure 2). This result is likely due to their diet, which primarily consists of small animals such as hares and hedgehogs. Notably, PFAS concentrations in weasel samples (W1–W19, 0.306–0.973 ng/g ww) were nearly twice those detected in hares and hedgehogs (Figure 2), underscoring their role in regional PFAS accumulation through trophic transfer.

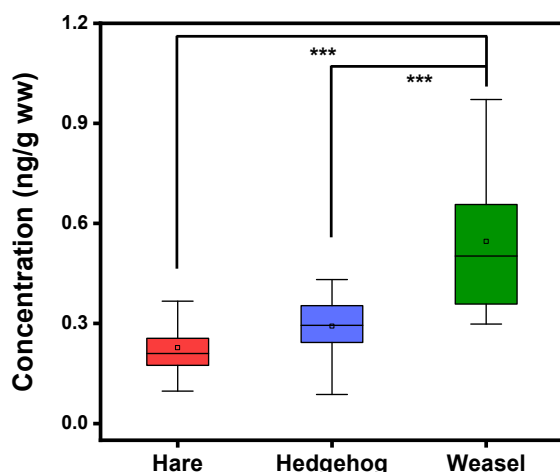


Figure 2. PFAS levels in different species (Hare, hedgehog, and weasel). Boxes indicate 25th, 50th, and 75th percentiles, and whiskers indicate 5th and 95th percentiles. The *** indicate significance at the level $p < 0.001$.

When comparing the Σ PFAS concentrations in terrestrial mammals reported in this study (0.086–0.973 ng/g ww) to those in other regions of China and worldwide, the levels observed here generally lower than those reported for chamois (19.9–30.1 ng/g ww), red deer (23.5–39.2 ng/g ww), roe deer (12.9–53.7 ng/g ww), hare (38.3 ng/g ww), wild boar (71.3–534 ng/g ww), wildcat (23.5–55.0 ng/g ww) in Germany [33]. Similarly, these concentrations are lower than those found in mammals from a desert oasis from New Mexico (0.25–79.6 ng/g ww) [34], terrestrial organisms from the south-western Europe and West Africa (10.24–128 ng/g ww) [35], and roe deer liver (<2.6–21.9 ng/g ww) from agrarian or forestry ecosystems in Germany [11]. However, the Σ PFAS concentrations from this study overlap with those reported for as well as caribou (0.189–0.271 ng/g ww) and wolf (0.74–0.87 ng/g ww) from Northwest Territories and western Nunavut from Canada [9]. The reported PFAS concentrations also were comparable or slightly lower with pika muscles (0.601–8.293 ng/g dw) in Tibetan Plateau [36], and wolf (1.32–5.39 ng/g ww), moose (0.45–0.70 ng/g ww), roe deer (0.56–3.01 ng/g ww), and Arctic fox (9.48–53.4 ng/g

ww) in Norwegian wildlife [8]. These comparison highlights the variability in PFAS accumulation across different species and geographic regions. These regional differences might be account for the different industrial history (e.g., less intensive historical production/use of certain PFAS), retention and bioavailability, and climatic conditions. Differences in geographic characteristics may contribute to variations in PFAS concentrations among species; Meanwhile, as a key component of terrestrial ecosystems, farmland soil acts as a critical reservoir and distribution center for PFASs. Sources such as sludge application, sewage irrigation, and improper waste disposal can directly introduce these persistent chemicals into soil system, further influencing their environmental fate and potential entry into food webs [37]. Furthermore, the accumulation and elimination of PFASs in wildlife are primarily governed by their interactions with proteins. In wildlife, physiological responses are dynamic and adaptive, often influenced by changes in biotic and abiotic environment factors—such as temperature, salinity, reproductive stage, and overall health status [38].

Due to the limited data on PFAS in terrestrial biota worldwide, a comprehensive global comparison of PFAS levels remains challenging. In this study, Σ_{15} PFAS concentrations were notably higher in carnivores (weasel, 0.306–0.973 ng/g ww) compared to omnivores (0.086–0.434 ng/g ww) and herbivores (0.121–0.386 ng/g ww), this is likely due to its higher trophic position and predatory diet, leading to the accumulation of PFAS biomagnified from its prey. These findings align with previous observations of carnivores, omnivores, and herbivores in Germany [33]. Additionally, as observed in Norway, where targeted PFAS concentrations in moose liver were lower than in marine mammals [8], this study provides valuable insight into the biomagnification potential of PFASs in terrestrial mammals. In addition, anthropogenic activities, regional pollution levels, and species-specific traits are critical factors determining the extent of PFAS accumulation in global biota [35,36]. Overall, the PFAS contamination levels in terrestrial mammals observed in this study are relatively low to moderate on an international scale, with regions such as Germany, Canada, and Norway reporting higher contamination. Beyond trophic class, ecological habitats also exerts a significant influence on PFAS levels across species [33].

As shown in Table S7, PFOA (MDL–0.240 ng/g ww) exhibited the highest mean concentration of 0.093 ng/g ww, making it the most abundant compound across all species. This was followed by PFPeA, PFPeS, PFOS, and PFBA which collectively accounted for 51.4–94.1% of the total Σ_{15} PFASs (Figure 1, Table S7). The high solubility of PFOA, PFOS, and PFBA (Table S1) likely explains its predominance, consistent with findings from previous studies [10,36]. As shown in Figure 1, PFHpA, PFNA, PFDoDA, PFTrDA, and PFTeDA were detected at low and

similar concentrations, contributing less than 5.76% of the total PFASs in mammals from the agricultural area. The environmental accumulation of PFASs, such as PFOS, may also result from abiotic [39] and biological degradation of precursors [40]. For larger molecules like PFTeDA and PFHxDA, steric hindrance in mammalian tissues likely contributes to their lower accumulation [10], suggesting that ongoing environmental degradation of PFAS precursors could be a secondary source contributing to the burden of terminal perfluoroalkyl acids in wildlife.

Additionally, a principal component analysis was then conducted to investigate potential contaminant patterns in terrestrial species. As showed in Figure 3, the score plot revealed that the samples from hedgehogs formed a distinct cluster at the bottom, while the samples from the other two terrestrial mammals were generally more dispersed. These suggests a relatively homogeneous that PFAS profile in hedgehogs, whereas PFAS levels in weasels showed greater variability. Specifically, hares tend to

accumulate PFODA, and PFTeDA, while weasels accumulate a wider range of PFAS, excluding PFODA, and PFTeDA. The comprehensive approach used in this study demonstrated that trophic class is a primary determinant of PFAS concentration, with clearly differences observed among herbivores, omnivores and carnivores in both PFAS and profiles. The pronounced differences may be attributed to variations in trophic class and ecological habitat. The greater dispersion of hare and weasel samples could be attributed to more variable individual diets (e.g., different plant types for hares, different prey compositions for weasels) and/or home ranges with varying local contamination. The differential loading of specific PFAS (e.g., PFODA, PFTeDA in hares) is also discussed in relation to potential source pathways or compound-specific accumulation behaviors linked to their feeding ecology. Furthermore, species-specific physiological processes—such as absorption, excretion, metabolic conversion rates, and distribution—as well as prey composition, also influence PFAS accumulation [33].

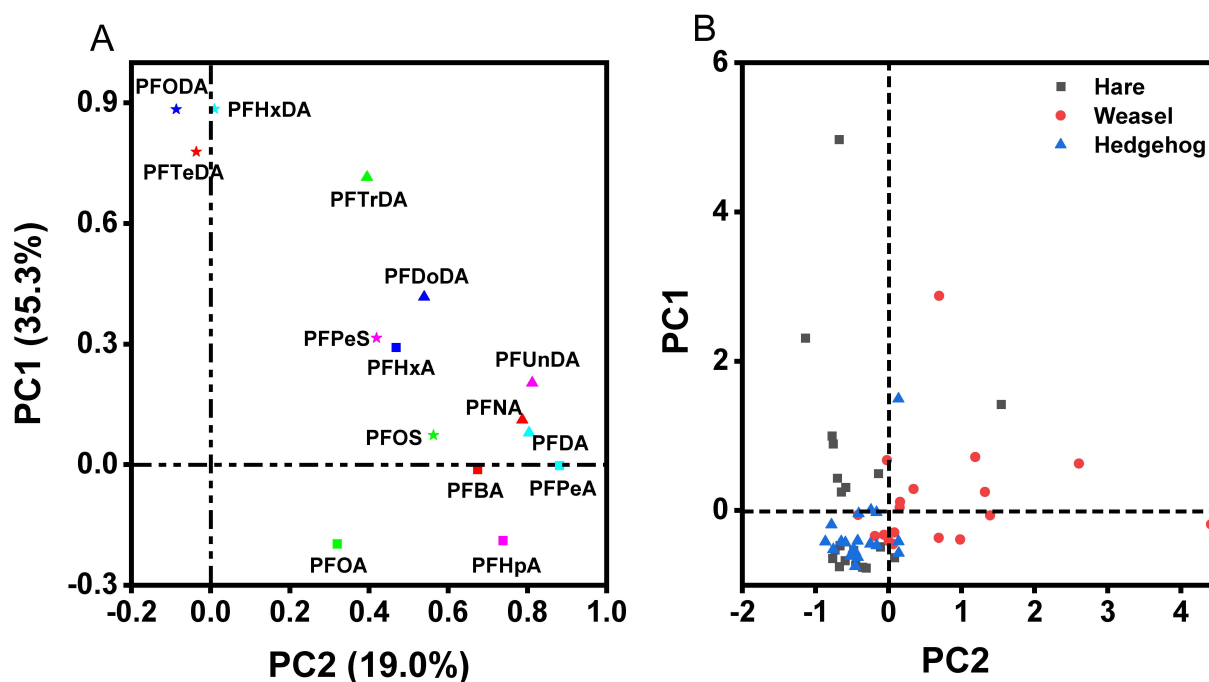


Figure 3. PCA plots with the composition of PFASs based on concentrations. (A): Factor scores on the basis of three terrestrial mammals, (B): factor loadings.

3.3. Transfer and Biomagnification of PFAS

The transfer of PFAS from herbivores (hares) and omnivores (hedgehogs) to carnivores (weasels) was examined in this study. The predator-prey relationships presented here are based on stable isotope analysis. It should be noted that direct dietary evidence (e.g., stomach content analysis) was not available, which represents a methodological limitation. Hares and hedgehogs are frequently exposed to PFAS-accumulating food sources, such as grass leaves, beetle, and mole-cricket, which subsequently transfer PFASs to their predator, such as

weasels. Biomagnification is more conclusive for assessing the bioaccumulation of chemicals and indicates when the biomagnification factor (BMF) between predator and prey exceeds 1, emphasizing the importance of identifying predator-prey relationships within the same food web [41]. Hedgehogs are opportunistic feeders with a diverse diet that varies depending on the local environment. They primarily prey on terrestrial organisms such as grasshoppers, beetles, crickets, mantises, and dragonflies. To date, no references have specifically documented terrestrial food items for hedgehogs and weasels. In this study, stable isotope analysis was conducted to investigate

predator-prey relationships, with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the three terrestrial species determined (Figure S14 and Table S9). The $\delta^{13}\text{C}$ difference between hares and hedgehogs was 0.7‰, indicating that both species share a similar food web. Based on these findings, biomagnification factors (BMFs) of PFASs among hares, hedgehogs, and weasels were assessed within the agricultural area. The evaluated BMFs for $\Sigma_{15}\text{PFASs}$ ranged from 0.84 to 4.38, with average BMF values > 1 indicating that PFASs can bioaccumulate in terrestrial mammals. Variations in BMFs between species likely result from differences in feeding metabolism, dietary habits, selective excretion abilities, and lipid composition [38,42]. The BMF values for hares to weasels (0.87–4.38) and hedgehogs to weasels (1.08–4.31) were slightly lower than those reported for PFASs in the plant–pika–eagle food chain, where BMFs were 6.55 (plant to pika) [36]. Among the three terrestrial species here, relatively high BMFs of PFPeA (3.11), PFHpA (4.38), PFOA (2.41), PFPeS (2.31), PFOS (2.79) were observed in hares–weasels, and values of PFBA (2.60), PFPeA (4.31), PFHpA (2.65), PFUnDA (2.45), PFPeS (2.42) in food chain of hedgehog–weasel (Table S11), which might be attributed to their octanol–water partition coefficients, octanol–air partition coefficient, as well as acid dissociation constant because they predominantly exist as nonvolatile anions in the environment or biota [36]. Conversely, lower BMFs were found between hares and weasels for PFHxDA (0.900), PFODA (0.868), and PFOA (0.843) (Table S10), although BMFs for ΣPFASs in this pair generally exceeded 1. These findings align with observations from other ecosystems, including the biomagnification from voles to owl eggs [43], PFAS transfer in the Lake Erie food web across fish, benthos, and zooplankton [44], and accumulation along a terrestrial food chain in a Nordic skiing area involving earthworms and bank voles [45]. It was worth noting that the BMFs of PFHxA, PFNA, PFTeDA, and PFPeS were comparable in both the hare–weasel and hedgehog–weasel food chain.

A negative relationship was observed between log BMF and both log K_{PW} and molecular volume for all PFASs (Figure 4). These findings are generally consistent with earlier studies. Specifically, the increase in BMFs with carbon chain length noted in this study corresponds to trends reported in marine organisms such as fish [46]. In general, longer-chain PFASs exhibit stronger protein binding affinity, which supports the relationships between bioaccumulation potential and carbon chain length reported in earlier research [10]. It is therefore reasonable that PFASs with higher log K_{PW} values demonstrate great bioaccumulation potential. However, the result was different with those PFASs from a study on the tropical amphibian species *Fejervarya limnocharis*, where no clear relationship was found between log BMF and log K_{PW} for PFAS [47].

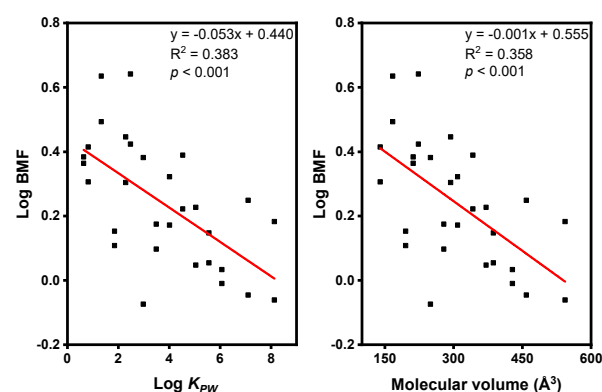


Figure 4. Correlations between log BMF and log K_{PW} and between log BMF and molecular volume ($\text{\AA}^3$) of PFAS.

The relationship between molecular volume of PFAS suggesting that molecular volume is an important factor influencing the bioaccumulation of chemicals in organisms. The accumulation of short-chain PFASs in mammals is likely attributed to a combination of the nonvolatility of ionized forms and high K_{OA} of their neutral forms, as well as the corresponding low rate of respiratory elimination [36]. PFAS with longer carbon chains generally exhibit stronger protein binding affinities [38], and greater molecular volumes [10]. In addition, biotransformation, elimination processes, together with the dietary habits of organisms significantly influence their biomagnification behavior. Hares, primarily herbivores, feed on grass roots, leaves, seeds, and buds [33]. Hedgehogs, on the other hand, consume insects such as grasshoppers, beetles, crickets, mantises, and dragonfly larvae. Weasels, as carnivores, have a diet that includes pika, hares, frogs, and other small vertebrates. Short chain precursors abundant in the environment can be metabolized into PFBA within organisms, further enhancing their bioaccumulation within the food chain [36].

3.4. Risk-Based Prioritization

To identify high risk PFAS, we adopted a risk-based prioritization model from a previous study [21] which integrated 18 PBT attributes along with the environmental exposure data. Unlike conventional PFAS risk assessment that rely solely on ecotoxicological or human health effects [48,49], this prioritization model incorporated multiple hazard properties. For persistence, all identified PFAS, except PFTeDA and PFBA, met three criteria, with BIOWIN 1, BIOWIN 3, and BIOWIN 5 were less than 0.5, 2.25, and 0.5. PFODA exhibited the highest persistence, classifying it as extremely persistence. Regarding bioaccumulation, 6 PFAS met the threshold of bioconcentration factor (BCF) > 2000 L/kg ($\text{Log}_{BCF} > 3.30$), with PFDA showing the highest bioaccumulation value ($\text{Log}_{BCF} = 4.14$; Table S11). For ecotoxicology, 5 PFAS met the aquatic toxicity criteria of LC50 or EC50 was less than

0.1 mg/L, with PFODA exhibiting the highest aquatic toxicity (Table S11), indicating a significant risk to aquatic organisms. For human health effects, 12 PFAS were associated with possible carcinogenicity, PFPeS with possible Eye irritation/corrosion, 9 PFAS with potential Skin irritation/corrosion, 6 PFAS showed potential skin sensitization, and 3 PFAS displayed acute toxicity to human, as indicated by an oral rat LD50 < 300 mg/kg (Table S11). None of the identified PFAS were found to have developmental toxicity, endocrine-disrupting effect, mutagenicity, and hepatotoxicity. ToxPi scores for 15 PFAS ranged from 0.1306 to 0.6645 (Table S12 and Figure 5), with PFTeDA having the highest ToxPi score (score = 0.6645, rank = 12/15), followed by PFODA (score = 0.6163, rank = 7/15). Longer carbon chain length PFAS generally had higher ToxPi scores ($r^2 = 0.764$, $p < 0.001$; Figure S15), possibly due to their greater PBT behavior to daphnid and green alga [21]. For instance, long-chain PFCA and PFSA exhibited greater bioaccumulation potential in aquatic food webs compared to short-chain PFCA [50]. PFSA with longer carbon chains also demonstrated higher toxicity to soil microorganisms [51] and greater developmental neurotoxicity to zebrafish [52].

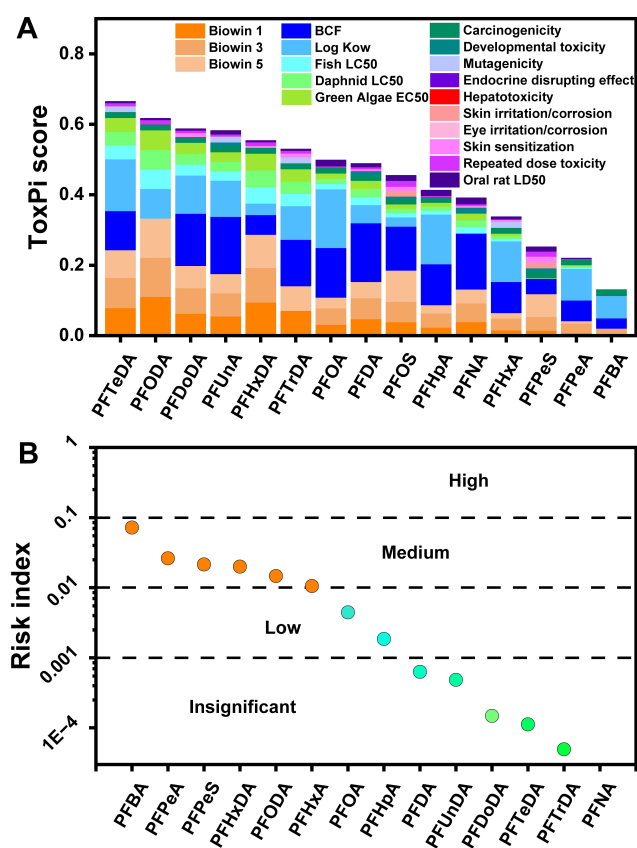


Figure 5. ToxPi scores (A) and risk index (B) of 15 PFAS quantified in the terrestrial samples.

Our study prioritized 4 PFAS with medium priority for terrestrial mammals in a typical agricultural region (Table S12 and Figure 5). Using DF and concentration magnitude for exposure assessment, we calculated

exposure values ranged from 0 to 1.0000 for 15 PFAS, and found 9 PFAS [i.e., PFPeA, PFOA, PFPeS, PFBA, PFOS, PFHxA, PFHxDA, PFODA, and PFHpA] with exposure values ≥ 0.01 (Table S12). These nine PFAS included the ubiquitous compounds such as PFOA, as well as PFOS, both of which had high detection frequencies greater than 80%. We calculated the risk index to prioritize PFAS in terrestrial mammal samples, and the values ranged from 0 to 0.3426 for 15 PFAS (Table S12 and Figure 5), with PFOA exhibited the highest priority. 4 PFAS (i.e., PFOA, PFPeA, PFPeS, and PFOS) exhibited a risk index greater than 0.1 and were categorized as high priority. PFPeA was included in the high priority list due to its high exposure normalized value. Additionally, 4 PFAS (i.e., PFHxDA, PFHxA, PFODA, and PFBA) exhibited a risk index greater than 0.01 and were categorized as medium priority.

4. Conclusions

This study demonstrates widespread contamination and clear biomagnification of PFAS in terrestrial mammals (hares, hedgehogs, and weasels) from a typical Chinese agricultural region. The research identified a diverse range of PFAS, with significantly higher concentrations observed in carnivorous weasels compared to herbivorous hares and omnivorous hedgehogs. A risk prioritization framework identified four PFAS (PFOA, PFPeA, PFPeS, and PFOS) as high priority due to their combined hazard potential and exposure levels. These findings provide critical insights into PFAS behavior in agricultural ecosystems, underscore the ecological risks posed to wildlife, and offer a science-based strategy for prioritizing PFAS management and monitoring in terrestrial environments. The integrated framework of non-target screening, trophic assessment, and risk-based prioritization developed here can be adapted for PFAS assessment in other terrestrial ecosystems. In this study, we acknowledged the moderate sample size per species and states that the opportunistic sampling for these terrestrial mammals over three years, while providing a valuable snapshot, could not systematically capture seasonal, interannual or gender variations, and suggest that future monitoring with structured design. Moreover, sampling in present study only deceased or injured animals might introduce a bias if PFAS body burdens are correlated with health status, and that this could affect the representativeness of our data for the overall healthy wild populations.

Supplementary Materials

The additional data and information can be downloaded at: <https://media.sciltp.com/articles/others/2609011052549804/GES-25120211-SM-FC.pdf>. Text S1: Quality assurance and quality control; Text S2: PBT prediction and ToxPi score calculation of PFAS, Supporting tables, and figures. Table S1: Details of abbreviations, names, formulas and logKow values for 18 PFAS and 9 isotope-labeled internal standards; Table S2:

Details of 59 sampled mammals; Table S3: Precursor and product ions of target PFAS and ISs in MRM mode. Table S4. The method detection limit (MDL) and the matrix spiked recoveries of PFASs were determined through LC-MS/MS analysis. Table S5. Details on the criteria and attributes used to calculate the ToxPi Score of PFASs. Table S6. Criteria for identification of PBT substances. Table S7. Descriptive statistics of detection frequency (DF) and [mean (range), ng/g wet weight] for PFAS calculated in terrestrial samples (n = 59). Table S8. Concentration (ng/g, ww) of target 15 PFAS in the terrestrial species collected from the agricultural growing area. Table S9. Stable isotope ratios for three terrestrial species in this study. Table S10. BMF values of PFAS in the hare–weasel and hedgehog–weasel food chain. Table S11. Persistence, bioaccumulation, ecotoxicological effects, and human health effects of PFASs used to calculate the ToxPi scores. Table S12. ToxPi scores, exposure, and risk indexes (RIs) of PFAS in samples of wild mammals. Figure S1. Map of the sampling areas and study region. Figure S2. Workflow for target, suspect and non-target screening of PFAS and risk-based prioritization. R, H, and W represent samples of hare, hedgehog, and weasel, respectively. Figure S3. MS/MS spectrum of C₃H₃F₃O₂. Figure S4. MS/MS spectrum of C₄H₂F₄O₂. Figure S5. MS/MS spectrum of C₅H₇F₃O₂S. Figure S6. MS/MS spectrum of C₄H₂F₆O₂. Figure S7. MS/MS spectrum of C₂H₂F₅NO₂S. Figure S8. MS/MS spectrum of C₅H₃F₅O₄. Figure S9. MS/MS spectrum of C₆H₆F₆O₂. Figure S10. MS/MS spectrum of C₄H₂F₉O₄P. Figure S11. MS/MS spectrum of C₅H₈F₅NO₅S₂. Figure S12. MS/MS spectrum of C₆H₄F₆O₃. Figure S13. MS/MS spectrum of C₆H₂F₁₀O₂. Figure S14. Correlations between carbon stable isotopes ($\delta^{13}\text{C}$) and nitrogen stable isotopes ($\delta^{15}\text{N}$) in three terrestrial mammal species. Figure S15. The relationship between ToxPi scores and the number of carbon atoms. References [53–58] are cited in the Supplementary Materials.

Author Contributions

C.Z.: Conceptualization, Investigation, Data curation, Software, Validation, Writing—original draft, Funding acquisition, Writing—review & editing. Z.Q.: Supervision, Resources, Project administration, Writing—review & editing. B.H.: Conceptualization, Software, Validation, Funding acquisition, Writing—review & editing. Y.L. (Yu Liu): Conceptualization, Software, Validation. X.H.: Investigation, Data curation. Y.L. (Yuejie Li): Writing—review & editing. J.Z.: Data curation. C.Y.: Writing—review & editing. Y.L. (Yine Liu): Conceptualization, Software, Validation, Funding acquisition. L.L.: Supervision, Resources, Project administration, Funding acquisition, Writing—review & editing. H.Y.: Writing—original draft, Writing—review & editing. Supervision, Project administration. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was approved by the Research Ethics Committee of Guangdong University of Technology (Approval No. GDUTXS2023093, approved on 13 March 2023).

Informed Consent Statement

Not applicable.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors used DeepSeek to language editing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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