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Toxicological effects of per- and polyfluoroalkyl substances (PFASs) on earthworms: Progress and prospects

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A R T I C L E I N F O

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A B S T R A C T

As a class of emerging persistent organic pollutants (POPs), per- and polyfluoroalkyl substances (PFASs) are widely detected in the soil environment, posing a significant threat to the soil ecosystem and human health. Therefore, it is necessary to study the ecotoxicological effects of PFASs in soil. In this study, we conducted a comprehensive review of the toxic effects of PFASs on earthworms at the individual and sub-individual levels, including survival status, body weight, reproduction, oxidative damage, genes, metabolism, and so on. Results showed that earthworms exposed to certain concentrations of PFASs display various pathological symptoms on their body surfaces, a decrease in body weight and reproductive rate, and even death. The LC_{50} values of PFOS to earthworms (365–1404 mg/kg) are consistently lower than those of PFOA (544–1307 mg/kg) under the same exposure condition, indicating a higher toxicity of PFOS compared to PFOA. At the sub-individual level, PFASs may induce oxidative stress, DNA damage, aberrant gene expression, and metabolic disruption in earthworms. PFOS induced disruption of the nervous and metabolic system, PFHxS disrupted energy balance and elicited inflammation, and PFBS induced cell apoptosis in earthworms. Compared to PFOS, PFHxS may induce a greater degree of oxidative stress and damage, and 6:2 Cl-PFESA (F—53B) exhibited a greater propensity to disrupt the extracellular matrix and induce cellular ferroptosis and apoptosis in earthworms. At environmentally relevant concentration levels, PFOA induces significant dysregulation of pathways related to amino acid, energy, and sulfur metabolisms within earthworms. Bioavailability and bioaccumulation capacity of PFASs are important factors in determining their toxicological effects in soil, which is influenced by the molecular structure of PFASs and the combined effects of various environmental factors, such as soil organic matter composition and content, pH, PFAS concentrations and exposure duration. Finally, existing research deficiencies and future directions about the toxicological research of PFASs on earthworms are proposed, aiming to offer reference for ecological risk assessment of PFASs-contaminated soil.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a group of anthropogenically synthesized persistent organic compounds in which some or all hydrogen atoms on the carbon backbone are replaced by fluorine atoms (Buck et al., 2011). Due to the hydrophobicity, oleophobicity, high surface activity, heat resistance, corrosion resistance, and other characteristics of PFASs, they are widely used in packaging, textile, leather, fire-fighting foams, shampoo, floor polishing, electroplating, and other industrial and civil fields (Wang et al., 2014). The presence of high-energy carbon-fluorine bonds in the molecular structure of PFASs makes them difficult to hydrolyze, photolyze and biodegrade, resulting in their environmental persistence (Olsen et al., 2007; Tang et al., 2024; Wang et al., 2022a; Wang et al., 2024b). In recent years, PFASs have been widely detected in environmental media such as soil, sediment, air and water, and have been found to migrate and transform to varying

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degrees in different environmental media (Jin et al., 2015; Shen et al., 2023; Wang et al., 2024b; Zhu et al., 2024). PFASs can be absorbed by plants, and enter animals through ingestion, respiration, and skin contact, and accumulate within the bodies, exerting toxicity on the endocrine, reproductive, immune, and nervous systems, ultimately posing harm to ecosystems and human health (Chen et al., 2022; Chen et al., 2024; Hong et al., 2024; Jian et al., 2017; Jin et al., 2020; Xiang et al., 2023). Since 2009, perfluorooctane sulfonate (PFOS) and its salts, perfluorooctane sulfonyl fluoride (PFOSF), perfluorooctanoic acid (PFOA) and its salts and related compounds have been successively listed as new persistent organic pollutants (POPs), and their production and use have been strictly controlled, attracting high attention from many countries and organizations worldwide (Lindstrom et al., 2011; Ministry of Ecology and Environment, 2019; UNEP/POPS/POPRC.15/2, 2019).

Soil is the Earth's precious natural resource and the core of the terrestrial ecosystem, serving as the essential foundation for human survival. Many studies have shown that PFASs can be detected in various soils from industrial pollution sites, residential areas, agricultural fields, and so on. The median concentrations of Σ_{93}PFASs were 2670, 1480, 1340 and 53 ng/g dw, respectively, in soils from four airport areas where fire training activities had taken place in Canada (Liu et al., 2022). The residual concentrations of Σ_{21}PFASs ranged from 0.244 to 13.6 ng/g dw in soils collected from residential areas in China in 2019 (Li et al., 2020). In 2023, the Σ_{17}PFASs in the agricultural soils (including open-field and greenhouse soils) from the Fen-Wei Plain, China, ranged from 0.669 to 3.26 ng/g dw (Shen et al., 2023). PFASs can alter the structure of soil microbial communities, inhibit soil enzyme activities, and suppress the growth of soil bacteria, consequently disrupting the normal function of soil (Xing et al., 2023). In addition, PFASs in soil can be absorbed and accumulated by plants and soil animals, subsequently leading to toxic effects at the physiological, biochemical, and molecular levels (Adu et al., 2023; Li et al., 2023; Munoz et al., 2020; Xu et al., 2022; Zhu et al., 2021). Overall, PFASs in soil can damage soil ecosystem, so it is necessary to conduct ecological risk assessments of PFASs-contaminated soils.

Earthworms, as a kind of invertebrate with the largest biomass in soil, with principal organs including the digestive system (mouth, pharynx, esophagus, crop, gizzard, intestine), nervous system (cerebral ganglia, ventral nerve cord), circulatory system (dorsal blood vessel, ventral blood vessel), respiratory system (body wall), excretory system (nephridia), and reproductive clitellum (Fig. 1) (Wang et al., 2009), play a crucial ecological role in soil formation, soil structure and fertility conservation, environmental protection, and so on (Jouquet et al., 2006). Due to the widespread distribution in soil and high susceptibility to pollutants, earthworms are often used as indicator organisms of soil pollution. This paper comprehensively reviews the research progress on the toxicity of PFASs to earthworms at the individual and sub-individual levels, with the main types of PFASs involved as shown in Table 1, and analyzes the toxicity influencing factors, and puts forward the future research trends in the hope of providing guidance for the assessment, diagnosis, and remediation of soils contaminated with PFASs.

2. Toxic effects at the individual level

Current studies on the toxicity of PFASs to earthworms at the individual level primarily focus on indicators such as survival, reproduction, weight change, pathological symptoms, and avoidance behavior. Furthermore, the selected target pollutants mainly include PFOA and PFOS.

Table 1
The basic information regarding PFASs discussed in this paper.

Full chemical name	Abbreviation	Molecular formula	CAS number
Trifluoroacetic acid	TFA	$C_2HF_3O_2$	76–05-11
Perfluoropropionic acid	PFPrA	$C_3HF_5O_2$	422–64-0
Perfluorobutanoic acid	PFBA	$C_4HF_7O_2$	375–22-4
Perfluoropentanoic acid	PFPeA	$C_5HF_9O_2$	335–67-1
Perfluorohexanoic acid	PFHxA	$C_6HF_{11}O_2$	307–24-4
Perfluoroheptanoic acid	PFHpA	$C_7HF_{13}O_2$	375–85-9
Pentadecafluorooctanoic acid	PFOA	$C_8HF_{15}O_2$	335–67-1
Perfluorononanoic acid	PFNA	$C_9HF_{17}O_2$	375–95-1
Perfluorobutanesulfonic acid	PFBS	$C_4HF_9O_3S$	375–73-5
Perfluorohexanesulphonic acid	PFHxS	$C_6HF_{13}O_3S$	355–46-4
Perfluorooctanesulfonic acid	PFOS	$C_8HF_{17}O_3S$	1763-23-1
6:2 Fluorotelomer carboxylic acid	6:2 FTCA	$C_8H_3F_{13}O_2$	53,826–12-3
6:2 Fluorotelomer sulfonic acid	6:2 FTSA	$C_8H_5F_{13}O_3S$	27,619–97-2
6:2 Fluorotelomer sulfonamidoalkyl betaine	6:2 FTAB	$C_{15}H_{19}F_{13}N_2O_4S$	34,455–29-3
6:2 Chlorinated polyfluoroalkyl ether sulfonate	6:2 Cl-PFESA	$C_8HClF_{16}O_4S$	73,606–19-6
N-ethyl perfluorooctane sulfonamide ethanol	N-EtFOSE	$C_{12}H_{10}F_{17}NO_3S$	1691-99-2
Acetic acid, 2,2-difluoro-2-[[2,2,4,5-tetra- fluoro-5-(trifluorometoxy)-1,3-dioxolan-4-l]oxy]-, ammonium salt	cC6O4	$C_6H_4F_9NO_6$	1,190,931–27-1

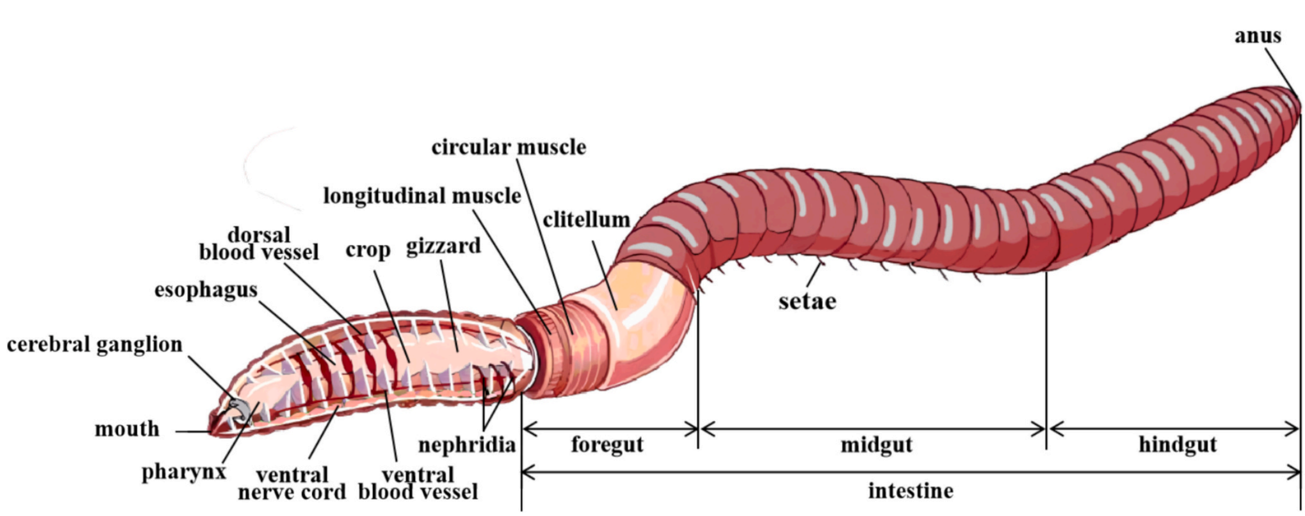

Fig. 1. The principal anatomical composition of the earthworms. Modified from Wang et al. (2009).

2.1. Survival status

The acute toxicity test for earthworms, a method in which the pollutants are exposed at a high dose once or multiple times within a short period, with the survival status as the endpoint, assesses the toxicity level of pollutants by determining the median lethal concentration (LC_{50}). Due to its short testing duration and easy operation, the acute toxicity testing method is widely used in ecotoxicological assessment of earthworms. The LC_{50} values of PFASs for earthworms in existing literature are shown in Table 2. The filter paper contact method determined the 24-h LC_{50} values of 25.7 to 32.4 μg/cm^2 for PFOA and 20.5 to 24.2 μg/cm^2 for PFOS, while the 48-h LC_{50} ranged from 19.3 to 26.7 μg/cm^2 for PFOA and 13.6 to 15.0 μg/cm^2 for PFOS. According to the classification criteria for determining the toxicity of chemicals to earthworms using the filter paper contact method (Roberts and Dorough, 1984), the LC_{50} values measured mostly ranged from 10 μg/cm^2 to 100 μg/cm^2, indicating high toxicity, whereas only one study measured that the 24 h-LC_{50} and 48 h-LC_{50} of PFOA for earthworms were 235 and 144 μg/cm^2 respectively, indicating moderate toxicity (Feng, 2016). Determined through the soil cultivation method, the 7-day LC_{50} range of PFOA and PFOS for earthworms (*Eisenia fetida*) was found to be 711 to 1307 mg/kg and 405 to 1404 mg/kg, respectively, while the 14-day LC_{50} values ranged from 544 to 1001 mg/kg for PFOA and 365 to 984 mg/kg for PFOS. According to the globally unified toxicity grading standard (ISO 11268-1, 2012), the LC_{50} values obtained through both artificial soil and natural soil methods fall within the range of 300 to 2000 mg/kg, indicating low toxicity. The 14d-LC_{50} of PFOA or PFOS for earthworms measured by soil test was lower than 7d-LC_{50}, and the 48 h-LC_{50} measured by filter paper method was also lower than 24 h-LC_{50}, indicating that the longer the exposure time, the greater the toxicity to earthworms. The LC_{50} of PFOS for earthworms was generally lower than that of PFOA, indicating that PFOS exhibited greater toxicity compared to PFOA. The fact that the LC_{50} of 6:2 Cl-PFESA for earthworms (7d-LC_{50}: 1118 mg/kg, 14d-LC_{50}: 816 mg/kg) was lower than that of PFOS

Table 2
The median lethal concentration (LC_{50}) of PFASs to earthworms.

Compound	Exposure medium	Earthworm species	Exposure concentration	Exposure time	LC_{50}(95 % Cl)	References
PFOA	Artificial soil	*Eisenia fetida*	500, 750, 1125, 1690, 2530 mg/kg	7 d 14 d	1307 mg/kg 1001 mg/kg	(Joung et al., 2010)
PFOS			100, 160, 256, 410, 655, 1050 mg/kg	7 d 14 d	405 mg/kg 365 mg/kg	
PFOA	Artificial soil	*Eisenia fetida*	50, 250, 400, 470, 540, 600, 800 mg/kg	14 d	760 mg/kg	(Zheng et al., 2016)
PFOS			50, 250, 400, 470, 540, 600 mg/kg	14 d	478 mg/kg	
PFOA	Natural soil	*Eisenia fetida*	560, 640, 720, 800, 880, 960 mg/kg	7 d 14 d	850 mg/kg 811 mg/kg	(Yuan et al., 2017)
	Filter paper		16, 20, 24, 28, 32, 36 μg/cm^2	24 h 48 h	32.4 μg/cm^2 26.3 μg/cm^2	
PFOS	Natural soil		320, 400, 480, 560, 640, 720 mg/kg	7 d 14 d	628 mg/kg 541 mg/kg	
	Filter paper		4, 8, 12, 16, 20, 24 μg/cm^2	24 h 48 h	21.3 μg/cm^2 15.0 μg/cm^2	
PFOA	Artificial soil	*Eisenia fetida*	100, 307.2, 786.4, 1000, 1268.3 mg/kg	7 d	965 mg/kg	(Zhu, 2015)
			120, 242.8, 491.5, 868,1268.3 mg/kg	14 d	933 mg/kg	
	Filter paper		10, 20, 30, 40, 50 μg/cm^2	24 h 48 h	25.7 μg/cm^2 19.3 μg/cm^2	
PFOA	Artificial soil	*Eisenia fetida*	50, 100, 200, 400, 800 mg/kg	7 d 14 d	711 mg/kg 544 mg/kg	(Feng, 2016)
	Filter paper		50, 100, 150, 200, 300 μg/cm^2	24 h 48 h	235 μg/cm^2 144 μg/cm^2	
PFOA	Artificial soil	*Eisenia fetida*	560, 640, 720, 800, 880, 960 mg/kg	7 d 14 d	817 mg/kg 793 mg/kg	(Xu et al., 2012)
PFOS	Artificial soil	*Eisenia fetida*	100, 491.5, 786.4, 1000, 1258 mg/kg	7 d 14 d	936 mg/kg 857 mg/kg	(Yao et al., 2013)
	Filter paper		7.5, 10, 30, 40,50 μg/cm^2	24 h 48 h	24.2 μg/cm^2 15.0 μg/cm^2	
PFOS	Artificial soil	*Eisenia fetida*	50, 250, 400, 470, 540, 600 mg/kg	14 d	478 mg/kg	(Zheng et al., 2013b)
PFOS	Artificial soil	*Eisenia fetida*	560, 640, 720, 800, 880, 960 mg/kg	14 d	955 mg/kg	(Xu et al., 2011)
	Natural soil		320, 400, 480, 560, 640, 720, 800 mg/kg	14 d	542 mg/kg	
	Filter paper		4, 8, 12, 16, 20, 24 μg/cm^2	24 h 48 h	20.5 μg/cm^2 13.6 μg/cm^2	
6:2 Cl-PFESA	Natural soil	*Eisenia fetida*	108, 215, 431, 646, 861, 1076, 1615, 2153 mg/kg	7 d 14 d	1118 mg/kg 816 mg/kg	(Ge et al., 2023)
PFOS			114, 228, 457, 685, 913, 1141, 1712, 2283 mg/kg	7 d 14 d	1404 mg/kg 984 mg/kg	
PFHxS	Artificial soil Natural soil	*Eisenia fetida*	1, 10, 150, 250, 500, 750, 1000 mg/kg	14 d	271 mg/kg 207 mg/kg	(Samarasinghe et al., 2023)
PFOA	Natural soil	*Aporrectodea caliginosa* (juvenile)	0.5, 2.3, 4.6, 9.2, 18.4 mg/kg	28 d	15.7 mg/kg	(Delor et al., 2023)
PFOS			0.3, 1.7, 3.5, 6.9, 13.9 mg/kg		31.7 mg/kg	
PFOA+PFOS			0.4, 0.7, 1.8, 3.6, 5.4, 7.3, 14.5, 29.0 mg/kg		9.91 mg/kg	

(7d-LC_{50}: 1404 mg/kg, 14d-LC_{50}: 984 mg/kg) suggested that the novel substitute 6:2 Cl-PFESA exhibit greater toxicity compared to PFOS (Ge et al., 2023), consistent with previous findings in studies involving humans (Liu et al., 2023), mice (Zhang et al., 2018), and zebrafish (Gui et al., 2023). Xu et al. (2011) reported that the 14d-LC_{50} of PFOS for earthworms measured by natural soil and artificial soil methods were 542 and 955 mg/kg, respectively. Similarly, Samarasinghe et al. (2023) reported that the 14d-LC_{50} of PFHxS for earthworms measured by natural soil and artificial soil methods were 207 and 271 mg/kg, respectively. These two studies suggested that the toxicity of PFOS and PFHxS to earthworms in natural soil may differ from that in artificial soil. It is well known that, unlike artificial soil, various factors such as concomitant chemical substances, microorganisms, and physicochemical properties of soil present in natural soil may influence the bioavailability and toxicity of PFASs. Therefore, when conducting toxicity experiments, it is recommended to preferentially use natural soil as the research medium as it closely resembles the natural environmental conditions, thereby providing more accurate results reflecting the actual toxicity caused by contaminated soil.

Some studies set up longer exposure time and lower exposure concentrations to investigate the subacute toxic effects of PFASs on earthworms. Feng (2016) conducted a 28-day PFOA exposure experiment using natural soil and revealed a significant decrease in the survival rate of earthworms at the concentration of 50 mg/kg. However, He et al. (2016) also conducted a 28-day PFOA exposure experiment using natural soils collected from Williamstown (New South Wales) and Edinburgh (South Australia), finding that even at the highest concentration of 100 mg/kg, all earthworms survived. Of particular concern was the significant reduction in earthworm (*Eisenia fetida)* survival observed following a 21/28-day exposure to environmentally relevant concentration (200 ng/g) of PFOA or PFOS (Han et al., 2023). In addition to using artificially contaminated soils, a few studies also directly used naturally contaminated soils for toxicity testing. Exposing earthworms to three different soil samples collected near an Australian firefighting training area with detected concentrations of PFOS at 1.83 mg/kg, 9.26 mg/kg, and 16.2 mg/kg, respectively for a duration of 30 days, resulted in complete mortality of earthworms in the highest concentration soil, while all earthworms survived in the other two soils with a reduction in body weight of approximately 50 % (Das et al., 2015). These indicate that the toxic effects have a great relationship with the composition and physicochemical properties of soils. Moreover, the natural environmental conditions are complex, which are often characterized by a combination of multiple pollutants, so the toxic effect on earthworms may be more serious.

In the toxicological study of earthworms, the most commonly selected earthworm is *Eisenia fetida*. This is primarily due to its high sensitivity to pollutants and strong reproductive capacity, as well as its short lifecycle. Only Zareitalabad et al. (2013) investigated the effects of PFOA and PFOS at three concentrations (1, 100, and 500 mg/kg) on endogeic earthworm (*Aporrectodea caliginosa*). The results showed that all earthworms survived at the concentration of 1 mg/kg, and the survival rate was less than 40 % at the concentration of 100 mg/kg, and all earthworms died at the concentration of 500 mg/kg. Delor et al. (2023) found that the 28d-LC_{50} values of PFOA, PFOS and PFOA&PFOS for juvenile endogeic earthworm (*Aporrectodea caliginosa*) were 15.7, 31.7, and 9.91 mg/kg, respectively. These 28-day LC_{50} values are more than ten times lower than the 7d/14d-LC_{50} values of PFOA and PFOS for *Eisenia fetida* (Table 2). We hypothesize that this difference may be attributed to the weaker resistance of juvenile worms to pollutant stress, variability in earthworm species, and differences in exposure duration. The co-exposure of PFOA and PFOS resulted in the LC_{50} lower than that of individual exposures, indicating that the combined toxicity of PFOA and PFOS to earthworms may exhibit synergistic effects. Additionally, in this study, the LC_{50} of PFOA for earthworms was lower than that of PFOS, contrary to the previously mentioned possibility of PFOA's acute toxicity to earthworms being less than that of PFOS. The likely reason for the opposite finding is that the LC_{50} value (31.7 mg/kg) of PFOS for earthworms obtained through linear fitting in this study falls outside the concentration range (0.3–13.9 mg/kg) set for the study, resulting in a significant margin of error. Apart from the aforementioned LC_{50} value, the LC_5 (6.8 mg/kg) and LC_{10} (8.41 mg/kg) of PFOA for earthworms were both greater than those of PFOS (LC_5: 3.01 mg/kg, LC_{10}: 5.47 mg/kg), which demonstrated that the acute toxicity of PFOS to earthworms was greater than that of PFOA. Overall, the response of different earthworm species exposed to PFASs may be different, but there is a lack of research data in this regard.

2.2. Weight change

Some studies have shown that PFASs can affect the growth of earthworms, with changes in body weight commonly used to assess growth toxicity. Zheng et al. (2016) conducted an acute toxicity exposure experiment using artificial soil to assess the effects of PFOA and PFOS on earthworms. They observed that the growth inhibition rate of surviving earthworms increased with increasing pollutant concentration during the 14-day experiment (50–800 mg/kg), exhibiting a clear dose-response relationship, similar to the results obtained in another 14-day PFOS exposure experiment (50–600 mg/kg) (Zheng et al., 2013b). Compared to the control group, Li (2013) and Xu et al. (2013) found that the weight of earthworms exposed to 10–120 mg/kg PFOS-contaminated artificial soil significantly decreased after 28 days. The 28-day PFOA exposure experiment also showed that the weight of surviving earthworms significantly decreased at concentrations of 10 and 50 mg/kg (Feng, 2016), as well as 25–100 mg/kg (He et al., 2016). Zareitalabad et al. (2013) found that the weight of surviving earthworms (*Aporrectodea caliginosa*) was significantly reduced when exposed to PFOA and PFOS at the concentration of 1 mg/kg and 100 mg/kg for 40 days. The weight loss may be attributed, on one hand, to earthworms employing a natural survival strategy of reducing feeding to avoid further toxin absorption. On the other hand, it could be due to the decline in physiological functions of earthworms under the toxic effects of pollutants. Further research is needed to elucidate the underlying mechanisms involved.

There is a lack of studies on the toxicity of other PFASs to earthworms, except for PFOA and PFOS. Zhao et al. (2014c) exposed earthworms to a composite soil contaminated with 11 different PFASs (PFCAs: PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA; PFSAs: PFBS, PFHxS, PFOS) at a concentration of 1 mg/kg for 14 days and found no significant change in earthworm weight. Karnjanapiboonwong et al. (2018) investigated the effects of PFBS, PFHxS, PFNA, and PFHpA on earthworms using a natural soil method, and no significant impact on earthworm mortality or weight was observed at concentrations of 1 mg/kg and below for these four PFASs after 21 days of exposure.

2.3. Pathological symptoms

Earthworms exposed to certain concentrations of PFASs may exhibit varying degrees of pathological symptoms. In the filter paper contact test, earthworms exhibited severe symptoms of epidermal ulceration, segmental breakage, and bleeding when exposed to 10–300 $\mu g/cm^2$ of PFOA (Feng, 2016; Zhu, 2015). In the artificial soil test, earthworms exposed to PFOA (100–1268 mg/kg) exhibited toxic symptoms such as intense reactions to dissection needles, sluggish movements, yellow body fluids exuding from the surface, congestion, body atrophy, segmental breakage, and reproductive ring swelling (Xu et al., 2012; Zhu, 2015). During the acute toxicity testing of PFOS on *Eisenia fetida*, similar pathological symptoms were observed as those seen under PFOA exposure (Xu et al., 2011; Yao et al., 2013). However, in Joung et al.'s experiment (2010), no significant pathological changes were observed in the surviving earthworms, even under high concentrations of 655 mg/kg PFOA and 1690 mg/kg PFOS exposure in a 14-day artificial soil test.

2.4. Reproduction

Currently, there is limited research on the reproductive toxicity of PFASs on earthworms. Feng (2016) studied the reproductive toxicity of PFOA on *Eisenia fetida* using a 28-day natural soil method. The results showed that the cocoon production and hatching rates of earthworms gradually decreased with increasing concentrations (1–50 mg/kg) of PFOA, and significant effects were observed at a concentration of 50 mg/kg. Han et al. (2023) found that compared to the control group, PFOS (0.2 mg/kg) significantly reduced the reproductive rate of earthworms, while PFOA (0.2 mg/kg) had no significant effect, indicating that the reproductive toxicity of PFOS to earthworms may be greater than that of PFOA. Furthermore, the PFAS concentration (0.2 mg/kg) in this study was similar to the maximum concentration found in non-site-polluted soils (Adu et al., 2023). Therefore, there is a critical need to prioritize the toxicity of environmentally relevant concentrations of PFAS to earthworms to safeguard biodiversity and soil ecological safety. Melo et al. (2022) found that earthworm reproduction was inhibited after 56 days of exposure to multi-contaminated soil in which the concentrations of PFOS and PFOA were 0.026 and 0.150 mg/kg, respectively, from a former military airport in Germany. For the novel substitute of PFOA in Italy, the fluorinated surfactant cC_6O_4, exhibited no significant effects on earthworm survival even after a 28-day exposure at the highest concentration of 1390 mg/kg (Bizzotto et al., 2024). However, reproduction, measured by juvenile production, appears to be a more sensitive toxicological endpoint than survival. After a 56-day exposure, the median effect concentration (EC_{50}) for earthworm reproduction was determined to be 10.4 mg/kg (Bizzotto et al., 2024).

2.5. Avoidance behavior

Since the International Organization for Standardization (ISO) drafted the method for assessing the behavioral effects of compounds on earthworms in 2005, the earthworm avoidance behavior test has been widely applied in the ecological risk assessment of contaminated soil. However, previous studies have reported limited information regarding earthworm avoidance behavior under PFAS contamination exposure. Yuan et al. (2017) found that earthworms exhibited significant avoidance behavior after 48 h of exposure to natural soil contaminated with 400 mg/kg PFOA and 160 or 320 mg/kg PFOS, suggesting that the habitat function of the soil has been disrupted by the contaminants. The study conducted by Xu et al. (2012) demonstrated that earthworms exposed to artificial and natural soils contaminated with 160 mg/kg PFOA also exhibited significant avoidance response after 48 h. In general, avoidance response is more sensitive and easier to measure than acute toxicity, making it a valuable tool for early diagnosis of soil pollution.

3. Toxic effects at the sub-individual level

Current research on the sublethal toxicity of PFASs to earthworms primarily focuses on oxidative stress, genetics, metabolism, digestive function, and lysosomal membrane damage. Indicators used to characterize these five types of toxicity can serve as biomarkers within earthworms for ecological risk assessment of soil pollution, including antioxidant system enzymes, cellulase, non-enzymatic substances in the antioxidant system, extent of DNA damage in coelomocytes, genes, cytochrome P450, endogenous metabolites, and lysosomal membrane stability.

3.1. Oxidative stress

Oxidative stress within earthworms is a toxic effect that arises when the production of excessive reactive oxygen species (ROS), which are byproducts of normal aerobic metabolism in organisms, exceeds the capability of their inherent antioxidant defense system to neutralize them (Sack et al., 2017). When exposed to PFASs, earthworms produce excessive ROS within their bodies. To prevent the potential damage caused by ROS, the antioxidant system operates to eliminate ROS and reduce its accumulation. However, as exposure time lengthens and pollutant concentrations increase, the earthworms' inherent antioxidant defense system becomes insufficient to rapidly clear the excessive ROS induced by the pollutants. This results in a disruption of the dynamic balance of ROS, leading to oxidative stress. Additionally, this may further induce lipid peroxidation, protein damage, DNA damage, and cell apoptosis in earthworms (Fig. 2). Therefore, the activity of antioxidant enzymes and the content of non-enzymatic substances in the antioxidant defense system are important indicators for evaluating the degree of oxidative stress within earthworms.

3.1.1. Antioxidant enzymes

The antioxidant enzymes that have received attention in previous studies on the toxicity of PFASs to earthworms include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione peroxidase (GSH-PX), and glutathione-S-transferase (GST). SOD, which is the most potent enzyme in antioxidant capacity, is a class of metalloenzymes that catalyze the dismutation reaction of superoxide anion radicals, generating hydrogen peroxide (H_2O_2) and oxygen (O_2), thereby mitigating the oxidative damage caused by superoxide anions and the resulting ROS (Ighodaro and Akinloye, 2018). SOD is known as the first intracellular detoxification enzyme. Due to its ability to catalyze various oxidation reactions using peroxides (ROOH) as electron acceptors, POD possesses the capability to eliminate H_2O_2, and is frequently selected as a biomarker in toxicological studies involving animals and plants (Passardi et al., 2007). CAT is an enzyme that protects cells from oxidative damage by converting H_2O_2 into water (H_2O) and further oxidizing it into O_2, playing a critical role in the biological defense system (Gill and Tuteja, 2010). GSH-PX is an endogenous antioxidant enzyme that utilizes glutathione (GSH) as an electron donor to catalyze the reduction of H_2O_2 and organic hydroperoxides to O_2 and H_2O (Moore and Sparling, 1995). CAT、POD and GSH-PX exhibit a synergistic effect, working together to facilitate the conversion of H_2O_2 generated by SOD-catalyzed dismutation reactions into harmless O_2 and H_2O, thereby effectively preventing the toxicity of H_2O_2 to the organism (Mittler, 2002). GST, the most important phase II detoxification enzyme in organisms, not only plays an antioxidative role by scavenging free radicals but also leads to the formation of nontoxic and easily excretable derivatives by catalyzing the conjugation of thiol group (-SH) on GSH with electrophilic groups from exogenous or endogenous pollutants (Sheehan et al., 2001; Wilce and Parker, 1994; Xu et al., 2013).

Previous research findings share a common characteristic where the antioxidant enzymes within earthworms exposed to certain PFAS-contaminated soil exhibit a pattern of initial promotion followed by inhibition with increasing exposure duration and concentration. Furthermore, the degree of inhibition becomes stronger with higher exposure concentration. For example, Xu et al. (2012) discovered that the SOD activity in earthworms exhibited a trend of “low promotion followed by high inhibition” with increasing exposure concentration, and the GSH-PX activity was generally suppressed after 42 days of exposure to PFOA concentration ranging from 10 to 120 mg/kg. Zhao et al. (2017) investigated the potential impact of PFOA on *Eisenia fetida* using an artificial soil method. Results showed that with increasing exposure duration (7–28 days) and concentration (5–40 mg/kg), SOD activity exhibited an overall trend of initial activation followed by inhibition, while POD, CAT, and GST displayed a pattern of initial inhibition followed by promotion and then inhibition again. Moreover, compared to the control, CAT activity decreased with increasing exposure concentration, and the SOD, POD, and GST activities reduced the most at the highest concentration of 40 mg/kg after 28 days of exposure, suggesting the greatest degree of oxidative stress occurring within the earthworms. Another study also found that the SOD, CAT, GST, and GSH-PX activity in earthworms exhibited a trend of initial activation

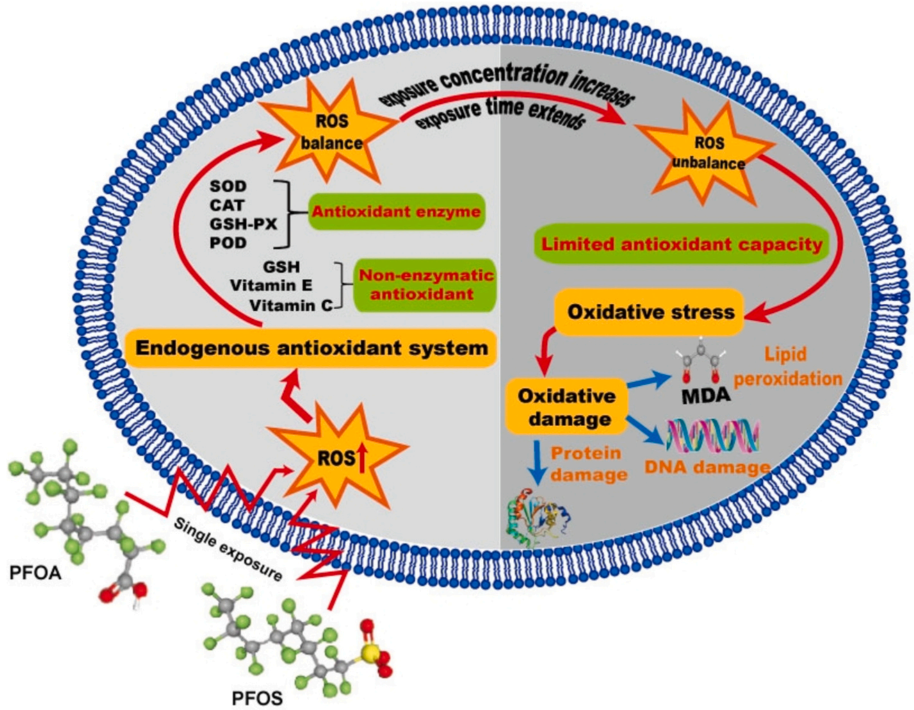

Fig. 2. The mechanism of oxidative stress and damage response in earthworms exposed to PFASs.

followed by inhibition with increasing PFOA exposure duration (3–42 days) and concentration (10–120 mg/kg) (Wang et al., 2021). Yuan et al. (2017) investigated the impact of PFOA and PFOS on antioxidant enzymes in earthworms. The results showed that, compared to the control group, earthworms exposed to PFOA-contaminated soil at concentration of 50 mg/kg exhibited a significant pattern of initial increase followed by a subsequent decrease in SOD activity with increasing exposure duration (3–14 days). Furthermore, the extent of reduction was significantly higher in the 50 mg/kg treatment compared to the 10 mg/kg treatment after 14 days. Earthworms exposed to PFOS-contaminated soil exhibited a trend in which SOD activity initially increased and then decreased with rising exposure concentrations (12.5–200 mg/kg) after 14 days of exposure.

There are a few reports on the impact of novel substitutes and short-chain homologs of PFOA and PFOS on earthworm antioxidant enzymes. Previous studies have shown an elevation in the activity levels of SOD, GST, POD, and CAT in earthworms exposed to the PFOA substitute 6:2 FTCA (62 ng/g), the PFOS substitute 6:2 FTAB (5.89 ng/g), and the typical PFOS precursor N-EtFOSE (0.009 nmol/g) through natural soil exposure methodology (Zhao et al., 2016b; Zong, 2022; Zong et al., 2022). Exposure to 6:2 fluorotelomer sulfonic acid (6:2 FTSA) and short-chain perfluorocarboxylic acids (TFA, PFPrA, PFBA, PFPeA, PFHxA, PFHpA) significantly increased SOD, POD, CAT, and GST activity in earthworms (Liu, 2021). Furthermore, compared to the treatments with perfluorocarboxylic acids (PFCAs) of the same or shorter perfluorocarbon chain length, the enzyme activity changes were more pronounced under the 6:2 FTSA treatment, suggesting that 6:2 FTSA may exhibit stronger toxicity to earthworms than corresponding or shorter PFCAs (Liu, 2021). In summary, the significant changes in antioxidant enzyme activity in earthworms exposed to sublethal doses of PFASs suggest that it can serve as an indicator of whether soil is contaminated with PFASs. However, the fluctuating and unstable nature of the increases or decreases in antioxidant enzyme activity under different concentrations and exposure duration suggests that antioxidant enzyme activity cannot accurately indicate the extent and type of PFASs contamination in soil.

3.1.2. *Non-enzymatic substances*

3.1.2.1. Glutathione (GSH). Glutathione (GSH) in earthworms can react with various oxygen free radicals to generate GSH-PX, which is capable of scavenging ROS, playing a crucial role in the synergistic antioxidant defense process. Under pollution stress, earthworms can generate ROS in their bodies. In the early stage, excessive ROS can be eliminated through the action of antioxidant enzymes and GSH. However, in the later stages, when antioxidant enzymes fail to function effectively and GSH is depleted, excessive ROS can cause oxidative stress. Previous studies have revealed a significant reduction in the GSH content in earthworms under PFOS contamination stress (Li, 2013; Xu et al., 2013). Furthermore, Wang et al. (2021) found that the GSH content in earthworms exposed to 40, 80, and 120 mg/kg PFOA-contaminated soil exhibited a significant decrease after 7 days, followed by a significant increase after 14 days. Ultimately, the GSH level returned to the control level at the end of the experiment. The significant decrease in the GSH content may be attributed to its consumption in response to overcoming excessive ROS generated under PFOA contamination stress, and the observed increase in the GSH level may be attributed to the activation of compensatory mechanisms by cells within the earthworms in response to oxidative stress. The eventual return to the normal level comparable to the control group might be associated with the adaptive response to prolonged exposure to pollution.

3.1.2.2. Malonaldehyde (MDA). Malonaldehyde (MDA) is the end product of lipid peroxidation when free radicals react with unsaturated fatty acids within biomembranes, inducing various forms of cellular damage to a certain extent (Du et al., 2015). The MDA content can directly reflect the degree of lipid peroxidation and indirectly indicate the level of free radicals, making it one of the better predictive indicators of oxidative damage (Xu et al., 2013). The study conducted by Zhao et al. (2017) demonstrated that the MDA content in earthworms significantly increased after 28 days of exposure to PFOA at concentrations of 5, 20, and 40 mg/kg, with the highest increase observed at 40 mg/kg. In Li's study (2013), it was found that there was no significant effect on the MDA content in earthworms after 7 days of exposure to

PFOS-contaminated soil (10–120 mg/kg), but significantly increased as the exposure duration increased. The MDA content in earthworms significantly increased under 6:2 FTSA contamination exposure, while there was no significant change in response to PFCAs with carbon chain lengths that were either equal to or shorter than 6:2 FTSA, suggesting that oxidative damage induced by 6:2 FTSA may be stronger than that caused by short-chain PFCAs (Liu, 2021). Generally speaking, in the early stage of exposure to PFASs, the earthworm's antioxidant system is capable of eliminating ROS induced by pollutants, successfully defending against oxidative stress, and preventing significant lipid peroxidation reactions. Therefore, there is no significant change in the MDA content. However, as the exposure duration increases and pollutant concentration rises, the amount of ROS produced exceeds the defense capacity limitations of the earthworm's antioxidant system, ultimately leading to lipid peroxidation and an increase in MDA content.

3.2. Genetic toxicity

3.2.1. DNA damage

Deoxyribonucleic acid (DNA) is a vital genetic material within organisms and is susceptible to damage from both internal and external factors. The DNA damage of coelomocytes in earthworms caused by exogenous pollutants has been extensively utilized in molecular ecotoxicological research on earthworms. Currently, the research on DNA damage to coelomocytes in earthworms caused by PFASs is primarily conducted using the comet assay which is a highly efficient method for assessing the extent of DNA damage at the single-cell level (Moller et al., 2020). The comet image of nuclear DNA in coelomocytes of healthy earthworms appears relatively intact, whereas those of damaged nuclear DNA exhibit a prominent phenomenon of "comet tailing" (Xu et al., 2013). Previous studies indicated that under PFOA and PFOS stress, coelomocyte DNA in earthworms exhibited significant tailing phenomena in comet images, and the length, distance, and DNA content of tails increased with the exposure concentration increasing (Feng, 2016; Li, 2013; Wang et al., 2021; Xu et al., 2013). Furthermore, another study revealed that non-lethal concentrations (10, 50, 250 mg/kg) of PFOS caused DNA damage to coelomocytes in earthworms during the acute exposure period (14 days), but DNA damage exhibited a recovery trend with prolonged exposure time during the sub-acute exposure period (28 and 42 days) (Zheng et al., 2013a). We speculate that the DNA damage was caused by the induction of DNA strand breaks and chromosomal aberrations due to excessive ROS generated within earthworms. The recovery of DNA damage may be associated with the ability of coelomocytes that suffered from low damage, to gradually restore themselves through intrinsic repair mechanisms.

3.2.2. Differential gene expression

Transcriptomics utilizes high-throughput sequencing to obtain genetic information sequences at the RNA level for studying intracellular gene transcription and transcription regulation patterns (Gong and Perkins, 2016; Lowe et al., 2017). The field of earthworm toxicology has extensively utilized transcriptomics to analyze the impact of pollutants on gene expression levels, thereby investigating the toxic mechanisms of pollutants on earthworms. Mayilswami et al. (2014) investigated the impact of chronic PFOS exposure (10 mg/kg, 8 months) on the expression of human homologs related to neuronal development in earthworms. They annotated the functions of over 2000 differentially expressed genes (DEGs) using BLAST and UniProt databases and validated the expression of differential genes associated with neuronal calcium sensor-2 (NCS-2) and nucleoside diphosphate kinase-1 (NDK-1) through the real-time quantitative PCR technique (RT-qPCR). Ultimately, they found that chronic PFOS exposure significantly altered the expression of genes related to calcium homeostasis and neuronal development. Another study reported abnormal gene expression related to cell apoptosis, reproduction, neuronal development, calcium homeostasis, and lipid metabolism in earthworms after exposure to 10 mg/kg PFOA-contaminated soil for 8 months (Mayilswami et al., 2016). In addition, Feng (2016) utilized RT-qPCR to determine that the gene expression of SOD and CAT in earthworms exhibited initial upregulation followed by downregulation after exposure to low-dose PFOA (0–10 mg/kg) for 28 days. In a study examining the varying toxicities of environmentally relevant concentrations (0.2 mg/kg) of PFOS and its substitutes PFBS, PFHxS, and 6:2 FTSA on earthworms, it was observed that after 56 days of exposure, PFOS induced disruption of the nervous and metabolic system, PFHxS disrupted energy balance and elicited inflammation, PFBS induced cell apoptosis, while 6:2 FTSA did not exhibit any adverse impacts on the transcriptome (Li et al., 2023). Exploring genes with high sensitivity and specificity toward PFAS exposure for further validation and development as biomarkers holds the potential to make novel contributions in the fields of ecotoxicological diagnostics and soil quality monitoring.

3.3. Metabolic toxicity

3.3.1. Exogenous metabolism

Cytochrome P450 (CYP450), as a metabolic enzyme, can respond to pollutants and participate in the metabolism and detoxification of exogenous contaminants (Lu et al., 2017). There is limited research on the effects of PFASs on CYP450 in earthworms, with only one study reporting the changes in CYP450 activity after exposure to PFOS (12.5–200 mg/kg) using a 14-day artificial soil method. The results indicated that compared to the control group, the CYP450 activity in earthworms exhibited an enhancement in the low-concentration group but inhibition in the high-concentration group. Furthermore, there was a clear dose-response relationship between the PFOS concentration and the CYP450 activity after 4 days (Yao et al., 2013). Whether CYP450 in earthworms can serve as a biomarker for the ecological risk assessment of PFASs contamination requires further extensive research to provide data support and elucidate the underlying mechanisms.

3.3.2. Endogenous metabolism

Metabolomics, an omics approach that has emerged after genomics, transcriptomics, and proteomics, can comprehensively and systematically reveal the toxic effects and mechanisms of exogenous disruptors on organisms by investigating the dynamic changes of endogenous metabolites and combining statistical methods in bioinformatics. Therefore, metabolomics technology plays an important role in the evaluation of long-term toxic effects of low-dose environmental pollutants and provides new ideas and methods for the screening of sensitive and specific biomarkers in earthworms (Griffin, 2004). Lankadurai et al. (2012, 2013) utilized ^{1}H NMR-based metabolomics technology and revealed significant alterations in the concentrations of 2-hexyl-5-ethyl-3-furansulfonate (HEFS), betaine, and ATP in earthworms exposed to PFOA or PFOS, verifying that PFOA and PFOS may interfere with fatty acid oxidation, energy metabolism and ATP synthesis, as well as disrupt the structure of biological membranes in earthworms. Another study employing integrated transcriptomics and untargeted metabolomics approaches found that PFOS and 6:2 Cl-PFESA primarily disrupt protein digestion and amino acid absorption, lipid metabolism, and immune responses in earthworms (Ge et al., 2023). Moreover, 6:2 Cl-PFESA exhibited a greater propensity to disrupt the extracellular matrix and induce cellular ferroptosis and apoptosis compared to PFOS, suggesting a potentially lower toxicity of PFOS relative to its novel substitute, 6:2 Cl-PFESA (Ge et al., 2023). Of particular concern is a study utilizing targeted metabolomics, which found that exposure to environmentally relevant concentration of PFOA (122 ± 9.2 ng/g) for 28 days resulted in significant dysregulation of pathways related to amino acid, energy, and sulfur metabolisms within earthworms, whereas the metabolic impact caused by PFOS (122 ± 15.2 ng/g) was minimal (Han et al., 2023). The finding that PFOA exerts a greater impact on earthworm metabolism than PFOS, contrary to the previously mentioned higher toxicity of PFOS compared to PFOA on individual indicators, is intriguing. We believe that further validation experiments are necessary to substantiate these conclusions and to elucidate their underlying mechanisms. Overall, PFASs may disrupt the metabolic activities of earthworms and the endogenous metabolites that exhibit significant changes in response to PFASs exposure may serve as potential biomarkers for diagnosing PFAS-contaminated soil.

3.4. Digestive toxicity

3.4.1. Cellulase

Cellulase is an important digestive enzyme present in earthworm gut tissues, and its activity can directly reflect the ability of earthworms to decompose soil organic matter (Luo et al., 2009). In toxicity studies on earthworms related to pesticide contamination, cellulase is commonly used as a biomarker. For instance, deltamethrin and acetochlor can inhibit the cellulase activity in earthworms (Sheehan et al., 2001; Shi et al., 2007). To date, there have been limited studies on the impact of PFASs contamination on cellulase in earthworms. Only one study conducted by Yuan et al. (2017) has found that cellulase activity in earthworms exhibited an overall pattern of initial inhibition followed by promotion under PFOA exposure, while it showed an initial promotion followed by inhibition under PFOS exposure. Further research is needed to investigate the reasons behind these phenomena.

3.4.2. Lysosomal membrane damage

Lysosome, a cellular organelle primarily involved in digestion processes within earthworms, is referred to as a specific biomarker of pollutant toxicity at the subcellular level (Weeks and Svendsen, 1996). Neutral red dye can rapidly accumulate in the lysosomes of coelomocytes in earthworms. When earthworms are subjected to pollution stress, the permeability of lysosomal membrane increases and stability decreases, leading to gradual leakage of neutral red dye into the cytoplasm (Svendsen et al., 2003). Therefore, the neutral red retention time (NRRT) measured through the neutral red assay is commonly employed to characterize the stability of lysosomal membranes. Wang et al. (2021) observed a decrease in NRRT values with increasing concentration (10–120 mg/kg) of PFOA and prolonged exposure time (3–42 days) during the artificial soil cultivation process. In other studies, the single and combined toxic effects of PFOA and arsenite, or PFOS and arsenate, on earthworms were studied using the artificial soil method, respectively. The results showed that NRRT values in all treatment groups were significantly lower than those in the control group after the exposure period (Wang et al., 2022b; Wang et al., 2022c). Therefore, the stability of earthworm lysosomal membrane may serve as a biomarker to indicate the toxic effects of PFASs on earthworms and the biomonitoring of soil contamination.

3.5. Comprehensive toxicity assessment

Single biomarker is often insufficient to provide a comprehensive assessment of the toxic effects of pollutants on earthworms. Therefore, several studies introduce biomarker index to provide a more comprehensive toxicity assessment. The “Integrated Biomarker Response” (IBR) is the area of a star plot of standardized biomarker responses and one of the most commonly used methods in field and laboratory studies (Sanchez et al., 2013). Li et al. (2023) evaluated the combined toxicity of PFOS, PFHxS, PFBS and 6:2 FTSA to earthworms by measuring physiological indices (ROS, SOD, CAT, GST, MDA, 8-OHdG) as well as the levels of gene expression (CRT, HSP70, TCTP, ANN) using the IBR method. The results showed that the toxicity of these pollutants to earthworms was in the order of 6:2 FTSA<PFBS<PFOS<PFHxS (Li et al., 2023). Similarly, two studies using weight change rate, antioxidant enzyme activities (SOD, CAT, GST, GPx), lipid peroxidation level, metallothionein content, and lysosomal membrane stability as biomarkers applied the IBR and “Biomarker Response Index” (BRI) to access the combined toxicity of arsenate (As(V)) and PFOS, or arsenite (As(III)) and PFOA in earthworms (Wang et al., 2022b; Wang et al., 2022c). The results indicated that the combined exposure to As(V) and PFOS, or As(III) and PFOA were more toxic to earthworms than individual exposures, with As(V) being more toxic than PFOS and As(III) more toxic than PFOA (Wang et al., 2022b; Wang et al., 2022c).

4. Key factors influencing the toxicity of PFASs to earthworms

The bioavailability of PFASs in soil is an important factor in determining their toxicological effects, which is influenced by the molecular structure of PFASs and the combined effects of various environmental factors, such as soil organic matter composition and content, pH, and aging time. A previous study found that the bioavailability of perfluoroalkyl acids (PFAAs) in peat soils was 3–10 times lower than in the plain farmland soil due to sequestration of PFAAs in soil (Zhao et al., 2016a). Surprisingly, in the peat soil with high organic carbon content (59 %), PFAAs were completely sequestered in soil, while sequestered PFAAs were still bioavailable to earthworms. Aging may lead to further sequestration of PFAAs in soils with relatively low SOM content. Moreover, the sequestration capacity of PFASs in different soils showed a changing pattern of increasing with decreasing pH, which is consistent with the finding that adsorption of PFAAs on humic substances and minerals decreased with increasing solution pH (Zhao et al., 2014a; Zhao et al., 2014b). Fabregat-Palau et al. (2021) found through model fitting that the higher the soil sand content, the smaller the soil-water partition coefficient, indicating that the weaker the adsorption capacity of the soil for PFASs. Burkhard and Votava (2022) compiled studies on the bioaccumulation of PFASs in earthworms and found that earthworms generally exhibit higher bioaccumulation potential in soils with a higher sand content. In addition, both the length of perfluorocarbon chain and the functionality of head group affected the distribution and desorption of PFASs in soil. Zhao et al. (2016a) found that the sequestration of PFAAs in soil was more pronounced with increasing perfluorocarbon chain length, and PFSAs were more easily sequestrated than PFCAs with the same carbon chain length. Although PFOS and its novel alternative 6:2 Cl-PFESA have the same carbon chain length, the larger chlorine atom replacing a fluorine atom and the addition of an ether bond makes the molecular volume of 6:2 Cl-PFESA larger than that of PFOS, which contributes to greater adsorption (Chen et al., 2018; Gomis et al., 2015).

Bioaccumulation capacity is also an important factor influencing the toxicity of organic pollutants. In general, the stronger the bioaccumulation capacity of a pollutant, the greater its biotoxicity. The ability of PFASs to bioaccumulate in earthworms depends mainly on the difference between functional groups and carbon chain lengths in their molecular structures. A review on the bioaccumulation of PFASs in earthworms suggests that the mean values of biota-soil accumulation factor (BSAF) for PFOS and PFOA were 0.167 and 0.0413, respectively (Burkhard and Votava, 2022). The main reason for the difference in bioaccumulation capacity between PFOA and PFOS may be related to the type of polar groups at their termini (Bai et al., 2023; Wang et al., 2024b). PFAAs readily accumulate in the blood, and serum albumin is their main carrier protein in the blood (Beale et al., 2022; Forsthuber et al., 2020). The type and nature of functional groups are key factors in determining the strength of the binding affinity of PFAAs to albumin, which is considered to be the main mechanism for their bioaccumulation and tissue distribution (Bai et al., 2023; Ng and Hungerbuehler, 2014). Evidence from several studies suggested that PFAAs carrying sulfonic acid groups have a stronger binding affinity for albumin than those carrying carboxylic acid groups for the same carbon chain length, and thus exhibit higher bioaccumulation capacity and toxicity (Bai et al., 2023; Chi et al., 2018; Gorrochategui et al., 2014; Wang et al., 2024b). Therefore, PFOS are usually more toxic than PFOA. Morever, carbon chain length is a key factor influencing the bioaccumulation and toxicity of PFAAs. As the carbon chain length increases, the hydrophobicity of molecular tail increases and the spatial resistance decreases, which in turn provides more potential binding sites. As a result, PFAAs with longer carbon chain usually show stronger affinity for serum proteins, which may lead to their higher bioaccumulation and toxicity (Bai et al., 2023; Bischel et al., 2011; Zhang et al., 2009). In fact, the review did also find that BSAFs of PFSAs in earthworms showed a general pattern of increasing with increasing carbon chain length (PFBS-PFDS) (Burkhard and Votava, 2022), and this phenomenon may be the key to the fact that the toxicity of PFOS and PFHxS was greater than that of PFBS in earthworms (Li et al., 2023).

There are insufficient data on the accumulation of novel PFASs other than PFAAs (PFCAs and PFSAs) in earthworms. Statistically, the BSAF of 6:2 FTSA in earthworms was much higher than that of $C_{4–7}$ PFCAs and $C_{4–7}$ PFSAs with shorter carbon chain lengths (Burkhard and Votava, 2022). Thus, 6:2 FTSA induced oxidative stress in earthworms to a significantly greater extent than short-chain PFCAs, probably mainly because of its sulfonic acid fraction and longer carbon chain length leading to higher bioaccumulation capacity. However, 6:2 FTSA was less toxic to earthworms than PFBS, PFOS and PFHxS (Li et al., 2023), which may be related to its lower bond energy of C—H bond in the molecule, resulting in lower molecular energy and thus easier degradation to short-chain PFCAs by biotic or abiotic processes (Lu et al., 2019; Zhao et al., 2021). In addition, Ge et al. (2023) found that PFOS was less toxic to earthworms than its novel substitute 6:2 Cl-PFESA. Although the bioaccumulation data of 6:2 Cl-PFESA in earthworms are not clear, it has been confirmed that the bioaccumulation capacity of 6:2 Cl-PFESA is stronger than that of PFOS in crucian carp (Shi et al., 2015), black-spotted frog (Cui et al., 2018), and freshwater alga (Liu et al., 2018). The accumulation and toxicity of PFOS and its novel substitute 6:2 Cl-PFESA in earthworms should be investigated in depth in future studies, especially the effects of the Cl substituent and ether bond of 6:2 Cl-PFESA on the bioaccumulation capacity and toxicity.

5. Current limitations and future perspectives

Although the detected concentration of PFASs in soil is relatively low, long-term exposure to low concentration can lead to various toxic effects on earthworms, which should not be overlooked in terms of their potential harm to the ecological environment and human health. In recent years, more and more countries have begun to pay attention to the problem of PFASs pollution in the soil environment. By sorting and summarizing relevant literature at home and abroad, we have found that ecotoxicological studies using earthworms as model organisms are becoming increasingly mature. However, the toxicological study of PFASs on earthworms is currently very limited, and there are still a series of issues and potential directions to be studied.

With the prohibition of traditional PFASs such as PFOA and PFOS in industrial production, a variety of novel PFASs have been detected in soil in recent years. The existing research on the toxicity of PFASs to earthworms mostly focused on PFOA and PFOS, while there is a significant paucity of studies on the toxicity of novel PFASs to earthworms. In the future, efforts should be directed toward filling the data gap on the foundational toxicity of emerging PFASs to earthworms. In addition, PFASs often coexist with other contaminants in actually contaminated soils, and their co-occurring toxicity does not appear to be a simple superposition of effects (Wang et al., 2022c). Current studies on the toxicity of PFASs to earthworms have mainly focused on the toxicity assessment of single PFASs. Therefore, when studying the toxicity of PFASs to earthworms, the co-existence of PFASs with other pollutants in actual soils should be considered, and exposure experiments for single and compound pollutants should be designed in combination with the key pollutant and their concentration levels. At the same time, the experiments should combine the laboratory conditions with the exposure scenarios of actual contaminated soils in order to more accurately assess the ecological risks of PFASs in complex soil environments.

Different earthworm species possibly lead to distinctive toxic outcomes. Currently, *Eisenia fetida* is most commonly used in soil ecotoxicology studies due to its high sensitivity to pollutants, high reproductive capacity and short life cycle (Wang et al., 2024a). In the previous studies on the toxicity of PFASs to earthworms, *Eisenia fetida* was usually chosen as the test earthworm. However, different species of earthworms may have different tolerances and sensitivities to pollutants due to differences in body size and immunity, etc. *Eisenia fetida* is considered to be more tolerant of contaminants than most earthworm species (Lowe and Butt, 2007). Moreover, the habitats of different species of earthworms may vary, e.g. *Eisenia fetida* and *Eisenia andrei* are not common in agricultural soils, whereas *Aporrectodea caliginosa* and *Lumbricus terrestris* are common in arable land (Bart et al., 2018). It is also important to note that current research on the toxicity of PFASs to earthworms predominantly centers on the whole organismal level, neglecting the imperative investigation from the perspective of different organs. It is suggested that future studies employ techniques such as "Mass Spectrometry Imaging" to examine the accumulation capacity of PFASs in different organs, thereby further elucidating the toxicological responses at the molecular level. In the future, utilizing different species of earthworms and various target organs as subjects in soil toxicology research could enhance the comprehensiveness and accuracy of toxicological studies, providing more effective scientific support for environmental protection and ecological safety.

The exposure concentration and duration of pollutants are key factors in determining the intensity of their toxic effects. PFASs are extremely environmentally persistent and chemically stable. Therefore, in general, the toxicity of PFASs to earthworms increases with increasing exposure concentration and duration. A comprehensive analysis of the literature showed that the 14d-LC_{50} of PFASs on earthworms was generally lower than the 7d-LC_{50}. In subacute toxicity studies, the effects of PFASs on oxidative stress and damage in earthworms were found to increase with increasing exposure concentration and duration. However, the relationship between the strength of the toxicity response of earthworms and the exposure concentration and exposure time should also take into account factors such as the sensitivity of the assessment index and the concentration threshold for the toxic effect. In addition, the exposure concentrations of PFASs in the existing studies were mostly in the mg/kg range, which were much higher than the concentrations of PFASs in the actual soil, and the exposure times were 7d, 14d and 28d, which could not simulate the toxic effects of PFASs on earthworms in the actual soil environment, which is a real-life situation of low concentration and long-term exposure. Therefore, investigating the chronic toxicity of PFASs to earthworms at environmentally relevant concentrations is a key focus for future research.

Conventional biomarkers, such as antioxidant enzymes and lipid peroxidation levels, typically respond to a wide range of pollutants. However, there is a lack of specific biomarkers for assessing the toxicity of PFASs in earthworms. The screening of biomarkers with specific responses to PFASs utilizing combined techniques of genomics, metabolomics, and multivariate statistics, is of more significant importance for the diagnosis and risk assessment of PFASs-contaminated soils. Specifically, multivariate statistical methods such as principal component analysis (PCA), partial least squares regression (PLS), and random forest (RF) can be employed to integrate and analyze multi-omics data, identifying genes or metabolites that are highly associated with PFAS exposure. Subsequently, the expression changes of the selected genes and metabolites can be validated under laboratory conditions, utilizing techniques such as gene knockout, overexpression, or RNA interference for biological function verification. Finally, potential biomarkers can be applied in natural environments to assess their environmental relevance.

Due to the extensive variety of PFASs, conducting earthworm toxicology tests for each PFASs individually would require a substantial workload. In future researches, endeavors should be made to explore the application of model calculations and machine learning in predicting the toxicity of PFASs to earthworms, such as constructing the quantitative structure-activity relationship (QSAR) model of toxic effects of PFASs on earthworms by machine learning. However, current foundational data on the toxicity of PFASs to earthworms are insufficient for modeling purpose. Therefore, future researches should prioritize filling these data gaps and exploring the relationship between chemical structure, physicochemical properties and toxicity of PFASs, to develop high-quality models that can quantify the impact of different chemical structures and key physicochemical factors on earthworm toxicity, providing critical insights for a comprehensive assessment of PFASs' toxicity to earthworms and promoting the green development of soil ecosystems.

CRediT authorship contribution statement

Cheng Qin: Writing – original draft, Conceptualization. **Chenxi Lu:** Writing – review & editing, Data curation. **Chang Lu:** Writing – review & editing. **Lixia Zhao:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Xiaojing Li:** Writing – review & editing. **Yang Sun:** Writing – review & editing, Conceptualization. **Liping Weng:** Writing – review & editing. **Yongtao Li:** Writing – review & editing.

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.

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Data availability

All the data are from the literatures and present in the paper.

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