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Review

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A review of the bioaccumulation and adverse effects of PFAS in free-living organisms from contaminated sites nearby fluorochemical production plants

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Abstract

Per- and polyfluoroalkyl substances (PFAS) encompass a large, heterogeneous group of chemicals of potential concern to human and environmental health. Based on information for some legacy PFAS, such as perfluorooctane sulfonate and perfluorooctanoate, there is an increasing awareness that they can represent a serious environmental risk. Although the environmental occurrence and fate of some legacy PFAS and their toxicity under controlled laboratory conditions have been investigated, to date, there is a dearth of information on the exposure and potential adverse effects of these compounds towards free-living organisms. The present review summarizes the findings of field studies investigating the accumulation and adverse effects induced by the exposure to environmental mixtures of both legacy and emerging PFAS in the wildlife living nearby fluorochemical production plants (FCP). Biomonitoring campaigns performed close to FCP, which can be considered as hotspots of PFAS contamination, can be very useful in exploring the fate and toxicity of these compounds towards free-living organisms. All studies showed that the bioaccumulation of both legacy and emerging PFAS in wildlife living near the FCP is higher compared to control sites and other areas worldwide. However, the investigation on adverse

effects returned contrasting results, suggesting the need for further studies to shed light on the toxicity and mechanism(s) of action of PFAS in free-living organisms.

Keywords: Polyfluoroalkyl substances, ecotoxicology, toxic effects, free-living organisms, contaminated sites

INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) represent a class of synthetic fluorinated alkyl compounds of growing environmental concern because of their presence in the environment and bioaccumulation in free-living organisms and humans. Because of their chemical-physical stability and surfactant properties, PFAS have been extensively used in a wide array of products[1,2]. These characteristics confer on them a high environmental persistence, bio-accumulative properties, or high environmental mobility, resulting in accumulation in both abiotic and biotic environmental matrices[3-6]. Two long-chain PFAS, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), have been identified as an intrinsic threat to human and ecosystem health[7]. Due to the increasing knowledge of the potential impact of long-chain PFAS exposure and the consequent regulatory restrictions, the industry has developed various alternative PFAS, whose properties or chemical structures are commonly treated as confidential information[8,9]. In addition, the production of long-chain PFAS has been phased out, and companies have shifted their production to short-chain fluorinated alternatives[10]. Short-chain PFAS are expected to be less persistent, bio-accumulative, and toxic than long-chain, legacy PFAS, but their lower technical performance potentially results in higher amounts of use and emissions into the environment[10,11]. Moreover, the increase of scientific knowledge on fluorinated compounds[6] and the improvement of analytical techniques[12] have allowed the identification of other PFAS never measured before in the environment, albeit they have been used for many years[13]. Short-chain fluorinated replacements and newly identified PFAS can be considered as emerging compounds, whose environmental presence, fate, and potential toxicity are totally (or largely) unknown. Several studies are investigating the presence of diverse emerging PFAS in different environmental matrices[8,14-16]. For instance, a recent study tentatively identified ten emerging chloroperfluoropolyether carboxylates in soils nearby two production plants[8]. Similarly, the acetic acid 2,2-difluoro-2-[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy, a substitute for PFOA commercially known as C6O4[17,18], has been detected in industrial wastewater (50-100 μg/L range) nearby a perfluoropolymer plant in Northern Italy[16], as well as in groundwater (≤ 3200 ng/L) and surface waters (≤ 300 ng/L) of the Po River basin (Northern Italy)[14,15].

Although several laboratory studies have investigated the exposure and toxicity of legacy PFAS (mainly PFOS and PFOA) on different model organisms reporting a wide array of adverse effects[19], limited information is currently available for emerging PFAS[20-22]. However, these studies have tested the toxicity of concentrations far from those measured or expected in the environment. In addition, the lack of information on many concentrations ranging from minimum to average or maximum, and adverse effects induced by the exposure to environmentally relevant PFAS mixtures in free-living organisms[23-26] preclude the opportunity to develop a solid and reliable environmental risk assessment of PFAS. For these reasons, a recent expert panel has suggested the urgent need to perform a comprehensive assessment of the PFAS environmental risks, including exposure and toxicity assessments for PFAS already present and/or emerging ones that will be released into ecosystems, as well as considering complex mixtures in prospective and retrospective assessments[10].

Reflecting the current environmental situation, field studies allow investigation of both the exposure and consequences of chemicals experienced by organisms in their native ecosystems and represent a tool to

implement PFAS risk assessment procedures. In particular, field biomonitoring studies aimed at investigating the presence of PFAS nearby production, i.e., highly contaminated sites, represent a unique opportunity to assess the fate and potential toxicity of specific PFAS or mixtures, as well as to identify the potentially hazardous compounds towards free-living organisms[16].

The present review aims to summarize the findings of field studies that explored the accumulation and consequences of free-living organisms induced by environmental exposure to PFAS nearby FCP. An *ad hoc* literature research was performed in the Google Scholar, Scopus, and Web of Science databases, focusing on scientific publications such as articles and proceedings of scientific conferences. Literature research was focused on papers published from 2000 to 2022, using different combinations of keywords. The keywords we included in our search were: PFAS, fluorochemical plant, effects, toxicity, and organisms.

ACCUMULATION AND EFFECTS OF PFAS IN FREE-LIVING ORGANISMS CLOSE TO FLUOROCHEMICAL PLANTS

Fluorochemical plants (FCP) where PFAS are synthesized or used in polymerization processes are considered among the most important sources of both legacy and emerging PFAS in the environment, as confirmed by the high environmental contamination measured in different matrices collected in their surroundings, including groundwater, freshwater, soils, and biota[15,16,27]. The literature research identified 14 papers that report the findings from field biomonitoring studies investigating the accumulation and/or effects induced by the exposure to legacy or emerging PFAS, either individually or in mixtures, in different free-living organisms from the surroundings of FCP [Table 1]. Two additional studies matching the selected keywords were extracted by the search[28,29] but were then excluded from this review because they have investigated the accumulation and impacts of PFAS far from an FCP.

Most field studies (n = 9; 64%) were performed in the surroundings of the Antwerp FCP (Belgium). Two studies explored the PFAS contamination and related effects nearby FCP in Northern Italy and China. Only one investigation was carried out in the USA. All these studies explored the accumulation of different PFAS in vertebrate (i.e., different bird species and mammals) or invertebrate (i.e., isopods, such as earthworms, slugs, millipedes, and woodlice) organisms. Only six studies (43%) investigated the effects induced by the exposure to PFAS, at different levels of the biological organization, from biochemical (e.g., changes in hepatic parameters and oxidative stress-related endpoints) to individual level (i.e., reproductive effort). These studies also made an effort to shed light on the relationships between the effects and concentrations of a specific compound. A study attempted to explore the impact due to the discharge of FCP at the community level through monitoring of changes in the macrobenthic community of the receiving river compared to a reference site upstream of the industrial discharge[30].

Overall, the accumulation and potential toxicity of PFAS mixtures towards free-living organisms have been investigated through the application of a before-after-control-impact/treatment (BACI) design, which is a common approach to evaluate the impacts of environmental perturbations on ecosystems where the allocation of treatment and control sites cannot be assigned randomly[31,32]. In particular, PFAS concentrations and potentially related effects have been measured in individuals sampled nearby FCP with respect to conspecifics from a reference site. In the next sections, the findings of studies on PFAS accumulation and/or effects divided by different taxonomic groups (i.e., mammals, birds, amphibians, and invertebrates) are discussed.

Table 1. List of studies investigating the accumulation (i.e., concentrations nearby the FCP) and/or effects (i.e., other measurements) induced by PFAS mixtures nearby fluorochemical plants (FCP) in different geographic areas

Measured PFAS	Country	Organism	Analyzed matrix	Concentrations nearby the FCP	Other measurements	Refs.
PFHxA, PFHpA, PFOA, PFNA, PFDA; PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFOS, FOSA, 8:2 FTS, C6O4	Italy	Great tit (*Parus major*); blue tit (*Cyanistes caeruleus*) and European starling (*Sturnus vulgaris*)	Eggs	*Parus major* PFCA: 10-14 (PFOA 3) ng/g ww; PFOS + FOSA + 8:2FTS: 2 ng/g ww; C6O4 2-4 ng/g ww *Cyanistes caeruleus* PFCA: 53-91 (PFOA 5-7) ng/g ww; PFOS + FOSA + 8:2FTS: 1 ng/g ww; C6O4 32-42 ng/g ww *Sturnus vulgaris* PFCA: 17-666 (PFOA 6-610) ng/g ww; PFOS + FOSA + 8:2FTS: 2-38 ng/g ww; C6O4 < LOD-3 ng/g ww	Effects were not evaluated	[16]
11 PFCA and 4 PFSA	Belgium	Great tit (*Parus major*)	Eggs and blood	In plasma of adults: PFCA: 8.54-94.9 pg/μL; PFSA: 43,428 pg/μL. In plasma of nestlings: PFCA: 12.2-24.1 pg/μL; PFSA: 14,514 pg/μL	Body condition (=); Total antioxidant capacity (+ in adults, - in nestlings), GPx (- in adults; = in nestlings), SOD (= in adults, + in nestlings), CAT (=), GSH (+ in adults, = in nestlings), protein carbonyls (+ in adults, = in nestlings), GSSG (- in adults, = in nestlings)	[23]
PFOS	Belgium	Great tit (*Parus major*); Northern lapwing (*Vanellus vanellus*); Mediterranean gull (*Larus melanocephalus*)	Eggs for each species and blood for *L. melanocephalus* only	*P. major*: 19-5635 ng/g ww; *V. vanellus*: 143-46,182 ng/g ww; *L. melanocephalus*: 150-916 ng/g ww for eggs and 118-943 ng/mL for blood	Only for *P. major*: total protein content (-)[#], cholesterol (=), triglyceride (-)[#] and uric acid (=) content	[24]
9 PFCA and 3 PFSA	Italy	Benthic communities and Trichoptera (*Hydropsyche modesta*)	River water	ΣPFSA: 333-8277 (PFOA: 267-6480) ng/L	Artificial Substrate Index (=), Amplified Fragment Length Polymorphism (+)	[30]
PFOA PFNA PFDA PFUnDA PFDoDA PFOS	Belgium	Wood mouse (*Apodemus sylvaticus*)	Liver	0.47-178.55 μg/g ww	Liver weight (+), peroxisomal β-oxidation activity (+), microsomal lipid peroxidation (+), mitochondrial fraction catalase activity (+), serum alanine aminotransferase (=), potassium level (=), triglyceride (=) and cholesterol (=) content	[33]
PFOS	Belgium	Wood mouse (*Apodemus sylvaticus*) and terrestrial invertebrates (earthworms, slugs, millipedes and woodlice)	Liver and kidneys for wood mouse. whole body for invertebrates	Liver: 787-22,355 ng/g ww; Kidneys: 13.7-4226 ng/g ww; Invertebrates: 28-9000 ng/g: ww	Effects were not evaluated	[34]
PFOS	Belgium	Great tit (*Parus major*) and blue tit (*Cyanistes caeruleus*)	Liver	*P. major*: 86-2788 ng/g ww *C. caeruleus*: 317-3322 ng/g ww	Significant positive correlation between PFOS concentration and serum alanine aminotransferase and Hematocrit; Significant negative correlations between PFOS concentration serum cholesterol and triglyceride content	[36]
PFOS	Belgium	Great tit (*Parus major*)	Blood and liver	Liver: 553-11,359 ng/g; Blood: 24-1625 ng/ml	Effects were not evaluated	[37]
8 PFCA and 4 PFSA	Belgium	Great tit (*Parus major*)	Eggs	PFHxS: 99.3 ng/g; PFOS: 10,380 ng/g; PFDS: 47.7 ng/g	Effects were not evaluated	[38]
11 PFCA and 4	Belgium	Great tit (*Parus major*)	Eggs	PFCA: 1.7-39 (PFOA	Start of egg laying (=), clutch size	[40]

PFSA				3.4-359) ng/g ww; PFSA: 315-48,056 (PFOS 5111-187,032) ng/g ww	(=), hatching success (=),breeding success (=), chick survival (=), eggshell thickness (-) and body condition (=)	
17 legacy PFAS and the novel 6:2 Cl-PFESA and HFPO-TA	China	Black-spotted frog (*Pelophylax nigromaculatus*)	Ovary, liver, testis, skin, lung, heart, intestine, stomach, muscle, carcass	ΣPFSA: 33.6 ± 24.4 ng/g ww	Effects were not evaluated	[42]
12 PFCAs, 4 PFSAs and 6:2 Cl-PFESA	China	Black-spotted frog (*Pelophylax nigromaculatus*)	Kidney, spleen liver, gonads, heart, lung, skin, stomach, intestine, muscle	ΣPFAS in the liver: 163.40 ng/g ww	Changes in body weight, liver weight, hepatosomatic index, triglyceride and cholesterol content were assessed but not compared with a reference site	[43]
11 PFCA and 4 PFSA	Belgium	Isopods (Oniscidae)	Pooled specimens	PFPeA < LOQ-292 ng/g ww; PFHpA < LOQ-313 ng/g ww; PFOA < LOQ-121 ng/g ww; PFDoDA < LOQ-729 ng/g ww; PFHxS < LOQ-26 ng/g ww; PFOS: 29-611 ng/g ww; PFDS: < LOQ-388 ng/g ww	Effects were not evaluated	[44]
PFHpA, PFOA, PFNA, PFDA; PFUnDA, PFDoDA	Ohio (USA)	Soil-earthworm (*Lumbricus terrestris*)	Pooled specimens	PFCA: 130-1600 (PFOA: 51-870) ng/g dw	Effects were not evaluated	[45]

Country: The country where the study was conducted; (+): significantly higher effects measured in organisms sampled close to the FCP compared to control site (or far from FCP); (-): significantly lower effects measured in organisms sampled close to the FCP compared to control site (or far from FCP); (=): no significant effects; #only in one-year-old individuals compared to older ones collected close the FCP; SOD: superoxide dismutase; GP: glutathione peroxidase; CAT: catalase; GSH: reduced glutathione; GSSG: oxidized glutathione. The abbreviations for PFAS compounds are reported within the main text.

Accumulation and effects in mammals

In 2004, the first pioneer study by Hoff and coauthors used a BACI approach to investigate the accumulation and potential adverse effects induced by exposure to six PFAS, namely PFOA, perfluorononanoate (PFNA), PFOS, perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), and perfluorododecanoate (PFDOA), towards adult individuals of the wood mouse (*Apodemus sylvaticus*) sampled in the nature reserve of Blokkersdijk, which is located nearby the FCP in Antwerp (Belgium), and at Galgenweel, a reference site selected 3 km from the FCP[33]. While PFNA, PFDA, PFUnDA, and PFDoDA were measured sporadically in the liver of mice caught in both sampling areas, the hepatic concentrations of PFOS in the mice from Blokkersdijk were higher (0.47-178.55 μg/g ww) compared to conspecifics from Galgenweel (0.14-1.11 μg/g ww). Although no sex dependency of PFOS levels was observed, the bioaccumulation of this compound increased with the individual age. Different biochemical hepatic endpoints, including peroxisomal β-oxidation activity, microsomal lipid peroxidation, and mitochondrial fraction protein content, as well as liver weight and relative liver weight, were significantly higher in Blokkersdijk mice compared to conspecifics from Galgenweel, showing a positive relationship with PFOS concentrations. However, the effects on serum triglyceride, cholesterol, or potassium levels were not PFOS-mediated effects, while the hepatic PFOS concentrations were negatively related to the serum alanine aminotransferase activity. These results first suggest that levels of a specific compound, such as PFOS, can represent a threat to the health status of individuals living nearby a highly contaminated site.

A similar BACI design was replicated in the same study areas to explore PFOS presence and transfer in a restricted terrestrial food chain, with the omnivorous wood mouse as the apical consumer[34]. Samples of

water, soil, fruits of common blackberry and European elder, and different invertebrates (see Section "Accumulation and effects in invertebrates") were collected to investigate the PFOS trophic transfer. Focusing on the concentrations accumulated in the wood mouse, as expected, the highest PFOS levels were measured in the liver from individuals collected at Blokkersdijk (range 787-22,355 ng/g ww). Although these levels were generally lower than those detected in the 2002 survey performed in the same study area[33], they were considered very high, as well as higher than those measured in other mammal species[35]. These data suggest that mice sampled nearby the FCP are directly exposed via the consumption of contaminated water, inhalation of the air, and ingestion of fine particles and/or suffer an indirect exposure via the consumption of PFOS-contaminated plants and invertebrates, resulting in high bioaccumulation[34].

Accumulation and effects in birds

The BACI design was also applied in biomonitoring studies of PFAS contamination nearby FCP using birds as sentinel organisms. The first investigation explored the accumulation of PFOS and related adverse effects in great tit (*Parus major*) and blue tit (*Cyanistes caeruleus*) nestlings from Blokkersdijk reserve (close to the FCP in Antwerp) and Fort IV (control area)[36]. The concentrations of PFOS measured in the liver isolated from nestling sampled in Blokkersdijk (86-2788 and 317-3322 ng/g ww for great and blue tits, respectively) were significantly higher compared to those measured in conspecifics from the control area (17-206 and 69-514 ng/g ww for great and blue tits, respectively). While no species- and sex-dependencies of hepatic PFOS concentrations were noted, they were significantly and positively correlated with the serum alanine aminotransferase activity and negatively with the serum cholesterol and triglyceride levels in both species. Moreover, in great tit individuals, hepatic PFOS concentrations were positively correlated with the relative liver weight, while in blue tits, the same relationship was noted with hematocrit levels. These results confirm the high bioaccumulation potential of PFOS in two bird species with different trophic strategies and the ability of this compound to alter biochemical parameters and organ development.

To avoid the previous invasive approach of measuring PFOS concentrations in bird nestlings, in 2007, Dauwe and coauthors demonstrated the reliability of using blood as a PFOS accumulation matrix in birds[37]. PFOS was measured in both the blood and liver of great tit individuals sampled in the same study area, getting values from 553 to 11,359 ng/g ww in the liver and from 24 to 1625 ng/mL in the blood. These PFOS levels are among the highest ever reported in free-living animals, and they exceeded, in most individuals, the hepatic benchmark concentrations for the protection of avian species[37]. A significant correlation in the PFOS concentrations measured in the liver and blood was found, suggesting the usefulness of blood as a non-destructive matrix for biomonitoring purposes[37].

While previous studies were specifically focused on PFOS, the investigation performed by Groffen and coauthors measured the accumulation of 12 perfluoroalkyl acids [PFAA, namely four perfluoroalkyl sulfonic acids (PFSA) and eight perfluoroalkyl carboxylic acids (PFCA)], as well as the contamination fingerprint, in the eggs of great tit individuals collected along a gradient away from the Antwerp FCP (from 1 to 70 km from the FCP)[38]. The median concentrations of PFOS, perfluorohexane sulfonate (PFHxS), perfluorodecane sulfonate (PFDS), and PFOA measured in great tit eggs collected in the surroundings of the FCP were 10,380 (extrapolated), 99.3, 47.7, and 19.8 ng/g ww, respectively. Although the concentrations of all compounds decreased with distance from the FCP, the levels measured in the eggs collected close to the FCP were higher than those reported in the eggs of free-living birds collected far from other contaminated sites[26,39]. PFOS was the main contributor to the PFSA and PFAA (range 63.4%-97.6%) profile at each site, while PFOA was the dominant PFCA close to the FCP and the nearest locations (range 41.0%-52.8%). PFDoDA (37.7%) prevailed in the farthest location. Interestingly, the results from this study show a slight decrease in PFOS levels compared to previous surveys performed in the same study area[37], likely due to the phasing out of this compound in the 2010s.

A further study by Lopez-Antia and coauthors investigated the accumulation of PFOS in the eggs of three bird species feeding primarily on invertebrates, namely the great tit, the northern lapwing (*Vanellus vanellus*), and the Mediterranean gull (*Larus melanocephalus*), sampled in 2006 nearby the Antwerp FCP[24]. Besides accumulation, the potential adverse effects induced by PFOS exposure in free-living birds were investigated by measuring the alterations of total protein content, cholesterol, triglyceride, and uric acid concentrations in the plasma of great tit adults only. PFOS levels measured in lapwing eggs (46,182 ng/g ww) were higher than those recorded in the other species, as well as with respect to previous surveys performed in the same area[36-38]. According to Groffen and coauthors, the levels of PFOS accumulated in eggs of lapwing and great tit decreased with distance from the FCP, up to 1700 m from the FCP[38]. In contrast, no differences in PFOS concentrations were noted in the eggs of the three species collected at 1700 and 5500 m from the FCP. Despite the high PFOS levels measured in the eggs of the great tit, reflecting high levels accumulated by mothers before transferring to their eggs, no changes in plasma parameters were noted in individuals collected at different distances from the FCP[24].

Additional information on the accumulation of 11 PFCA [i.e., perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), PFOA, PFNA, PFDA, PFUnDA, PFDoDA, perfluorotridecanoate (PFTrDA), and perfluorotetradecanoate (PFTeDA)] and 4 PFSA [perfluorobutane sulfonate (PFBS), PFHxS, PFOS, and PFDS] in the plasma of adults and nestlings of the great tit collected in the study area previously described[24,36-38] was reported by Lopez-Antia and coauthors[23]. In addition, antioxidant and oxidative stress parameters, including total antioxidant capacity, reduced glutathione, superoxide dismutase, catalase and glutathione peroxidase activity, and protein carbonyls, were measured in red blood cells of great tits to check for PFAS-related alterations and to assess potential causal relationships. Five of the eleven PFAA were detected in the highest ever reported concentrations in bird plasma, confirming that Antwerp FCP is a hotspot of PFAS contamination. In contrast to previous studies on birds, this investigation reported that females had higher mean concentrations (and detection frequencies) for PFOS and PFUnDA than males. Moreover, the findings suggest that maternal transfer and dietary intake represent the main routes of exposure for nestlings to PFOS, but not to other compounds. A positive correlation between PFAA concentrations and protein damage was noted in adult birds, while in nestlings, a positive correlation was observed with higher activity of antioxidant enzymes (i.e., glutathione peroxidase and catalase). These results suggest that exposure to different PFAA might imbalance the oxidative status and induce the onset of oxidative damage in free-living birds. In particular, the imbalance of the oxidative status observed in nestlings might translate into detrimental oxidative damage to cellular macromolecules in juvenile or adult individuals. Thus, because of their "sedentary" habits during early post-natal periods, nestlings can be considered as sentinels of local contamination able to point out early-warning signals of biological effects.

The study by Groffen and coauthors investigated the relationships between the concentrations of 4 PFSA and 11 PFCA in the eggs of great tits collected along a distance gradient from a pollution source and diverse reproductive parameters (i.e., the start of egg laying, clutch size, hatching success, fledging success and total breeding success, eggshell thickness, and body condition of the nestlings)[40]. High concentrations of PFOS (range 5111-187,032 ng/g ww), PFDoDA (range 1.1-133 ng/g ww), PFTrDA (range below the limit of quantification to 156 ng/g ww), and PFTeDA (range below the limit of quantification to 22 ng/g ww) measured close to the FCP were associated with a reduced hatching success of nests, with at least one egg hatched, thinner eggshells, and increased survival of the hatched chicks. High concentrations of PFDA were associated with reduced hatching success, especially in nests where no eggs hatched, an earlier start of egg laying, and a reduction of total breeding success, mainly caused by the failure in hatching. These findings suggest that exposure to high levels of PFAS can affect the reproduction of birds, with potential negative

consequences for the populations of species living in the surroundings of FCP.

Lastly, the recent study by Morganti and coauthors focused on the contamination due to both legacy and emerging PFAS in birds breeding in the surroundings of a perfluoropolymer factory site in the Upper Po Plain (Northern Italy)[16]. In this study, the accumulation of 13 PFAS, including PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFOS, perfluorooctane sulfonamide (FOSA), 8:2 fluorotelomer sulfonate (8:2 FTS), and C6O4, was investigated in the eggs of great and blue tits and European starlings (*Sturnus vulgaris*) breeding close to the factory and compared to a reference site located in a rural site in Northern Italy, far from any known point source of PFAS release. Overall, the PFAS concentrations in starling and great tit eggs collected nearby the FCP were at least one order of magnitude higher than those collected in the reference site. An unprecedented contamination level of PFOA and C6O4 was recorded in the eggs of these passerine birds. PFOA concentrations in starling eggs collected around the FCP were two orders of magnitude higher than those measured in the tit samples, suggesting species-specific differences in accumulation and maternal transfer of this compound, likely due to different trophic strategies. It was already known that PFOA, used for many years in polytetrafluoroethylene production, heavily contaminates the soil through atmospheric deposition[41]; therefore, soil organisms were likely enriched with this compound. As the European starling mainly feeds on terrestrial organisms (e.g., earthworms), which accumulate PFOA, the accumulation of this species was higher compared to that of the great tit. Differently, C6O4 is a recently introduced compound and it has not yet heavily contaminated the soil, so its presence in the air through aerosol emissions can result in deposition on local vegetation and the contamination of insects feeding on that vegetation[16]. This study returned crucial information on the presence and accumulation of emerging PFAS, confirming the role of FCP as hotspots of contamination of these emerging contaminants. However, further studies are necessary to understand the potential toxicity of these new compounds.

Accumulation in amphibians

Only one study investigated the accumulation of PFAS in amphibians collected nearby a production site[42]. Specifically, the occurrence, tissue distribution, and bioaccumulation of two novel PFAS, chlorinated polyfluorinated ether sulfonic acid (6:2 Cl-PFESA) and hexafluoropropylene oxide trimer acid (HFPO-TA), were measured in black-spotted frogs (*Pelophylax nigromaculatus*) collected in cities with or without the presence of fluorochemical industries in China. Frogs sampled in cities with large-scale FCP showed higher hepatic levels of ΣPFAS (i.e., 31.22 ng/g ww in Huantai and 54.28 ng/g ww in Changshu) than those from cities without FCP (i.e., 7.68 ng/g ww in Quzhou and 9.91 ng/g ww in Zhoushan). Sex- and age-specific differences in PFAS accumulation were noted, with females showing lower hepatic levels than males, while older frogs accumulated lower levels than younger ones. Concerning tissue distribution, skin, liver, and muscle contributed nearly 80% to the whole-body burden of 6:2 Cl-PFESA in males, while, in females, the ovaries alone accounted for 58.4%.

A recent study performed in a Chinese fluorochemical industrial area[43] demonstrated the accumulation of PFAS in the liver of the black-spotted frog (*Rana nigromaculata*; mean ΣPFAS = 163.40 ng/g ww). Measured physiological indices, namely body weight (BW), liver weight, hepatosomatic index, and triglyceride and cholesterol content, resulted in a positive correlation with the hepatic PFAS concentrations, particularly with PFOA, PFOS, and 6:2 Cl-PFESA. In particular, hepatic PFAS levels were positively correlated with body weight, liver weight, and triglyceride content, while PFOS concentrations were only significantly positively correlated with triglyceride content. These results suggest that PFAS exposure might disrupt the lipid metabolism of the black-spotted frog. Moreover, PFAS levels measured in male frogs were higher compared to females and showed stronger correlations with lipid metabolism-related indices, suggesting that males might be more sensitive to PFAS than females. The PFAS mechanism of action on lipid

metabolism suggested by the field study was confirmed through a companion laboratory study, which found that PFAS might act as lipid metabolism-disrupting chemicals in frogs[43].

Accumulation and effects in invertebrates

D'Hollander and coauthors first investigated the accumulation of PFOS in earthworms (family Lumbricidae), slugs (order Stylommatophora), millipedes (class Diplopoda), and woodlice (order Isopoda) collected at Blokkersdijk, close to the FCP in Antwerp (Belgium)[34]. PFOS was detected in all invertebrate taxonomic groups ranging from 28 to 9000 ng/g ww. In addition, the same study measured the concentrations of PFOS in soil (68 ng/g dry weight, dw) and water (22 ng/L) to derive the field biota-to-soil accumulation factors (BSAF), which ranged from 0.11 to 68 for earthworms. At the same time, these data allowed the estimation of the biomagnification factors for the wood mouse (concentration in the liver/concentration in berries), which were as high as 302, while biomagnification factors for invertebrates were lower (≤ 2).

In recent years, Groffen and coauthors explored if the concentrations of 15 PFAA, measured in isopods (Oniscidae) collected at the Antwerp (Belgium) FCP and in four other areas representing a gradient in the distance from the PFAS source (1-11 km), were related to the concentrations in the soil and the eggs of the great tit collected in the same areas[44]. PFOS and PFPeA were the main PFAA measured in isopods, with mean concentrations of 253 and 108 ng/g ww, respectively. As observed in other studies, the PFAA concentrations decreased with increasing distance from the FCP. As the PFAA concentrations measured in isopods correlated with the concentrations in the soils, as well as in bird eggs, this study demonstrated that isopods can be considered as excellent bioindicators of PFAA contamination in soil and eggs.

Recently, a field study was conducted to elucidate the distribution and partitioning of perfluoroalkyl carboxylic acids (PFCAs; C7-12) in a terrestrial ecosystem nearby a fluoropolymer manufacturing facility in Ohio[45]. Surface soil (0-6 cm), plants, and earthworms (*Lumbricus terrestris*) were collected from a field located within a one-mile radius of the FCP. The spatial distribution of PFCAs at this site suggested that both atmospheric deposition and groundwater recharge contributed to the contamination. Soil and plant tissue contamination consisted mainly of PFOA (range 77%-97% of the total PFCA), whereas longer-chain PFCA, such as PFUnDA (18%) and PFDoDA (32%), accounted for relatively higher proportions in earthworms than in soil. However, PFOA in earthworms was the major compound found at concentrations that ranged from 51 to 860 ng/g dw, with a mean value of 270 ng/g dw. The measured concentrations were higher than those reported for corresponding matrices in other locations worldwide, suggesting that fluorochemical manufacturing contributed to the contamination. BSAF (soil-earthworm) was calculated and compared to values derived from literature data and controlled laboratory exposure studies. The estimated BSAF of PFCA in earthworms increased with perfluorocarbon chain length (the values increased by approximately 0.07-0.58 log units for each additional CF_2 group), but the values were substantially lower than those in the controlled laboratory exposure studies. These results suggest that PFCA are efficiently transferred from soil to soil-dwelling invertebrates and that the bioavailability and bioaccumulation factors calculated from field studies can provide more realistic information on PFAS environmental transport and partitioning.

Lastly, Rusconi and coauthors evaluated the impact of a fluoropolymer factory plant in Northern Italy on a river macrobenthic community by a multidisciplinary approach based on combined chemical, ecological, and genetic analysis[30]. First, a BACI approach was implemented to investigate the stream macrobenthic community composition up- and downstream of the FCP discharge point, whereby the concentrations upstream of FCP discharge were lower than those downstream. The mean sum of 12 PFAA (i.e., PFBA,

PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFHxS, and PFOS) measured upstream was 35 ng/L (range 0-120 ng/L), while downstream was 2050 ng/L (range 333-8042 ng/L)[30]. Second, the toxicological implication at the population scale was explored by investigating changes in population genetics of a native benthic organism (*Hydropsyche modesta*, Trichoptera), with the aim to highlight sub-lethal effects that can accumulate through generations and lead to fitness reduction and, potentially, population extinction. The overall ecological quality of the sites was calculated through the STAR_ICMi multimetric index, which analyzes the community structure by calculating and integrating different biotic indices, such as ASPT, Log10(Sel_EPTD + 1), 1-GOLD, total number of families, number of EPT families, and Shannon-Wiener Index[46]. The multimetric index did not show any difference between the two sampling points, which were both classified as moderate. Nevertheless, there was some evidence of an ecological impact since the community composition upstream of the discharge point showed the presence of more sensitive taxa, such as Ephemeroptera (e.g., *Choroterpes* sp.), while the downstream site was dominated by a larger number of taxa generally resistant to moderate organic pollution loads such as Diptera taxa and *Echinogammarus* sp., potentially caused by genetic drift between exposed and non-exposed populations. However, genetic drift cannot be causally linked to a specific effect induced by PFAS.

CONCLUSION

The present review summarizes the findings from field studies investigating the accumulation and adverse effects induced by exposure to PFAS in free-living organisms collected close to PFAS hotspots such as fluorochemical plants. All studies included in this review revealed that organisms belonging to different taxonomic groups, from invertebrates to vertebrates, are exposed to and accumulate higher concentrations of single or mixtures of PFAS than individuals living in areas far from a focal contamination source. Biomonitoring activities in contaminated sites can play a crucial role in environmental risk assessment of PFAS. On the one hand, they can allow monitoring of the presence and the trend of single (or more focal compounds depending on the production or use by the FCP) PFAS over time, contributing to evaluating the efficacy of bans or mitigation actions for specific compounds. On the other hand, they can allow the early identification of new compounds (i.e., emerging PFAS) produced or used by industry and predict their environmental fate.

Moreover, as the concentrations accumulated by the organisms living (or breeding) nearby contaminated sites such as FCP are generally high, adverse effects at different levels of the biological organization might occur with high probability compared to other areas, even though no effect or even opposite effects could be observed due to acclimation and developed tolerance of free-living organisms. Thus, field biomonitoring activities can represent a valuable tool to explore the toxic effects and the mechanism(s) of action PFAS, as well as the potential causal relationships between PFAS concentrations and adverse effects, under realistic scenarios.

Our *ad hoc* search found few studies that focused on these topics, which could reflect the difficulty in sampling free-living organisms nearby contaminated sites, limited research attention given to these organisms, and/or the fact that our literature search strategy was not comprehensive (e.g., not including grey literature or literature in languages other than English). Surprisingly, no study has investigated bioaccumulation and adverse effects induced by PFAS contamination close to FCP using fish as biological indicators. This gap of knowledge could be due to the lack of water streams close to FCP or difficulties in sampling fish nearby the output sewage of FCP. However, considering that PFAS are commonly found in water, sediments, and biota collected in aquatic ecosystems worldwide, further research on bioaccumulation and effects of PFAS induced to fish living close FCP should be a priority. Future research synthesis on this topic should be performed using a comprehensive systematic review approach to reveal the full extent of

currently available evidence. Despite few exceptions, these studies returned crucial data to enlarge the understanding of the consequences of exposure to both legacy and emerging PFAS for wildlife at individual and population levels. To better understand the link between the effects at sub-individual, individual, and population levels, further biomonitoring studies could rely on evolutionary toxicology techniques, which might assess the transgenerational effects and the genetic variation within a population induced by the exposure to a substance (or mixtures of substances) at sub-lethal concentrations[47]. However, to perform effective biomonitoring, sensitivity/tolerance of the biological indicator and the underlying mechanisms should be established.

In conclusion, to obtain a comprehensive environmental risk assessment of both legacy and emerging PFAS, field studies aimed at investigating the exposure levels and the effects of PFAS in wildlife populations living nearby contaminated sites need to be performed using longitudinal and multigenerational approaches. To reach this goal, it is crucial that, at the global level, countries invest in facilities and human resources to perform analyses to enlarge the knowledge of the distribution, fate, and toxicity of PFAS contamination.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Parolini M, De Felice B, Rusconi M, Morganti M, Polesello S, Valsecchi S

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All authors declared that there are no conflicts of interest.

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Not applicable.

REFERENCES

1. Kissa E. Fluorinated surfactants and repellents (Vol. 97); 2001. New York: Marcel Dekker, Inc.
2. Moody CA, Field JA. Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams. *Environ Sci Technol* 2000;34:3864-70. DOI
3. Lanza HA, Cochran RS, Mudge JF, et al. Temporal monitoring of perfluorooctane sulfonate accumulation in aquatic biota downstream of historical aqueous film forming foam use areas. *Environ Toxicol Chem* 2017;36:2022-9. DOI PubMed
4. de Vries P, Slijkerman DME, Kwadijk CJAF, et al. The toxic exposure of flamingos to per- and Polyfluoroalkyl substances (PFAS) from firefighting foam applications in Bonaire. *Mar Pollut Bull* 2017;124:102-11. DOI PubMed
5. Navarro I, de la Torre A, Sanz P, et al. Uptake of perfluoroalkyl substances and halogenated flame retardants by crop plants grown in biosolids-amended soils. *Environ Res* 2017;152:199-206. DOI PubMed
6. Evich MG, Davis MJB, McCord JP, et al. Per- and polyfluoroalkyl substances in the environment. *Science* 2022;375:eabg9065. DOI

PubMed PMC
7. Wickham GM, Shriver TE. Emerging contaminants, coerced ignorance and environmental health concerns: the case of per- and polyfluoroalkyl substances (PFAS). *Sociol Health Illn* 2021;43:764-78. DOI PubMed
8. Washington JW, Rosal CG, McCord JP, et al. Nontargeted mass-spectral detection of chloroperfluoropolyether carboxylates in New Jersey soils. *Science* 2020;368:1103-7. DOI PubMed PMC
9. Wagner WE, Gold SC. Legal obstacles to toxic chemical research. *Science* 2022;375:138-41. DOI PubMed
10. Ankley GT, Cureton P, Hoke RA, et al. Assessing the ecological risks of per- and polyfluoroalkyl substances: current state-of-the science and a proposed path forward. *Environ Toxicol Chem* 2021;40:564-605. DOI PubMed PMC
11. Lindstrom AB, Strynar MJ, Libelo EL. Polyfluorinated compounds: past, present, and future. *Environ Sci Technol* 2011;45:7954-61. DOI PubMed
12. Jia S, Marques Dos Santos M, Li C, Snyder SA. Recent advances in mass spectrometry analytical techniques for per- and polyfluoroalkyl substances (PFAS). *Anal Bioanal Chem* 2022;414:2795-807. DOI PubMed
13. Glüge J, Scheringer M, Cousins IT, et al. An overview of the uses of per- and polyfluoroalkyl substances (PFAS). *Environ Sci Process Impacts* 2020;22:2345-73. DOI PubMed PMC
14. ARPAV; 2019. Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto. Il composto cC6O4 nel Po.
15. ARPAV; 2020. Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto. Sostanze perfluoro alchiliche (PFAS).
16. Morganti M, Polesello S, Pascariello S, et al. Exposure assessment of PFAS-contaminated sites using avian eggs as a biomonitoring tool: a frame of reference and a case study in the Po River valley (Northern Italy). *Integr Environ Assess Manag* 2021;17:733-45. DOI PubMed
17. ECHA; 2019. European chemicals agency: difluoro{[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy}acetic acid.
18. Ng C, Cousins IT, DeWitt JC, et al. Addressing urgent questions for PFAS in the 21st century. *Environ Sci Technol* 2021;55:12755-65. DOI PubMed PMC
19. Podder A, Sadmani AHMA, Reinhart D, Chang NB, Goel R. Per and poly-fluoroalkyl substances (PFAS) as a contaminant of emerging concern in surface water: a transboundary review of their occurrences and toxicity effects. *J Hazard Mater* 2021;419:126361. DOI PubMed
20. Bernardini I, Matozzo V, Valsecchi S, et al. The new PFAS C6O4 and its effects on marine invertebrates: first evidence of transcriptional and microbiota changes in the Manila clam Ruditapes philippinarum. *Environ Int* 2021;152:106484. DOI PubMed
21. Fabrello J, Ciscato M, Masiero L, et al. New compounds, old problems. The case of C6O4 - a substitute of PFOA - and its effects to the clam Ruditapes philippinarum. *J Hazard Mater* 2021;420:126689. DOI PubMed
22. Fabrello J, Targhetta F, Ciscato M, et al. First evidence of in vitro effects of C6O4 - a substitute of PFOA-on haemocytes of the clam Ruditapes philippinarum. *Toxics* 2021;9:191. DOI PubMed PMC
23. Lopez-Antia A, Groffen T, Lasters R, et al. Perfluoroalkyl acids (PFAAs) concentrations and oxidative status in two generations of great tits inhabiting a contamination hotspot. *Environ Sci Technol* 2019;53:1617-26. DOI PubMed
24. Lopez-Antia A, Dauwe T, Meyer J, Maes K, Bervoets L, Eens M. High levels of PFOS in eggs of three bird species in the neighbourhood of a fluoro-chemical plant. *Ecotoxicol Environ Saf* 2017;139:165-71. DOI PubMed
25. Blévin P, Angelier F, Tartu S, et al. Perfluorinated substances and telomeres in an Arctic seabird: cross-sectional and longitudinal approaches. *Environ Pollut* 2017;230:360-7. DOI PubMed
26. Custer CM, Custer TW, Delaney R, Dummer PM, Schultz S, Karouna-Renier N. Perfluoroalkyl contaminant exposure and effects in tree swallows nesting at clarks marsh, Oscoda, Michigan, USA. *Arch Environ Contam Toxicol* 2019;77:1-13. DOI PubMed
27. Valsecchi S, Rusconi M, Mazzoni M, et al. Occurrence and sources of perfluoroalkyl acids in Italian river basins. *Chemosphere* 2015;129:126-34. DOI PubMed
28. Hoff PT, Van Campenhout K, Van de Vijver K, et al. Perfluorooctane sulfonic acid and organohalogen pollutants in liver of three freshwater fish species in Flanders (Belgium): relationships with biochemical and organismal effects. *Environ Pollut* 2005;137:324-33. DOI PubMed
29. Lopez-Antia A, Kavelaars MM, Müller W, Bervoets L, Eens M. Understanding PFAAs exposure in a generalist seabird species breeding in the vicinity of a fluorochemical plant: Influence of maternal transfer and diet. *Environ Pollut* 2021;271:116355. DOI PubMed
30. Rusconi M, Marziali L, Stefani F, et al. Evaluating the impact of a fluoropolymer plant on a river macrobenthic community by a combined chemical, ecological and genetic approach. *Sci Total Environ* 2015;538:654-63. DOI PubMed
31. Eberhardt LL. Quantitative ecology and impact assessment. *J Environ Manag* 1976;4:27-70. DOI
32. Green RH. Sampling design and statistical methods for environmental biologists. New York: John Wiley & Sons; 1979. DOI
33. Hoff PT, Scheirs J, Van de Vijver K, et al. Biochemical effect evaluation of perfluorooctane sulfonic acid-contaminated wood mice (Apodemus sylvaticus). *Environ Health Perspect* 2004;112:681-6. DOI PubMed PMC
34. D'Hollander W, De Bruyn L, Hagenaars A, de Voogt P, Bervoets L. Characterisation of perfluorooctane sulfonate (PFOS) in a terrestrial ecosystem near a fluorochemical plant in Flanders, Belgium. *Environ Sci Pollut Res Int* 2014;21:11856-66. DOI PubMed
35. Greaves AK, Letcher RJ, Sonne C, Dietz R, Born EW. Tissue-specific concentrations and patterns of perfluoroalkyl carboxylates and sulfonates in East Greenland polar bears. *Environ Sci Technol* 2012;46:11575-83. DOI PubMed
36. Hoff PT, Van de Vijver K, Dauwe T, et al. Evaluation of biochemical effects related to perfluorooctane sulfonic acid exposure in organohalogen-contaminated great tit (Parus major) and blue tit (Parus caeruleus) nestlings. *Chemosphere* 2005;61:1558-69. DOI

PubMed

37. Dauwe T, Van de Vijver K, De Coen W, Eens M. PFOS levels in the blood and liver of a small insectivorous songbird near a fluorochemical plant. *Environ Int* 2007;33:357-61. DOI PubMed
38. Groffen T, Lopez-Antia A, D'Hollander W, Prinsen E, Eens M, Bervoets L. Perfluoroalkylated acids in the eggs of great tits (Parus major) near a fluorochemical plant in Flanders, Belgium. *Environ Pollut* 2017;228:140-8. DOI PubMed
39. Custer CM, Custer TW, Dummer PM, et al. Legacy and contaminants of emerging concern in tree swallows along an agricultural to industrial gradient: Maumee River, Ohio. *Environ Toxicol Chem* 2020;39:1936-52. DOI PubMed
40. Groffen T, Lasters R, Lopez-Antia A, Prinsen E, Bervoets L, Eens M. Limited reproductive impairment in a passerine bird species exposed along a perfluoroalkyl acid (PFAA) pollution gradient. *Sci Total Environ* 2019;652:718-28. DOI PubMed
41. Davis KL, Aucoin MD, Larsen BS, Kaiser MA, Hartten AS. Transport of ammonium perfluorooctanoate in environmental media near a fluoropolymer manufacturing facility. *Chemosphere* 2007;67:2011-9. DOI PubMed
42. Cui Q, Pan Y, Zhang H, et al. Occurrence and tissue distribution of novel perfluoroether carboxylic and sulfonic acids and legacy per/polyfluoroalkyl substances in black-spotted frog (pelophylax nigromaculatus). *Environ Sci Technol* 2018;52:982-90. DOI PubMed
43. Lin H, Liu Z, Yang H, et al. Per- and polyfluoroalkyl substances (PFASs) impair lipid metabolism in rana nigromaculata: a field investigation and laboratory study. *Environ Sci Technol* 2022;56:13222-32. DOI PubMed
44. Groffen T, Eens M, Bervoets L. Do concentrations of perfluoroalkylated acids (PFAAs) in isopods reflect concentrations in soil and songbirds? *Sci Total Environ* 2019;657:111-23. DOI PubMed
45. Zhu H, Kannan K. Distribution and partitioning of perfluoroalkyl carboxylic acids in surface soil, plants, and earthworms at a contaminated site. *Sci Total Environ* 2019;647:954-61. DOI PubMed
46. Buffagni A, Erba S, Furse MT. A simple procedure to harmonize class boundaries of assessment systems at the pan-European scale. *Environ Sci Policy* 2007;10:709-24. DOI
47. Rusconi M, Bettinetti R, Polesello S, Stefani F. Evolutionary toxicology as a tool to assess the ecotoxicological risk in freshwater ecosystems. *Water* 2018;10:490. DOI