

Review

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Review

# PFOA, PFOS, PFBA, PFBS, ADONA and GenX: Toxicological Profile for Freshwater Ecosystems

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#### **Abstract**

Per- and polyfluoroalkyl substances (PFAS) represent a diverse group of chemicals widely used in industrial and consumer products due to their unique physicochemical properties. This review critically examines the ecotoxicological profiles of six key PFAS—PFOA, PFOS, PFBA, PFBS, GenX, and ADONA—focusing on their effects on standard aquatic model organisms (*Daphnia magna*, *Raphidocelis sub*capitata, and *Aliivibrio fischeri*). The data highlight that legacy compounds such as PFOA and PFOS generally show greater toxicity, particularly with reproductive and growth endpoints in *D. magna* and growth inhibition in R. subcapitata, at lower concentrations compared to their short-chain analogues (PFBA, PFBS). GenX and ADONA, introduced as replacements for long-chain PFAS, have been the subject of far fewer studies. Available data, mainly from regulatory sources, suggest lower acute and chronic toxicity for these alternatives under standard test conditions. However, the lack of comprehensive data—especially regarding sub-lethal, chronic, and mixture effects—precludes firm conclusions about their environmental safety. The findings underscore the need for further ecotoxicological research on emerging PFAS and call for integrating bioassays with chemical analyses to better assess risks associated with PFAS mixtures in aquatic ecosystems.

**Keywords:** ecotoxicity; PFAS; water

### 1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a wide class of anthropogenic compounds that have been manufactured since the late 1940s . The universe of PFAS was depicted by the new OECD definition, drafted in 2021 and based on the OECD 2018 PFAS List [1]in addition to the recent non target screening studies, which renewed the previous definition [2] addressing some gaps and ambiguous descriptions. The revision's goal is not to broaden the PFAS world, but to accurately outline it and provide a reference point for all users. As a result, PFASs are defined as fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), i.e., with a few noted exceptions, any chemicals with at least a perfluorinated methyl group (-CF2-) [1], [3]

High electronegativity, low polarizability and small size of the fluorine atoms which result in a strong C-F bond, weak intermolecular interaction, low surface energy and the formation of a sheath around the backbone structure guarantee the thermal and chemical stability, electrical inertness in addition to hydrophobic and lipophobic surfactant properties [4]. These different physicochemical features have led to PFAS being used in a wide range of industrial applications as well as consumer items. In the chemical industry, PFAS are used as processing aids in the polymerization of fluoropolymers (such as PTFE and PVDF), as wetting agents in metal plating, and as film formers in aqueous foam producing foams in fire-fighting foam (AFFFs). Significant amounts of PFAS have been

used as surface protectors in textile items (raincoats, snowsuits, umbrellas, tents, and awnings), food contact material (plates, popcorn bags, pizza boxes, food containers, and non-stick cookware), upholstery, and leather products due to their water repellency and stain resistance [2], [5–7]

Due to their persistence and tendency to bioaccumulate, these compounds have become widespread throughout various environmental compartments, including rivers, lakes, groundwater, sediments, soils, air, and living organisms [8–10]. Concerns over their potential toxicity and related health risks have driven several researchers to undertake ecotoxicological studies using a range of biological models. Intergenerational research using two waterflea species (*D. magna* and *Moina macrocopa*) found that chronic exposure to both PFOA and PFOS in the parent generation resulted in substantial reductions in reproductive and population growth rates in their offspring [11]. Acute, developmental ad transgenerational toxicity on zebrafish was observed, testing single toxicity and mixtures of different compounds [12–14]. A 21-day reproduction test in *D. magna* at sub-lethal GenX concentrations resulted in substantial reductions in the number of offspring with exposure doses of 8.13 mg/L or higher [15]; similar findings were also found with sub-lethal exposure to legacy PFAS contaminants (PFOS and PFOA[16,17]

However, there is little known regarding their combined toxicity to aquatic organisms. In the present study, a single exposure of PFOA, PFOS, PFBA, PFBS to *Daphnia magna*, *Raphidocelis subcapit*ata and *Aliivibrio fischeri* were investigated. Subsequently, the collected data were refined in order to determine the theoretically combined toxicity using the concentration addition model, as demonstrated by [18]. This calculation simulates the toxicity-shift in the replacement legacy PFAS phase by observing the toxicity pattern of a PFAS mixture in which the various components are present in variable proportions.

Historically, nearly all manufacturers utilized ammonium or sodium perfluorooctanoate (APFO and NaPFO) as processing aids in the emulsion polymerization of polytetrafluoroethylene (PTFE), perfluorinated ethylene-propylene copolymer (FEP), perfluoroalkoxy polymer (PFA), and specific fluoroelastomers; and employed ammonium perfluorononanoate (APFN) in the emulsion polymerization of polyvinylidene fluoride (PVDF) [19]. However, during the recent transition, most of the producers have developed their own alternatives: GenX from DuPont and ADONA from 3M/Dyneon [6]

GenX chemicals act as substitutes for the longer-chain PFOA, which had been phased out in the United States by 2015 after an agreement between manufacturers and the U.S. Environmental Protection Agency (EPA) under the PFOA Stewardship Program initiated in 2006. GenX chemicals are used in the production of fluoropolymers, which have several industrial uses throughout the medical, automotive, electronics, aerospace, energy, and semiconductor sectors [20]

# 2. Target Molecules and Model Organisms: Selection Criteria

The subject of this review is six compounds, highly significant in terms of production volumes, environmental contamination (both diffuse and point-source pollution), and toxicological concerns, supported by substantial available data. In particular, perfluorooctanoic acid (PFOA) and its sulfonic analogue perfluorooctane sulfonic acid (PFOS) were selected. PFOA began to be produced in the 1940s, initially as a by-product and later as a commercial product for various industrial applications, including non-stick coatings and fire-fighting foams. PFOS production started in the 1950s, mainly for use in fire-fighting foams, water-repellent treatments, and paper and textile coatings. Additionally, the review includes perfluorobutanoic acid (PFBA) and perfluorobutane sulfonic acid (PFBS), both of which have seen increased use since the early 2000s as replacements for long-chain PFAS compounds, following the phase-out of PFOA and PFOS due to environmental and health concerns. PFBA and PFBS, thanks to their shorter chains, have been marketed as alternatives considered to have a lower bioaccumulation potential, although their environmental persistence remains a critical issue. Finally, two other substances were considered, namely, two substitutes for long-chain PFASs (with similar industrial performance and desired lower potential for bioaccumulation and toxicity): GenX and ADONA. GenX, or hexafluoropropylene oxide dimer acid



(HFPO-DA), has been produced since around 2009 as a replacement for PFOA in fluoropolymer manufacturing. ADONA, or ammonium 4,8-dioxa-3H-perfluorononanoate, has been manufactured since the early 2000s. However, recent studies have raised concerns about their environmental persistence, mobility, and possible health effects, indicating that these substitutes also warrant careful monitoring and risk assessment.

This literature review focused on gathering data on the model organisms most widely used to assess potential risks to aquatic ecosystems. The use of these organisms has been prescribed or recommended by international regulations since the 1990s. Their adoption has been facilitated by the relative simplicity of the test procedures, as well as the availability of reagents, test kits, and equipment compliant with established standards and guidelines. Specifically, the study focused on decomposers (belonging to the detritus-based food web), primary producers (at the first trophic level), and primary consumers, with the aim of defining a minimum battery of tests that could represent the aquatic ecosystem. Among decomposers, the bioluminescent bacterium *A. fischeri* was selected; for primary producers, the unicellular alga *R. subcapitata* was chosen; and for primary consumers, the cladoceran crustacean *D. magna* was considered. Table 1 lists the compounds considered in this review and details their acronym, CAS number, molecular formula, average molar mass and structural formula.

**Table 1.** Acronym, preferred name, CAS, molecular formula and average molar mass of the molecules considered in this review (U.S. EPA ECOTOXicology Knowledgebase).

Acronym	Preferred name	CAS number	Molecular formula	Average mass (g/mol)	Structural formula
PFOA	Perfluorooctanoic acid	335-67-1	C8HF15O2	414.07	HO F F F F F F F F
PFOS	Perfluorooctanesulfonic acid	1763-23-1	C8HF17O3S	500.13	F
PFBA	Perfluorobutanoic acid	375-22-4	C4HF7O2	214.04	F F F OH

PFBS	Perfluorobutanesulfonic acid	375-73-5	C4HF9O3S	300.09	HO S F F F F
HFPO- DA (Gen-X)	Perfluoro-2-methyl- 3-oxahexanoic acid (also known as hexafluoropropylene oxide dimer acid)	13252-13- 6	C6HF11O3	330.05	HO F F F
ADONA	4,8-Dioxa-3H- perfluorononanoic acid	919005- 14-4	C7H2F12O4	378.07	HO F F

#### 3. Results

The results of the critical literature review were summarized in tables, each providing information for a specific compound and a selected model organism.

# 3.1. Toxicity Quantified with the Crustacean Daphnia Magna

#### 3.1.1. PFOA

Table 2 reports the main toxicological findings for PFOA; the unit of measurement was standardized and all original values expressed in mg/L.

**Table 2.** Experimental findings obtained after the exposure (various time length) of *D. magna* to PFOA. The available endpoints were included (beside immobilization).

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confide nce interval	Ref.
2	Intoxication	Immobi le	EC05	182 mg/L	NR - NR	[21]
2	Intoxication	Immobi le	EC10	195 mg/L	NR - NR	[21]
2	Intoxication	Immobi le	EC50	67.2 mg/L	31.3 – 88.5	[22]

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confide nce interval	Ref.
21	Mortality	Lifespa n	EC10	11.12 mg/L	NR - NR	[23]
21	Reproduction	Mean spawns per female	EC10	7.02 mg/L	NR - NR	[23]
1	Intoxication	Immobi le	EC50	219.87 mg/L	209.52 – 229.81	[24]
2	Intoxication	Immobi le	EC50	211.59 mg/L	184.68 - 254.24	[24]
2	Intoxication	Immobi le	EC50	239 mg/L	190 - 287	[21]
2	Intoxication	Immobi le	EC50	109 mg/L	NR - NR	[25]
2	Intoxication	Immobi le	EC50	476.52 mg/L	375.32 <i>-</i> 577.72	[11]
1	Intoxication	Immobi le	EC50	675.05 mg/L	559.62 <i>-</i> 790.50	[11]
2	Intoxication	Immobi le	EC50	223.60 mg/L	188.40 - 264.59	[22]
2	Intoxication	Immobi le	EC50	110.7 mg/L	NR - NR	[26]
2	Mortality	Mortalit y	LC50	268.73 mg/L	225.67 - 313.04	[22]
2	Mortality	Mortalit y	LC50	139.0 mg/L	NR - NR	[26]
2	Mortality	Mortalit y	LC50	137 mg/L	NR - NR	[25]

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confidence interval		Ref.
2	Mortality	Mortalit y	LC50/	120.91 mg/L	NR NR	-	[27]
2	Mortality	Mortalit y	LC50	201.85 mg/L	134.68 302.50	-	[23]
2	Intoxication	Immobi le	LOEC	500 mg/L	NR NR	-	[11]
1	Intoxication	Immobi le	LOEC	1000 mg/L	NR NR	-	[11]
21	Growth	Length	LOEC	22.61 mg/L	NR NR	-	[27]
21	Reproduction	Time to first progen y	LOEC	15.11 mg/L	NR NR	-	[27]
21	Reproduction	Progen y counts/ number s	LOEC	10.10 mg/L	NR NR	-	[27]
1	Intoxication	Immobi le	LOEC	186.33 mg/L	NR NR	-	[24]
2	Intoxication	Immobi le	LOEC	227.74 mg/L	NR NR	-	[24]
21	Reproduction	Fecundi ty	LOEC	0.16 mg/L	NR NR	-	[26]
21	Reproduction	Fecundi ty	LOEC	4 mg/L	NR NR	-	[26]
21	Reproduction	Time to pregna	LOEC	4 mg/L	NR NR	-	[26]

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confice nce interva		Ref.
		ncy/gra vidity					
21	Reproduction	Time to first progen y	LOEC	4 mg/L	NR NR	-	[26]
21	Growth	Length	LOEC	0.16 mg/L	NR NR	-	[26]
21	Reproduction	Time to first progen y	LOEC	12.5 mg/L	NR NR	-	[11]
21	Reproduction	Progen y counts/ number s	LOEC	25 mg/L	NR NR	-	[11]
21	Growth	Length	LOEC	50 mg/L	NR NR	-	[11]
21	Reproduction	Fecundi ty	LOEC	0.41 mg/L	NR NR	-	[28]
21	Reproduction	Time to first progen y	LOEC	10.10 mg/L	NR NR	-	[27]
1	Intoxication	Immobi le	NOEC	165.63 mg/L	NR NR	-	[24]
2	Intoxication	Immobi le	NOEC	207.04 mg/L	NR NR	-	[24]
21	Growth	Length	NOEC	0.032 mg/L	NR NR	-	[26]

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confidence interv		Ref.
21	Reproduction	Time to pregna ncy/gra vidity	NOEC	0.8 mg/L	NR NR	-	[26]
21	Reproduction	Fecundi ty	NOEC	0.032 mg/L	NR NR	-	[26]
21	Reproduction	Time to first progen y	NOEC	0.8 mg/L	NR NR	-	[26]
21	Reproduction	Fecundi ty	NOEC	37.97 mg/L	NR NR	-	[23]
21	Reproduction	Fecundi ty	NOEC	0.8 mg/L	NR NR	-	[26]
21	Reproduction	Progen y counts/ number s	NOEC	37.97 mg/L	NR NR	-	[23]
21	Reproduction	Time to first progen y	NOEC	37.97 mg/L	NR NR	-	[23]
21	Reproduction	Progen y counts/ number s	NOEC	12.5 mg/L	NR NR	-	[11]
21	Reproduction	Progen y counts/	NOEC	50 mg/L	NR NR	-	[11]

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confide nce interval		Ref.
		number s					
21	Reproduction	Time to first progen y	NOEC	6.25 mg/L	NR NR	-	[11]
21	Growth	Length	NOEC	25 mg/L	NR NR	-	[11]
21	Mortality	Surviva l	NOEC	50 mg/L	NR NR	-	[11]
1	Intoxication	Immobi le	NOEC	500 mg/L	NR NR	-	[11]
2	Intoxication	Immobi le	NOEC	250 mg/L	NR NR	-	[11]
21	Reproduction	Time to first progen y	NOEC	6.71 mg/L	NR NR	-	[27]
21	Growth	Length	NOEC	15.11 mg/L	NR NR	-	[27]
21	Reproduction	Progen y counts/ number s	NOEC	6.71 mg/L	NR NR	-	[27]
21	Reproduction	Time to first progen y	NOEC	10.10 mg/L	NR NR	-	[27]
21	Mortality	Mortalit y	NR- ZERO	6.25 mg/L	NR NR	-	[11]

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Conficence interval		Ref.
2	Intoxication	Immobi le	EC50	181 mg/L	166 198	-	[29]*
21	Growth	Length	EC50	> 88.6/ mg/L	NR/ NR/	-	[30]
21	Reproduction	Fecundi ty	EC50	39.6/ mg/L	36.7/ 42.5/	-	[30]
1	Intoxication	Immobi le	EC50	298 mg/L	<ul><li>278</li><li>321</li></ul>	-	[29]*
2	Intoxication	Immobi le	EC50	480 mg/L	NR NR	-	[31]
21	Growth	Length	EC50	> 88.6 mg/L	NR NR	-	[31]
21	Reproduction	Time to first progen y	EC50	39.6 mg/L	NR NR	-	[31]
1	Intoxication	Immobi le	EC50	599 mg/L	NR NR	-	[31]
2	Intoxication	Immobi le	EC50	156.9 mg/L	NR NR	-	[32]*
21	Mortality	Mortalit y	LC50	> 100 mg/L	NR NR	-	[33]*
21	Mortality	Mortalit y	LC50/	> 88.6/ mg/L	NR/ NR/	-	[30]
2	Mortality	Mortalit y	LC50	226.70 mg/L	NR NR	-	[32]
21	Reproduction	Fecundi ty	LOEC	44.2/ mg/L	NR/ NR/	-	[30]

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confidence interv		Ref.
21	Reproduction	Time to first progen y	LOEC	44.2 mg/L	NR NR	-	[31]
2	Reproduction	Progen y counts/ number s	LOEC	100 mg/L	NR NR	_	[33]
1	Reproduction	Progen y counts/ number s	LOEC	10 mg/L	NR NR	-	[33]
21	Reproduction	Time to first progen y	LOEC	100 mg/L	NR NR	-	[33]
21	Reproduction	Progen y counts/ number s	LOEC	32 mg/L	NR NR	-	[33]
21	Growth	Length	LOEC	88.6/ mg/L	NR/ NR/	-	[30]
21	Mortality	Mortalit y	NOEC	88.6/ mg/L	NR/ NR/	-	[30]
21	Growth	Length	NOEC	44.2/ mg/L	NR/ NR/	-	[30]
21	Reproduction	Time to first	NOEC	20 mg/L	NR NR	-	[31]

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confide nce interval		Ref.
		progen y					
21	Growth	Length	NOEC	44.2 mg/L	NR NR	-	[31]
21	Mortality	Surviva l	NOEC	88.6 mg/L	NR NR	-	[31]
21	Intoxication	Immobi le	NOEC	> 88.6 mg/L	NR NR	-	[31]
21	Reproduction	Progen y counts/ number s	NOEC	20 mg/L	NR NR	-	[31]
2	Reproduction	Progen y counts/ number s	NOEC	32 mg/L	NR NR	-	[33]
1	Reproduction	Progen y counts/ number s	NOEC	3.2 mg/L	NR NR	-	[33]
21	Reproduction	Progen y counts/ number s	NOEC	10 mg/L	NR NR	-	[33]
21	Reproduction	Time to first progen y	NOEC	32 mg/L	NR NR	-	[33]

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confide nce interval	Ref.
21	Reproduction	Fecundi ty	NOEC	20.0/ mg/L	NR/ - NR/	[30]
2	Intoxication	Immobi le	NOEC	125 mg/L	NR - NR	[29]
1	Intoxication	Immobi le	NOEC	125 mg/L	NR - NR	[29]
21	Mortality	Surviva 1	NOEC	> 100 mg/L	NR - NR	[33]
21	Mortality	Surviva 1	NR	NR/ mg/L	4.31/ - 88.6/	[30]
21	Reproduction	Abort	NR	NR/ mg/L	4.31/ - 88.6/	[30]
21	Reproduction	Progen y counts/ number s	NR	NR/ mg/L	4.31/ - 88.6/	[30]
21	Intoxication	Immobi le	NR	NR/ mg/L	4.31/ - 88.6/	[30]

<sup>(\*)</sup> Values refer to CAS 3825-26-1, perfluorooctanoic acid ammonium salt.

# 3.1.2. PFOS

Table 3 reports the main toxicological findings for PFOS, the analogue sulfonated of PFOA; the unit of measurement was standardized and all original values expressed in mg/L.

**Table 3.** Experimental findings obtained after the exposure (various time length) of *D. magna* to PFOS. The available endpoints were included (beside immobilization).

Exposu re time (day)	Response	Respon se measur ement	Parameter	Value	Confide nce interval	Ref.
1	Intoxication	Immobi le	EC50	76.82 mg/L	62.09 - 91.56	[11]
2	Intoxication	Immobi le	EC50	37.36 mg/L	30.72 - 43.99	[11]
2	Intoxication	Immobi le	EC50	23.41 mg/L	NR - NR	[34]
2	Mortality	Mortalit y	LC50	49.27 mg/L	NR - NR	[34]
1	Mortality	Mortalit y	LC50	156.67 mg/L	132.21 - 179.03	[35]
2	Mortality	Mortalit y	LC50	116.52 mg/L	99.32 - 145.01	[35]
2	Intoxication	Immobi le	LOEC	25 mg/L	NR - NR	[11]
21	Reproduction	Progen y counts/ number s	LOEC	2.5 mg/L	NR - NR	[11]
21	Reproduction	Time to first progen y	LOEC	2.5 mg/L	NR - NR	[11]
21	Reproduction	Progen y counts/ number s	LOEC	2.5 mg/L	NR - NR	[11]

Exposu re time (day)	Response	Respon se measur ement	Parameter	Value	Confide nce interval		Ref.
21	Growth	Length	LOEC	0.008 mg/L	NR NR	-	[34]
21	Reproduction	Fecundi ty	LOEC	0.04 mg/L	NR NR	-	[34]
21	Reproduction	Progen y counts/ number s	LOEC	0.04 mg/L	NR NR	-	[34]
21	Reproduction	Fecundi ty	LOEC	5.30 mg/L	NR/ NR/	-	[28]
21	Reproduction	Time to pregna ncy/gra vidity	LOEC	1 mg/L	NR NR	-	[34]
21	Reproduction	Time to first progen y	LOEC	1 mg/L	NR NR	-	[34]
1	Intoxication	Immobi le	LOEC	50 mg/L	NR NR	-	[11]
21	Mortality	Surviva l	NOEC	5 mg/L	NR NR	-	[11]
21	Reproduction	Progen y counts/ number s	NOEC	1.25 mg/L	NR NR	-	[11]
21	Growth	Length	NOEC	5 mg/L	NR NR	-	[11]

Exposu re time (day)	Response	Respon se measur ement	Parameter	Value	Confide nce interval	Ref.
21	Reproduction	Progen y counts/ number s	NOEC	1.25 mg/L	NR - NR	[11]
21	Reproduction	Time to first progen y	NOEC	1.25 mg/L	NR - NR	[11]
21	Reproduction	Progen y counts/ number s	NOEC	0.008 mg/L	NR - NR	[34]
21	Reproduction	Time to pregna ncy/gra vidity	NOEC	0.2 mg/L	NR - NR	[34]
21	Reproduction	Fecundi ty	NOEC	0.008 mg/L	NR - NR	[34]
2	Growth	Growth rate	NOEC	36/ mg/L	NR/ - NR/	[36]
21	Reproduction	Time to first progen y	NOEC	0.2 mg/L	NR - NR	[34]
21	Reproduction	Fecundi ty	NOEC	0.53 mg/L	NR/ - NR/	[28]
2	Intoxication	Immobi le	NOEC	12.5 mg/L	NR - NR	[11]
1	Intoxication	Immobi le	NOEC	25 mg/L	NR - NR	[11]

Exposu re time (day)	Response	Respon se measur ement	Parameter	Value	Confide nce interval	Ref.
2	Mortality	Mortalit y	EC10	58.57 mg/L	12.16 – 100.56	[37]
21	Mortality	Lifespa n	EC10	4.17 mg/L	NR - NR	[23]
21	Reproduction	Mean spawns per female	EC10	2.26 mg/L	NR - NR	[23]
1	Mortality	Mortalit y	EC10	90.62 mg/L	89.51 – 91.72	[37]
2	Intoxication	Immobi le	EC50	67.2 mg/L	31.3 - 88.5	[22]
1	Intoxication	Immobi le	EC50	193 mg/L	177 - 209	[29]
1	Mortality	Mortalit y	EC50	> 100.56 mg/L	NR - NR	[22]
2	Intoxication	Immobi le	EC50	63 mg/L	58 - 69	[29]
2	Intoxication	Immobi le	EC50	79.35 mg/L	NR - NR	[38]
2	Mortality	Mortalit y	EC50	67.41 mg/L	36.47 – 100.56	[37]
2	Mortality	Mortalit y	EC90	69.62 mg/L	12.15 – 100.56	[37]
1	Mortality	Mortalit y	EC90	> 100.56 mg/L	NR - NR	[37]
[22] N2	Mortality	Mortalit y	LC50/	22.77 mg/L	NR - NR	[27]

Exposu re time (day)	Response	Respon se measur ement	Parameter	Value	Confide nce interval	Ref.
2	Mortality	Mortalit y	LC50	78.09 mg/L	54.38 <i>-</i> 112.13	[23]
2	Mortality	Surviva 1	LC50	130 mg/L	112 - 136	[22]
21	Mortality	Mortalit y	LC50	9.1 mg/L	7.3 - 11.5	[29]
21	Mortality	Mortalit y	LC50	42.9 mg/L	31.7 - 56.4	[22]
21	Reproduction	Progen y counts/ number s	LOEC	0.67 mg/L	NR - NR	[27]
2	Mortality	Mortalit y	LOEC	26.52 mg/L	24.86 – 27.63	[39]
21	Reproduction	Time to first progen y	LOEC	0.67 mg/L	NR - NR	[27]
21	Growth	Length	LOEC	1.01 mg/L	NR - NR	[27]
21	Reproduction	Time to first progen y	LOEC	0.67 mg/L	NR - NR	[27]
21	Mortality	Mortalit y	LOEC	26.52 mg/L	22.5 <i>-</i> 25.0	[39]
21	Reproduction	Progen y counts/	LOEC	5 mg/L	NR - NR	[29]

Exposu re time (day)	Response	Respon se measur ement	Parameter	Value	Confide nce interval	Ref.
		number s				
21	Mortality	Surviva l	LOEC	10 mg/L	NR - NR	[29]
2	Intoxication	Immobi le	LOEC	100 mg/L	NR - NR	[38]
21	Reproduction	Progen y counts/ number s	LOEC	16 mg/L	NR - NR	[38]
21	Reproduction	Fecundi ty	LOEC	50 mg/L	NR - NR	[22]
21	Mortality	Surviva l	LOEC	50 mg/L	NR - NR	[22]
21	Reproduction	Progen y counts/ number s	LOEC	50 mg/L	NR - NR	[22]
21	Intoxication	Immobi le	LOEC	50 mg/L	NR - NR	[40]
21	Mortality	Mortalit y	MATC	18.79 mg/L	NR - NR	[39]
21	Reproduction	Fecundi ty	NOEC	25 mg/L	NR - NR	[22]
21	Mortality	Mortalit y	NOEC	5.3 mg/L	2.5 - 9.2	[22]
21	Reproduction	Progen y counts/	NOEC	25 mg/L	NR - NR	[22]

Exposu re time (day)	Response	Respon se measur ement	Parameter	Value	Confide nce interval		Ref.
		number s					
21	Mortality	Surviva l	NOEC	25 mg/L	NR NR	-	[22]
21	Intoxication	Immobi le	NOEC	25 mg/L	NR NR	-	[40]
2	Reproduction	Progen y counts/ number s	NOEC	10 mg/L	NR NR	-	[29]
21	Reproduction	Fecundi ty	NOEC	7.43 mg/L	NR NR	-	[23]
21	Reproduction	Time to first progen y	NOEC	7.43 mg/L	NR NR	-	[23]
21	Reproduction	Progen y counts/ number s	NOEC	7.43 mg/L	NR NR	-	[23]
2	Intoxication	Immobi le	NOEC	66 mg/L	NR NR	-	[38]
21	Reproduction	Progen y counts/ number s	NOEC	8 mg/L	NR NR	-	[38]
2	Mortality	Mortalit y	NOEC	12.71 mg/L	11.2 11.8	-	[39]

Exposu re time (day)	Response	Respon se measur ement	Parameter	Value	Confide nce interval	Ref.
21	Growth	Length	NOEC	0.67 mg/L	NR - NR	[27]
2	Intoxication	Immobi le	NOEC	20 mg/L	NR - NR	[29]
1	Intoxication	Immobi le	NOEC	100 mg/L	NR - NR	[29]
21	Mortality	Surviva 1	NOEC	12.71 mg/L	12.38 – 13.04	[39]
21	Reproduction	Abort	NOEC	12.71 mg/L	12.38 <b>–</b> 13.04	[39]
21	Mortality	Mortalit y	NOEC	12.71 mg/L	12.38 – 13.04	[39]
21	Reproduction	Abort	NOEC	12.71 mg/L	12.38 <b>–</b> 13.04	[39]
21	Growth	Length	NOEC	12.71 mg/L	12.38 <b>–</b> 13.04	[39]
21	Reproduction	Fecundi ty	NOEC	12.71 mg/L	12.38 – 13.04	[39]
21	Reproduction	Time to first progen y	NOEC	12.71 mg/L	12.38 – 13.04	[39]
21	Growth	Weight	NOEC	12.71 mg/L	12.38 - 13.04	[39]
21	Reproduction	Progen y counts/ number s	NOEC	1 mg/L	NR - NR	[29]

Exposu re time (day)	Response	Respon se measur ement	Parameter	Value	Confide nce interval	Ref.
2	Intoxication	Immobi le	NOEC	0.8 mg/L	0.6 - 1.3	[22]
21	Mortality	Surviva 1	NOEC	5 mg/L	NR - NR	[29]
21	Reproduction	Time to first progen y	NOEC	10 mg/L	NR - NR	[29]
1	Reproduction	Progen y counts/ number s	NOEC	10 mg/L	NR - NR	[29]
21	Reproduction	Progen y counts/ number s	NOEC	10 mg/L	NR - NR	[29]
2	Mortality	Surviva 1	NOEC	33.1 mg/L	32.8 - 34.1	[22]

<sup>(\*)</sup> Values refer to CAS 2795-39-3, potassium perfluorooctanesulfonate.

# 3.1.3. PFBA

Table 4 reports the main toxicological findings for PFBA, the perfluorinated carboxylic acid with 4 carbon atoms; the unit of measurement was standardized and all original values expressed in mg/L.

**Table 4.** Experimental findings obtained after the exposure (various time length) of *D. magna* to PFBA. The available endpoints were included (beside immobilization).

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confide nce interval	Ref.
2	Intoxication	Immobi le	EC05	3014 mg/L	NR - NR	[17]

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confidence interval		Ref.
2	Intoxication	Immobi le	EC10	3470 mg/L	NR NR	-	[17]
2	Intoxication	Immobi le	EC10	> 1006 mg/L	NR NR	-	[41]
2	Intoxication	Immobi le	EC50	5251 mg/L	3889 6614	-	[17]
2	Intoxication	Immobi le	EC50	> 1006 mg/L	NR NR	-	[41]
1	Intoxication	Immobi le	EC50	> 4280.8 mg/L	NR NR	-	[24]
2	Intoxication	Immobi le	EC50	> 4280.8 mg/L	NR NR	-	[24]
2	Intoxication	Immobi le	EC50	181.51 mg/L	0.841 0.856	-	[24]
1	Intoxication	Immobi le	EC50	185.14 mg/L	0.858 0.871	-	[24]
2	Mortality	Mortalit y	LC50	> 1006 mg/L	NR NR	-	[41]
1	Intoxication	Immobi le	LOEC	192.64 mg/L	NR NR	-	[24]
2	Intoxication	Immobi le	LOEC	181.93 mg/L	NR NR	-	[24]
1	Intoxication	Immobi le	NOEC	181.93 mg/L	NR NR	-	[24]
2	Intoxication	Immobi le	NOEC	177.65 mg/L	NR NR	-	[24]
2	Mortality	Mortalit y	NR- ZERO	45 mg/L	NR NR	-	[42]

#### 3.1.4. PFBS

Table 5 reports the main toxicological findings for PFBS, the perfluorinated sulfonic acid with 4 carbon atoms; the unit of measurement was standardized and all original values expressed in mg/L.

**Table 5.** Experimental findings obtained after the exposure (various time length) of *D. magna* to PFBS. The available endpoints were included (beside immobilization).

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confide nce interval	Ref.
1	Intoxication	Immobi le	EC50	2598.36 mg/L	1754.37 - 3871.53	[43]
2	Intoxication	Immobi le	EC50	2236.68 mg/L	1754.37 - 3871.53	[43]
2	Intoxication	Immobi le	LOEC	1748.98 mg/L	NR - NR	[43]
21	Mortality	Mortalit y	LOEC	1928.06 mg/L	1766.70 - 2135.66	[44]
21	Growth	Length	LOEC	1022.61 mg/L	972.25 – 1096.61	[44]
21	Reproduction	Fecundi ty	LOEC	1022.61 mg/L	972.25 – 1096.61	[44]
21	Reproduction	Fecundi ty	NOEC	515.93 mg/L	487.15 – 559.10	[44]
21	Mortality	Mortalit y	NOEC	1022.61 mg/L	972.25 – 1096.61	[44]
21	Growth	Length	NOEC	515.93 mg/L	487.15 - 559.10	[44]
2	Intoxication	Immobi le	NOEC	907.79 mg/L	NR - NR	[43]

#### 3.1.5. Gen-X

Table 6 reports the main toxicological findings for the compound Gen-X, perfluoroether carboxylic acid derivative; the unit of measurement was standardized and all original values expressed in mg/L.

**Table 6.** Experimental findings obtained after the exposure (various time length) of *D. magna* to Gen-X. The available endpoints were included (beside immobilization).

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confide nce interval	Ref.
2	Mortality	Mortalit y	EC50	> 102 mg/L	NR/ - NR/	[45]
2	Mortality	Mortalit y	LC50	183.14 mg/L	NR - NR	[46]
2	Mortality	Mortalit y	LC50	307.70 mg/L	NR - NR	[46]
2	Mortality	Mortalit y	LC50	156.24 mg/L	NR - NR	[46]
21	Mortality	Surviva l	LOEC	8.13 mg/L	NR - NR	[45]
21	Mortality	Mortalit y	LOEC	16.2 mg/L	NR - NR	[45]
21	Mortality	Surviva l	NOEC	4.17 mg/L	NR - NR	[45]
21	Growth	Length	NOEC	> 33.0 mg/L	NR - NR	[45]
21	Reproduction	Time to first progen y	NOEC	> 33.0 mg/L	NR - NR	[45]
21	Mortality	Mortalit y	NOEC	8.13 mg/L	NR - NR	[45]
21	Mortality	Surviva l	NOEC	> 33.0 mg/L	NR - NR	[45]

# 3.2. Toxicity Quantified with the Unicellular Green Alga Raphidocelis Subcapitata

# 3.2.1. PFOA

Table 7 reports the main toxicological findings for PFOA; the unit of measurement was standardized and all original values expressed in mg/L.

**Table 7.** Experimental findings obtained after the exposure (various time length) of *R. subcapitata* to PFOA. The endpoints refer to the growth rate.

Exposure time (day)	Response	Response measure ment	Parameter	Value		Confidenc e interval	Ref.
NR	Populatio n	Abundan ce	EC10	19.72 mg/L		NR - NR	[47]
NR	Populatio n	Abundan ce	EC20	35.47 mg/L		NR - NR	[47]
3	Populatio n	Populatio n growth rate	EC50	96.2/ mg	g/L	88.6/ - 113.7/	[48]
NR	Populatio n	Abundan ce	EC50	96.75 mg/L		NR - NR	[47]
NR	Populatio n	Abundan ce	EC90	474.67 mg/L		NR - NR	[47]
2	Populatio n	Populatio n growth rate	EC10	> 5 mg/L	500	NR - NR	[49]
2	Populatio n	Populatio n growth rate	EC50	> 5 mg/L	500	NR - NR	[49]
4	Populatio n	Biomass	EC50/	> 1 mg/L	100	NR - NR	[31]
3	Populatio n	Biomass	EC50/	> 1 mg/L	100	NR - NR	[31]
3	Populatio n	Populatio n growth rate	EC50	> 1 mg/L	100	NR - NR	[31]

Exposure time (day)	Response	Response measure ment	Parameter	Value	Confidenc e interval	Ref.
4	Populatio n	Populatio n growth rate	EC50	> 100 mg/L	NR - NR	[31]
3	Populatio n	Populatio n growth rate	LOEC	369.67 mg/L	NR - NR	[31]
4	Populatio n	Populatio n growth rate	LOEC	22.70 mg/L	NR - NR	[31]
3	Populatio n	Biomass	LOEC	369.67 mg/L	NR - NR	[31]
4	Populatio n	Biomass	LOEC	22.70 mg/L	NR - NR	[31]
3	Populatio n	Populatio n growth rate	NOEC	200 mg/L	NR - NR	[31]
4	Populatio n	Populatio n growth rate	NOEC	6.25 mg/L	NR - NR	[31]
3	Populatio n	Biomass	NOEC	400 mg/L	NR - NR	[31]
3	Populatio n	Populatio n growth rate	NOEC	180.67 mg/L	NR - NR	[31]
4	Populatio n	Biomass	NOEC	11.37 mg/L	NR - NR	[31]
4	Populatio n	Populatio n growth rate	NOEC	11.37 mg/L	NR - NR	[31]
3	Populatio n	Biomass	NOEC	180.67 mg/L	NR - NR	[31]

Exposure time (day)	Response	Response measure ment	Parameter	Value	Confidenc e interval	Ref.
4	Populatio n	Biomass	NOEC	100 mg/L	NR - NR	[31]

<sup>(\*)</sup> Values refer to CAS 3825-26-1, perfluorooctanoic acid ammonium salt.

#### 3.2.2. PFOS

Table 8 reports the main toxicological findings for PFOS; the unit of measurement was standardized and all original values expressed in mg/L.

**Table 8.** Experimental findings obtained after the exposure (various time length) of *R. subcapitata* to PFOS. The endpoints refer to the growth rate.

Exposu re time (day)	Response	Response measurement		Parame ter	Value	Confide nce interval	Ref.
2	Population	Population rate	growth	EC10	17 mg/L	13 - 23	[49]
3	Population	Population rate	growth	EC50	35.0 mg/L	34.2 <i>-</i> 35.5	[48]
2	Population	Population rate	growth	EC50	109 mg/L	80 - 149	[49]

### 3.2.3. PFBA

Table 9 reports the main toxicological findings for PFBA; the unit of measurement was standardized and all original values expressed in mg/L.

**Table 9.** Experimental findings obtained after 15 minutes exposure of *A. fischeri* to different molecules of PFAS. The endpoint is luminescence (linked to metabolism) inhibition.

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confide nce interval	Ref.
2	Population	Populat ion growth rate	EC10	62 mg/L	42 - 92	[49]

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confide nce interval	Ref.
2	Population	Populat ion growth rate	EC50	1830 mg/L	1500 - 2230	[49]

#### 3.2.4. PFBS

Table 10 reports the main toxicological findings for PFBS; the unit of measurement was standardized and all original values expressed in mg/L.

**Table 10.** Experimental findings obtained after the exposure (various time length) of *R. subcapitata* to PFBS. The endpoints refer to the growth rate.

Exposu re time (day)	Respon se	Response measure ment	Param eter	Value	Confide nce interval	Ref.
3	Populat ion	Populatio n growth rate	EC50	> 20250 mg/L	NR - NR	[50]
2	Populat ion	Populatio n growth rate	EC10	299 mg/L	117 - 767	[49]*
2	Populat ion	Populatio n growth rate	EC50	> 1000 mg/L	NR - NR	[49]*

<sup>\*</sup> Values refer to CAS number 29420493, potassium perfluorobutanesulfonate.

# 3.2.5. Gen-X

Table 11 reports the main toxicological findings for Gen-X; the unit of measurement was standardized and all original values expressed in mg/L.

**Table 11.** Experimental findings obtained after the exposure (various time length) of *R. subcapitata* to Gen-X. The endpoints refer to the growth rate.

Exposu re time (day)	Response	Respon se measur ement	Parame ter	Value	Confide nce interval	Ref.
3	Population	Populat ion growth rate	EC50	> 107/ mg/L	NR/ - NR/	[45]
3	Population	Abunda nce	EC50	> 107/ mg/L	NR/ - NR/	[45]
3	Population	Biomas s	EC50	> 107/ mg/L	NR/ - NR/	[45]
3	Population	Biomas s	NOEC	> 107/ mg/L	NR/ - NR/	[45]
3	Population	Populat ion growth rate	NOEC	> 107/ mg/L	NR/ - NR/	[45]
3	Population	Abunda nce	NOEC	> 107/ mg/L	NR/ - NR/	[45]

# 3.3. Toxicity Quantified with the Luminescent Bacteria Aliivibrio fischeri

# 3.3.1. Different Compounds

Table 12 reports the main toxicological findings for the considered molecules; the unit of measurement was standardized and all original values expressed in mg/L.

**Table 12.** Experimental findings obtained after the exposure (various time length) of *R. subcapitata* to Gen-X. The endpoints refer to the growth rate.

CAS	Exposu	Response	Respons	Paramete	Value	Confide	Ref.
number	re time		e	r		nce	
	(min)		measure			interval	
			ment				
335671	15	Metabolis m	Lumines cent	EC50	524 mg/L	NR - NR	[48]

CAS number	-	Response	Respons e measure ment	Paramete r	Value	Confide nce interval	Ref.
			inhibitio n				
335671	30	Metabolis m	Lumines cent inhibitio n	EC50	570.19 mg/L		[51]
1763-23- 1	15	Metabolis m	Lumines cent inhibitio n	EC50	>500 mg/L	NR - NR	[48]
375-73-5	15	Metabolis m	Lumines cent inhibitio n	EC50	17520 mg/L	NR - NR	[48]

Regarding ADONA, the lack of bibliographic references led us to consult the ECHA database, specifically the substance registration dossier. Regarding acute short-term toxicity on aquatic invertebrates, exposure of D. magna for 48 hours resulted in an EC50 greater than 100 mg/L. A 21-day exposure produced an EC50 of 100 mg/L. For the alga R. *subcapitata*, after 96 hours of exposure, the EC50 was 100 mg/L. A 72-hour exposure led to both an EC10 and an EC50 greater than 1,000 mg/L.

#### 3. Discussion

The results of bioassays on the aquatic model organisms: *D. magna, R. subcapitata,* and *A. fischeri* reported in the tables above provide a clear overview of the toxicological profiles of PFOA, PFOS, PFBA, PFBS, GenX, and ADONA.

For *D. magna*, legacy compounds (PFOA and PFOS) showed lower effect concentration values compared to the analogue short-chain molecules (PFBA, PFBS) and the newer substitutes (GenX, ADONA). In particular, after a chronic exposure, reproductive endpoints (e.g., fecundity, time to first progeny) appeared to be especially sensitive, with LOEC and NOEC values for PFOS and PFOA often in the low mg/L or even sub-mg/L range. These findings highlight the potential for significant biological effects at concentrations below normal acute thresholds. On the contrary, short-chain PFAS like PFBA and PFBS generally required higher concentrations to produce comparable responses, supporting the perception of lower bioaccumulation potential but not excluding environmental persistence concerns.

In *R. subcapitata*, growth inhibition data confirmed the greater toxicity of PFOS and PFOA with respect to the alternatives. PFOS, for example, exhibited EC10 values near or below 20 mg/L, while PFBA, PFBS, and GenX displayed EC50 values typically above several hundreds of mg/L. This

pattern suggests a lower inherent hazard for these shorter-chain or substitute compounds in primary producers, although high variability in sensitivity across studies emphasizes the need for standardized testing protocols.

The *A. fischeri* assays confirmed the lower sensitivity of this assay (although it should represent the decomposers: marine luminescent bacteria are increasingly considered a controversial model), with EC50 values exceeding 500 mg/L for both PFOA and PFOS, and significantly higher for PFBS. While this may suggest a reduced acute hazard at lower concentrations, the potential for sub-lethal effects or mixture toxicity is still an open issue.

Comparing PFOA and PFOS, which belong to the class of long-chain per- and polyfluoroalkyl substances (PFAS) but differ in their functional group, this structural difference play we can a different environmental behavior and toxicological profile can be highlighted.

Based on the results of bioassays on the model organisms, PFOS generally showed greater toxicity compared to PFOA. For D. magna, chronic and reproductive endpoints showed that PFOS caused effects at lower concentrations (lower NOEC and LOEC values), suggesting a higher potential to interfere with sensitive biological processes such as reproduction. This is consistent with PFOS greater tendency for bioaccumulation and stronger binding to proteins.

In *R. subcapitata*, PFOS also exhibited lower EC10 and EC50 values than PFOA, indicating a stronger inhibitory effect on algal growth. This phenomenon can be correlated with the higher hydrophobicity and stronger membrane affinity of sulfonic PFASs, which can disrupt cellular processes in primary producers more effectively than carboxylate ones.

For *A. fischeri*, although both compounds required relatively high concentrations to produce acute luminescence inhibition, PFOS typically presented slightly lower EC50 values compared to PFOA. This pattern, while less pronounced in bacteria, suggests the generally higher toxicity potential of sulfonic PFAS.

From a mechanistic perspective, the stronger acidic character and lower pKa of PFOS contribute to its higher persistence and bioaccumulation potential. This, combined with its stronger adsorption to sediments and biota, explains its enhanced ecotoxicological profile relative to PFOA [52] [53]

Overall, the comparison highlights that PFOS poses a higher ecological hazard than PFOA across trophic levels, supporting the recent policy actions that have prioritized the phase-out of sulfonic PFASs due to their elevated risk to aquatic ecosystems.

PFBA and PFBS represent the short-chain analogues of PFOA and PFOS, respectively, sharing a C4 perfluorinated backbone but differing in their functional groups—carboxylic for PFBA and sulfonic for PFBS. This structural variation, although subtle, influences their environmental fate and ecotoxicological profiles.

In *D. magna*, PFBS generally showed slightly higher toxicity than PFBA, although both compounds exhibited relatively low toxicity compared to their analogue long-chain molecules. Chronic endpoints (e.g., reproduction, growth) showed higher NOEC and LOEC values for PFBA, consistent with its greater water solubility and lower bioaccumulation potential. Although PFBS can be considered less bioaccumulable than PFOS, it showed greater capacity to cause adverse effects at comparable concentrations, likely due to the stronger acidic nature of its sulfonic group.

In *R. subcapitata*, both compounds required relatively high concentrations to affect algal growth, but PFBS tended to inhibit growth at lower concentrations compared to PFBA. This suggests that, as observed in long-chain PFAS, the sulfonic acid group confers greater potential for cellular interaction and disruption, even in short-chain variants.

For *A. fischeri*, acute toxicity tests demonstrated minimal differences between PFBA and PFBS, with both compounds showing low toxicity in terms of luminescence inhibition. This reflects the general trend that short-chain PFAS are less disrupting to bacterial processes at environmentally relevant concentrations.

Considering a mechanistic approach, the difference in functional groups explains the slightly higher ecotoxicity of PFBS: sulfonic acids tend to bind more effectively to biological surfaces and

proteins compared to carboxylic acids, enhancing their interaction with aquatic organisms despite their small molecular size.

While both PFBA and PFBS are considered lower risk compared to long-chain PFAS, PFBS shows a slightly higher ecotoxicological concern. This confirms the growing attention to short-chain sulfonic PFAS in environmental monitoring and regulatory frameworks.

In contrast to the relatively well-documented toxicity profiles of traditional PFAS such as PFOA, PFOS, and their short-chain analogues, data on GenX (hexafluoropropylene oxide dimer acid, HFPO-DA) and ADONA (3H-perfluoro-3-[(3-methoxy-propoxy)propanoic acid]) are extremely scarce, particularly regarding their ecotoxicological effects on standard aquatic organisms such as *D. magna*, *R. subcapitata*, and *A. fischeri*. The toxicological studies presented in the scientific literature refer mainly on mammals or to marine ecosystems.

The few studies available suggest that GenX, despite being introduced as a safer alternative to long-chain PFAS, may still present a measurable toxicity. Chronic tests with *D. magna* and algal growth inhibition tests indicate that GenX can exert sub-lethal effects at concentrations in the low mg/L range, although its acute toxicity appears lower than that of PFOA. Likewise, effects on *A. fischeri* luminescence are reported only at relatively high concentrations, reinforcing the idea that acute microbial toxicity is limited.

For ADONA, the situation is even more critical: literature data are almost absent (for this reason, the ECHA registration dossiers [54]were considered in this review) providing basic toxicity information. These data show high NOEC and EC50 values (typically >100 mg/L) for *D magna* and *R. subcapitata*, suggesting low acute and chronic toxicity. However, the absence of peer-reviewed studies prevents drawing reliable assessments, and significant gaps remain regarding potential long-term effects, bioaccumulation potential, or sub-lethal impacts.

In conclusion, the lack of comprehensive ecotoxicological data for GenX and ADONA highlights a key challenge in evaluating the safety of emerging PFAS substitutes. Further studies are required to fill knowledge gaps and ensure that the adoption of these alternatives does not pose new environmental risks.

# 5. Conclusions

This review highlights a significant variability in the ecotoxicological profiles of the considered PFASs, including both legacy compounds and their modern substitutes. The comparison between PFOA and PFOS confirms a generally higher toxicity of the sulfonic compound at the various trophic levels (based on the tests carried out on the model organisms *D. magna*, *R. subcapitata*, and *A. fischeri* ) at relatively low concentrations. Likewise, among short-chain analogues, PFBS appears more toxic than PFBA, although both exhibit a reduced toxicity with respect to their analogue long-chain molecules.

Interestingly, despite having been produced (as alternatives) since the early 2000s, GenX and ADONA, which have been introduced as alternatives, are characterized by a lack of ecotoxicological data. The few studies available, mainly deriving from regulatory dossiers rather than from scientific literature, show lower levels of acute and chronic toxicity under standard testing conditions. Nevertheless, this evidence remains too scarce to support definitive conclusions about their safety for aquatic ecosystems, especially concerning sub-lethal, long-term, or multigenerational effects.

The results of this review highlight the need for comprehensive and standardized ecotoxicological investigations on emerging PFAS, including GenX and ADONA. The integration of effect-based bioassays beside chemical analyses (aimed at their quantification in the different environmental matrices) could yield to a more realistic assessment of the ecological risks posed by complex PFAS mixtures, i.e., the actual situation in surface water, soil, groundwater.

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## **Abbreviations**

The following abbreviations are used in this manuscript:

ADONA Ammonium 4,8-Dioxa-3H-Perfluorononanoate Error! Reference source not found.

AFFF Aqueous Film-Forming Foam
APFO Ammonium Perfluorooctanoate
CAS Chemical Abstracts Service
EC Effect Concentration

FEP Fluorinated Ethylene Propylene

HFPO-DA Hexafluoropropylene Oxide Dimer Acid (Or GenX)

LC Lethal Concentration

LOEC Lowest Observed Effect Concentration

MATC Maximum Acceptable Toxicant Concentration

NaPFO Sodium Perfluorooctanoate
NOEC No Observed Effect Concentration
PFAS Per- And Polyfluoroalkyl Substances

PFBA Perfluorobutanoic Acid
PFBS Perfluorobutane Sulfonate
PFOA Perfluorooctanoic Acid
PFOS Perfluorooctane Sulfonate
PTFE Polytetrafluoroethylene
PVDF Polyvinylidene Fluoride

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