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Advancing the understanding of PFAS-induced reproductive toxicity in key model species

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Perfluoroalkyl substances (PFASs) are widely used and resistant to degradation, leading to their frequent detection in both humans and animals. These substances are believed to be associated with a range of toxic effects. Reproductive toxicity warrants greater attention due to its potential impact on offspring development. This article reviews and summarizes the literature from the past fifteen years on the studies of reproductive toxicity in model organisms induced by PFASs. This article organizes the studies according to the five most commonly used model organisms including mice, rats, zebrafish, *Oryzias*, and *Caenorhabditis elegans*. By reviewing the literature, it was found that PFASs primarily induced reproductive toxicity through gonadal damage, disruption of sex hormones, and effects on offspring development. Moreover, mammals, rats and mice possess many conserved signaling pathways with humans, making them valuable models for studying various diseases and metabolic pathways. Zebrafish and *Oryzias* are well-suited for examining chronic toxicity at environmentally relevant exposure levels, with their high-throughput screening capacity enabling efficient and low-cost assessment of transgenerational effects. In summary, this study systematically reviews the reproductive toxicity of PFASs in model organisms, offering a scientific foundation for optimizing model selection, exploring intervention strategies, and shaping future research directions.

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Environmental significance

Using model organisms to study the reproductive toxicity of PFASs provides a powerful tool for understanding their environmental significance. Model organisms exposed to PFASs often exhibit reproductive impairments, such as reduced fertility, developmental abnormalities, and hormonal disruptions. PFAS exposure in model organisms has been linked to transgenerational effects, where reproductive and developmental abnormalities persist in offspring even without direct exposure. Model organism studies also provide mechanistic insights into how PFASs may affect human reproductive health, particularly in communities with high exposure levels. These studies highlight the broader ecological and human health risks associated with their persistence in the environment.

1. Introduction

PFASs are a group of synthetic chemicals that have been in use since the 1940s. In 2023, the EPA established a regulatory definition for PFASs, specifying that any compound containing the structures R-(CF_2)-CF(R′)R″, R-CF_2OCF_2-R′, or $CF_3C(CF_3)$ R′R″ is classified as a PFAS.[1] Their low surface tension, low viscosity, and water- and oil-repellent properties make them widely applicable in various fields, including chemicals, firefighting, construction, machinery, and aerospace. Due to their persistence and widespread use, PFASs have been detected in various environmental media worldwide, including the atmosphere, water sources,[2] and soil.[3] They have also been found in human bodies across multiple regions, such as North America,[4] Europe,[5] and East Asia,[6] significantly affecting human safety and quality of life. In the 2007 revision of the Stockholm Convention on Persistent Organic Pollutants, the widely used perfluorooctane sulfonates (PFOSs) and perfluorooctanoic acid (PFOA) were included. Since then, various new PFASs and alternatives have been produced. Given their relatively recent entry into the environment, toxicity data remain limited; nevertheless, their potential risks should not be underestimated.

During the study of PFASs, researchers found that PFASs can cause a range of toxic effects in organisms, including hepatotoxicity,[7] neurotoxicity,[8] immunotoxicity,[9] and reproductive toxicity,[10] among others. Damage to the reproductive system can

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not only cause pathological changes in the gonads of adult male and female organisms, but also lead to hormonal imbalances by altering hormone levels in the body. This can directly affect the health of the next generation and severely impact the reproductive success of the species. In a study by Bogdanska *et al.*,[11] the tissue distribution of perfluorobutane sulfonate (PFBS) in mice was characterized, revealing higher concentrations of PFBS in male reproductive organs, particularly in the testes. Therefore, reproductive organs are key target organs for PFASs, and studying their toxic effects on the reproductive system is crucial for understanding the health impacts of PFASs.

However, investigating the reproductive toxicity of PFASs requires appropriate methods and platforms. In the early stages, researchers primarily relied on traditional biological methods, such as model organism approaches[12] and cell culture methods,[13] to explore the toxicological characteristics of various compounds. With the advancement of technology and the development of computer science, new research methods have emerged, including genomics,[14] microarray analysis,[15] and molecular dynamics (MD) simulations,[16] and techniques that leverage large analytical instruments, such as mass spectrometry imaging.[17] Although a variety of emerging methods are continuously being developed, model organism approaches remain the most direct and intuitive research method. At present, they are still indispensable in fields such as biology and environmental toxicology.

Model organisms provide an effective platform for researchers to study the toxic effects of pollutants, investigate their mechanisms of toxicity, and explore their metabolic pathways. Several species, including mice, rats, zebrafish, *Oryzias*, nematodes, fruit flies, and African clawed frogs, are widely recognized as excellent model organisms, with well-established research systems. Since model organisms are complete living systems, they provide a more comprehensive and holistic approach to observing the toxic effects of pollutants, compared to cell or tissue models, by enabling the study of toxicity endpoints at the organismal level. With the completion of the Human Genome Project and the arrival of the post-genomic era, model organism research strategies have gained increased emphasis. The structure and function of genes can be studied in appropriate model organisms, and similarly, suitable species can be chosen to simulate human physiological and pathological processes. However, due to the diversity of model organisms across various levels of the biosphere, selecting the most appropriate model for studying the reproductive toxicity of pollutants, while achieving research objectives in the most efficient and cost-effective manner, may present a challenge for some researchers.

In recent years, growing attention has been given to the reproductive toxicity of PFASs in review articles. Notably, Shi *et al.*[18] adopted an epidemiological perspective to provide an in-depth analysis of the effects of PFASs on human reproductive

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health. González-Álvarez *et al.*[19] provided a detailed account of the effects of PFASs on the female reproductive system, with no discussion of male reproductive outcomes. Their review was primarily epidemiological in nature. In the section addressing reproductive toxicity in model organisms, the classification was organized according to toxicological endpoints, but it lacked a synthesis based on the species of the model organisms. Chambers *et al.*[20] compared the differential reproductive effects of long-chain and short-chain PFASs, with a primary focus on humans and mammals. Despite increasing attention to PFAS-induced reproductive toxicity, a systematic overview from the perspective of multiple model organisms is still lacking. While the 3Rs principle remains a cornerstone of ethical animal research, fully replacing model organisms in current experimental systems continues to pose substantial challenges. Domínguez-Oliva *et al.*[21] emphasized that the systemic toxicity, metabolic processes, and crosstalk among the endocrine, immune, and nervous systems induced by viruses or drugs can only be faithfully recapitulated in intact organisms. Furthermore, long-term effects such as carcinogenicity, reproductive toxicity, and aging-related phenotypes require observation over extended periods—ranging from months to years—which cannot be adequately modeled using *in vitro* systems or organoids.

Based on the aforementioned issues, as well as existing literature reviews, some studies focus solely on the multifaceted toxic effects of PFAS exposure in a single model organism, while others concentrate on the specific toxicity of particular PFASs in model organisms. However, there is a lack of comprehensive exploration and synthesis of the common reproductive toxic effects of multiple PFASs across various model organisms. Therefore, this article conducts a comprehensive search of research published in the past fifteen years in the Web of Science database, using a combination of keywords such as "Perfluor*", "PFAS", "PFOS", "PFOA", "model organism", "reproductive toxicity", "ovary", and "testis." After excluding studies unrelated to the topic, such as those focusing on developmental toxicity or neurotoxicity, the remaining research primarily involves five model organisms: two widely used mammalian models—mice and rats; two aquatic models—zebrafish and medaka; and one hermaphroditic model organism—*Caenorhabditis elegans*. To minimize the risk of omission, additional searches were conducted by combining the terms "mice," "rat," "zebrafish," "*Oryzias*," and "*Caenorhabditis elegans*" with the previously mentioned keywords. Furthermore, quality control was applied to the included studies, with particular attention to issues such as exposure concentrations exceeding solubility limits and the appropriateness of concentration gradients. Finally, this review categorizes the retrieved literature according to five representative model organisms and briefly summarizes additional studies involving other species. It also compiles and discusses substances that may mitigate PFAS-induced reproductive toxicity.

In conclusion, this review employs model organisms as a research framework to systematically synthesize existing evidence of their application in toxicity assessment. By addressing the current gap in model-organism-based mechanistic studies, it provides novel and multidimensional insights into the reproductive toxicity of PFASs.

2. Reproductive toxicity induced by PFASs in various model organisms

2.1 Reproductive effects in mice

Mice are the most widely used and extensively studied mammalian experimental animals in laboratory settings. They offer several advantages, including early maturity, high reproductive capacity, small size, ease of care and management, a docile temperament, and heightened sensitivity to external stimuli.[22] Moreover, while there are structural differences in the reproductive systems of mice and humans, both species' reproductive cycles are regulated by sex hormones such as estrogen and progesterone. Many genes and signaling pathways that regulate gonadal physiological processes are conserved between the two.[23] This indicates that the mouse model can offer valuable insights into the study of human reproductive diseases. Significant progress has also been made in research on the reproductive toxicity of PFASs in mice.

2.1.1 Damage to female gonads. PFOS exposure has been found to markedly decrease the numbers of mature follicles and corpora lutea in female mice, while increasing atretic follicles, thereby impairing follicular development and ovulation. It also leads to a prolonged estrous cycle beginning in the third month of exposure.[24] A subsequent study,[25] which increased the exposure concentration and shortened the exposure duration, used oral administration and found that exposing mice to PFOS led to a reduction in ovulation. A recent study[26] using an *in vitro* mouse oocyte maturation system has shown that exposure to PFOS and perfluorohexane sulfonate (PFHxS) for 14 hours can induce chromosomal abnormalities, abnormal F-actin organization, prolonged spindle formation, and asymmetric division in oocytes. These meiotic defects contribute to the impaired developmental potential of oocytes.

PFOA is also a widely studied type of PFAS. Chen *et al.*[27] reported that PFOA significantly increased the number of resorbed embryos, while markedly reducing the number of corpora lutea and the ratio of corpora lutea area to total ovarian area. Zhang *et al.*[28] reported that even at lower doses (1 and 5 mg per kg per day), 28 days of gavage treatment with PFOA can impair oocyte maturation, as indicated by a reduced blastocyst rupture rate and a lower first polar body extrusion rate. Additionally, the proportion of the DNA damage marker γ-H2AX was significantly increased in oocytes following PFOA exposure. These alterations led to abnormal spindle formation and chromosomal alignment during oocyte maturation. In a similar timeframe, PFOA has been shown to significantly affect follicular dynamics, characterized by a reduction in primordial follicles and an increase in primary and preantral follicles, suggesting that it may accelerate folliculogenesis and thereby contribute to premature reproductive aging.[29]

Other PFASs have been less extensively studied, and they are collectively summarized here. Cao *et al.*[30] found that gavage

exposure to PFBS in mice resulted in a significant increase in atretic follicles, along with a notable decrease in early antral, antral, and secondary follicles, suggesting that PFBS disrupts normal folliculogenesis. In an *in vitro* oocyte maturation system, PFNA treatment was shown to significantly suppress germinal vesicle breakdown (GVBD) and polar body extrusion (PBE) in mouse oocytes.[31] Moreover, PFNA induced abnormal metaphase I (MI) spindle assembly and disrupted the formation of metaphase II (MII) spindles in most oocytes with polar bodies. A recent study[32] exposed mice to sublethal doses of PFHxS (25.1 mg kg^{-1} and 62.5 mg kg^{-1}) through a single intraperitoneal injection. The results revealed that exposure to 62.5 mg kg^{-1} of PFHxS prolonged the estrous cycle and reduced the ovulation rate in female mice. As the PFHxS concentration increased, the number of oocytes gradually decreased, and intracellular calcium levels also declined, suggesting that PFHxS interferes with calcium homeostasis in COCs and oocytes, which is essential for oocyte maturation. These findings indicate that PFHxS impacts the estrous cycle and ovulation in female mice.

2.1.2 Damage to male gonads. The widely used PFOS has always been a research hotspot. Previously, Qu *et al.*[33] found that after exposing male mice to 10 mg kg^{-1} of PFOS *via* gavage for 5 weeks, sperm count decreased and both the absolute and relative testicular weights were significantly reduced. Histological examination of tissue sections revealed pathological changes, including vacuolation in spermatogonia, spermatocytes, and interstitial cells. Zhang *et al.*[34] used the drinking water exposure method to treat mice with PFOS at doses $\geq$0.5 mg kg^{-1} (cumulative dose) for 90 days. They found histopathological changes in the testicular tissue, including disorganization and vacuolization of germ cells, a decreased sperm concentration, reduced sperm motility, and an increase in sperm abnormalities, indicating reproductive toxicity. However, the increase in testicular and epididymal coefficients was in contrast to the findings of Qu *et al.* Two later studies, consistent with the findings of Qu *et al.*, showed that oral PFOS exposure significantly decreased the weights of the testes, epididymides, and seminal vesicles, as well as their respective organ indices in mice. Furthermore, a pronounced thinning of the epididymal epithelium was observed, indicating that PFOS exposure causes germ cell loss within the seminiferous tubules.[35,36] Li *et al.*[37] exposed mice to 1 and 5 μg per g body weight of PFOS *via* oral administration for 21 days. The study found a decrease in epididymal sperm motility, as evidenced by reduced sperm swimming speed.

PFOA has been demonstrated to induce histopathological alterations in the testicular architecture of mice, including dilation of seminiferous tubule lumens, disruption of the spermatogenic cell layer with cleft formation, and expansion of interstitial spaces due to tubule contraction. In addition, apoptotic foci were observed among spermatogenic cells.[38] Regarding hexafluoropropylene oxide (HFPO), Peng *et al.*[39] also used gavage to treat mice and found that exposure to 1, 5, 10, or 20 mg kg^{-1} of HFPO for 28 days led to disruption of the germ cell layer in the seminiferous tubules, along with vacuolation of the supporting cells. A subsequent study[40] using the same treatment approach further demonstrated that PFOA significantly reduced sperm motility and count, caused structural damage to the vas deferens, and markedly decreased the thickness of the seminiferous epithelium, while leaving the diameter of the seminiferous tubules unaffected.

As for other PFASs, oral administration of PFNA by Singh *et al.*[41] revealed that the compound disrupts spermatogenesis by altering germ cell populations at various developmental stages, thereby impairing overall germ cell differentiation and reducing the number of elongated spermatids. In addition, PFNA-treated mice showed uneven and varied degenerative changes in the seminiferous tubules of the testes. The damaged seminiferous tubule epithelium exhibited vacuolization, sperm chromatin condensation at the edges, and the presence of giant cells, along with the detachment of germ cells in the tubule lumen. These findings suggest that spermatogenesis is impaired. HFPOs have also been found to damage the seminiferous tubules in mice.[39] In the hexafluoropropylene oxide dimer acid (HFPO-DA) gavage treatment group, disorganization of the germ cell layer and vacuolization were observed. The high-dose HFPO-DA group (20 mg kg^{-1}) showed detachment of germ cells. Additionally, at all tested concentrations of hexafluoropropylene oxide trimer acid (HFPO-TA) and hexafluoropropylene oxide tetramer acid (HFPO-TeA) (0.05, 0.1, 0.5, or 1 mg kg^{-1}), an accumulation of immature germ cells was observed in the seminiferous tubules.

The blood-testis barrier (BTB) is a structure within the male testes formed by tight junctions between Sertoli cells. By establishing the BTB, Sertoli cells help maintain the microenvironment essential for spermatogenesis, protecting germ cells from toxic effects.[42] Exposure to endocrine-disrupting chemicals (EDCs) can significantly affect the formation of the BTB and spermatogenesis. Zhang *et al.*[35] used biotin tracers to evaluate the integrity of the BTB. They found that in mice exposed to PFOS (10 mg per kg per day for 28 days *via* gavage), the biotin tracer crossed the BTB and entered the seminiferous tubule lumen, indicating that PFOS exposure compromised the integrity of the BTB. Another study[40] provided evidence that PFOA disrupts spermatogenesis by compromising the integrity of the blood-testis barrier (BTB), as indicated by an increase in red biotin fluorescence within the seminiferous tubule lumen. Peng *et al.*[39] found at the histological level that HFPOs could produce similar effects at lower doses. Exposure to HFPO-DA at 1 mg per kg per day and higher doses, as well as HFPO-TA and HFPO-TeA at 0.05 mg per kg per day and higher doses, caused significant damage to the integrity and function (permeability) of the BTB in mice.

2.1.3 Transgenerational toxicity. Earlier research[43] found that oral exposure to PFOS during pregnancy increased fetal mortality. Hines *et al.*[44] exposed mice to PFOS *via* gavage at doses ranging from 0 to 5 mg kg^{-1} for 17 days and observed that the offspring had significantly lower body weights compared to the control group. A later study[45] found similar results. The transgenerational toxicity of PFOA closely resembles that of PFOS. Yahia *et al.*[46] discovered that gavage exposure to 5 and 10 mg kg^{-1} of PFOA for 18 days led to a reduction in neonatal survival rates and decreased body weight in the surviving offspring. Similarly, a later research study[47] involving gavage

exposure of pregnant female mice found that PFOA exposure significantly reduced embryo weight. Another study[38] showed that continuous gavage exposure to 5 mg per kg BW of PFOA for 21 days in pregnant mice had a detrimental effect on the testicular development of male offspring. This exposure caused significant testicular damage, including increased cell apoptosis, enlarged seminiferous tubule lumen, cracks in the germ cell layer, and widened gaps in the seminiferous tubules.

Among other PFASs, PFBS is thought to cause developmental defects in the reproductive organs of female offspring, manifested by a significant delay in vaginal opening and first estrus, prolonged estrous cycles, and reductions in the size of the ovaries and uterus, as well as decreased numbers of follicles and corpora lutea.[48] Xia *et al.*[49] studied a relatively less common PFAS, 6:2 FTOH. Pregnant mice were orally administered 6:2 FTOH at doses of 5, 25, and 125 mg per kg bw, which led to disrupted testicular architecture in their offspring. This exposure decreased the expression of tight junction proteins in Sertoli cells (SCs), impaired the formation and maturation of the blood-testis barrier (BTB), reduced sperm motility, increased the incidence of sperm abnormalities and developmental defects, and induced testicular inflammation. Zhou *et al.*[50] studied perfluoroheptanoic acid (PFHpA) and found that gavage exposure to PFHpA for 16 days during pregnancy resulted in a decrease in sperm count, sperm concentration, and total cell count in male offspring. Histological analysis of H&E-stained tissue sections showed that the high-dose PFHpA treatment group exhibited changes in seminiferous tubule morphology, disorganized epithelium, and a reduction in germ cells. These effects were similar to those observed with PFOA.

2.1.4 Disruption of sex hormone levels. Disruption of sex hormone levels is a significant indicator of reproductive toxicity. PFOS has been shown to decrease serum levels of estradiol (E2) and progesterone, along with significant reductions in luteinizing hormone (LH), follicle-stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH),[24] and testosterone (T)[33] during the proestrus phase in mice. Additionally, a study[25] found that 7 days of oral exposure to 10 mg kg^{-1} of PFOS in adult female mice led to a decrease in serum P, LH, and hypothalamic GnRH levels. This resulted in prolonged pregnancy, reduced corpora lutea, and a decrease in ovulation.

As for PFOA and other PFASs, Chen *et al.*[27] found that on day 7 of exposure, gavage treatment with 10 mg per kg per day of PFOA significantly increased serum E2 levels, with no significant effect on serum P levels. However, by day 13 of exposure, treatment with 5 and 10 mg per kg per day of PFOA significantly reduced serum P levels. A subsequent study by Yang *et al.*[29] found that oral exposure to 5 mg kg^{-1} of PFOA for 10 days significantly reduced P and pregnenolone levels in female mice, while exposure to 1 mg kg^{-1} of PFOA increased T levels. One study on PFBS[30] reported that gavage exposure to 200 mg per kg per day of PFBS for 14 days led to a decrease in serum E2 and P levels in mice (Table 1).

2.1.5 Summary. Overall, existing studies on reproductive toxicity in mice have focused on eight types of PFASs: PFOS, PFOA, PFBS, PFHxS, PFHpA, PFNA, 6:2 FTOH, and HFPOs. Among these, PFOS and PFOA are the most extensively studied, while research on other PFASs remains relatively limited. The reproductive toxicity caused by PFASs is most visibly reflected in changes in gonadal weight, with most PFASs resulting in a reduction in the GSI. Additionally, gonadal damage is observed through techniques such as tissue sectioning. In female mice, PFASs induce ovarian damage, characterized by impaired follicular development, reduced ovulation, slower oocyte maturation, meiotic defects, and chromosomal abnormalities. In male mice, PFASs induce testicular damage, which is reflected in changes in the testicular organ-to-body weight ratio, altered sperm motility, reduced sperm count, and disruption of the BTB.

At the molecular level, this histological damage may result from changes in the body's sex hormone levels. For instance, PFOS has been shown to decrease the levels of E2, T, LH, and P, while PFOA has been found to induce varying trends in E2 and T levels across different studies. These alterations not only impair the reproductive system and disrupt endocrine function in the parental generation, but also affect the health of subsequent generations, leading to increased embryo mortality, impaired reproductive organ development, and other issues that threaten population viability.

Common exposure methods for PFASs in mice include oral gavage and dietary administration, with single intraperitoneal injection being less frequently used. Among these, oral gavage provides more precise control over the administered dose, ensuring complete delivery without variability due to individual behavioral differences in model organisms. In terms of the dosage that induces reproductive toxicity, the effective dose range for PFOS is 0.1–10 mg kg^{-1} d^{-1}, with exposure durations varying from 7 days to 4 months. In comparison, the effective dose for PFOA is higher, ranging from 1–20 mg kg^{-1} d^{-1}, with exposure periods spanning from 7 to 28 days. Among other PFASs, the effective dose for PFBS is relatively high, reaching 200 mg kg^{-1} d^{-1}, while PFHxS also shows a high effective dose of 62.5 mg kg^{-1} d^{-1}. In contrast, HFPO-TA and HFPO-TeA exhibit lower effective doses, with the lowest observed effect concentration being as low as 0.05 mg kg^{-1} d^{-1}.

In summary, no significant differences in reproductive toxicity were observed across the various compounds; however, differences in dosage were noted. For example, within the same class of compounds, those with longer carbon chains generally exhibit greater toxicity, with lower concentrations required to induce effects. PFBS is a perfluorinated sulfonic acid with a four-carbon chain, exhibiting an effective dose of 200 mg kg^{-1} d^{-1}. PFHxS, a six-carbon perfluorinated sulfonic acid, has an effective dose of 62.5 mg kg^{-1} d^{-1}, while the highest effective dose for PFOS, an eight-carbon perfluorinated sulfonic acid, is only 10 mg kg^{-1} d^{-1}. Furthermore, for PFASs with different functional groups, the effective dose range can vary even when the carbon chain length is the same. For instance, among the eight-carbon compounds, PFOS exerts stronger toxic effects than PFOA. Therefore, when conducting toxicity studies using model organisms, it is crucial to first understand the properties of different PFASs and perform preliminary toxicity tests to establish an appropriate concentration range.

Table 1 Summary of the reproductive toxicity of eight PFASs in mice

Toxicity	Female reproductive system damage		Male reproductive system damage		Transgenerational toxicity		Hormonal level changes	
Compounds	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose
PFOS	Reduced mature follicles and corpora lutea	0.1 mg kg^{-1} d^{-1}, 4 months[24]	Decreased GSI	10 mg kg^{-1} d^{-1}, 5 weeks[33]	Reduced embryo weight	0–5 mg kg^{-1} d^{-1}, 17 days[44]	Reduced serum E2, P, and LH in females	0.1 mg kg^{-1} d^{-1}, 4 months[24]
	Reduces ovulation	10 mg kg^{-1} d^{-1}, 14 days[25]		10 mg kg^{-1} d^{-1}, 28 days[35]		1 and 3 μg per g BW per day, 14 days[45]		10 mg kg^{-1} d^{-1}, 14 days[25]
				5 and 10 mg kg^{-1} d^{-1}, 30 days[36]				
	Impaired oocyte development	300 mg kg^{-1}, 14 h[26]	Increased GSI	0.5 mg kg^{-1} d^{-1}, 90 days[34]	Increased fetal mortality with prenatal exposure	6 mg kg^{-1} d^{-1}, 12 days[43]	Reduced T levels	10 mg kg^{-1} d^{-1}, 5 weeks[33]
			Testicular histological damage	10 mg kg^{-1} d^{-1}, 5 weeks[33]				
				0.5 mg kg^{-1} d^{-1}, 90 days[34]				
				10 mg kg^{-1} d^{-1}, 28 days[35]				
			Reduced sperm quality and quantity	10 mg kg^{-1} d^{-1}, 5 weeks[33]				
				0.5 mg kg^{-1} d^{-1}, 90 days[34]				
				5 and 10 mg kg^{-1} d^{-1}, 30 days[36]				
				1 and 5 μg g^{-1} d^{-1}, 21 days[37]				
			BTB damage	10 mg kg^{-1} d^{-1}, 28 days[35]				
PFOA	Increased absorbed embryos and reduced corpora lutea	2.5, 5, and 10 mg kg^{-1} d^{-1}, 7 or 13 days[27]	Reduced sperm quality and quantity	5 and 20 mg kg^{-1} d^{-1}, 28 days[40]	Reduced embryo weight	5 and 10 mg kg^{-1} d^{-1}, 18 days[46]	Reduced P and progesterone in females; increased T levels	5 and 10 mg kg^{-1} d^{-1} and 13 days[27]
	Impaired oocyte maturation	1 and 5 mg per kg per day, 28 days[28]	Testicular histological damage	5 mg kg^{-1} d^{-1}, 21 days[38]		5 mg kg^{-1} d^{-1}, 10 days[47]		1 and 5 mg kg^{-1}, 10 days[29]
	Accelerated folliculogenesis and reproductive senescence	1 and 5 mg kg^{-1}, 10 days[29]		1, 5, 10, and 20 mg kg^{-1} d^{-1}, 28 days[39]	Testicular structural damage in offspring	5 mg kg^{-1} d^{-1}, 21 days[38]	Elevated serum E2	10 mg kg^{-1} d^{-1}, 7 days[27]
				5 and 20 mg kg^{-1} d^{-1}, 28 days[40]	Reduced neonatal survival rates	5 and 10 mg kg^{-1} d^{-1}, 18 days[46]		
			BTB damage	5 and 20 mg kg^{-1} d^{-1}, 28 days[40]				

Table 1 (*Contd.*)

Toxicity	Female reproductive system damage		Male reproductive system damage		Transgenerational toxicity		Hormonal level changes	
Compounds	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose
PFNA	Impaired oocyte maturation	300 mg kg^{-1}, 14 h[31]	Disrupted spermatogenesis Abnormal seminiferous tubule histology	2 and 5 mg kg^{-1} d^{-1}, 14 days[41] 2 and 5 mg kg^{-1} d^{-1}, 14 days[41]				
PFBS	Follicular disruption	200 mg kg^{-1} d^{-1}, 14 days[30]			Delayed estrus onset in offspring Impaired female reproductive organ development in offspring	200 and 500 mg kg^{-1} d^{-1}, 20 days[48] 200 and 500 mg kg^{-1} d^{-1}, 20 days[48]	Decreased E2 and P levels	200 mg kg^{-1} d^{-1}, 14 days[30]
PFHxS	Prolonged estrous cycles and reduced ovulation	25.1 and 62.5 mg kg^{-1}, single intraperitoneal injection[32]						
PFHpA					Reduced sperm quantity in male offspring Altered testicular morphology in offspring	0.0015, 0.015, and 0.15 mg per kg bw per day, 16 days[50]		
6:2 FTOH					Testicular damage in offspring Abnormal sperm development in offspring	5, 25, and 125 mg per kg bw , 10 days[49]		
HFPOs			Testicular histological damage BTB damage	1, 5, 10, and 20 mg per kg per day, 28 days[39]				

2.2 Reproductive effects in rats

Rats are also extensively used as model organisms. The genomes of both rats and mice have been fully sequenced, and a large number of gene knockout and transgenic models are available. These models are valuable tools for investigating the role of specific genes in reproductive processes.[24] Compared to mice, rats are larger, making them easier to handle (*e.g.*, for chip implantation). Their physiological characteristics are also more readily observed, and they display a certain degree of adaptability and aggression. As a result, rats have increasingly become a valuable model for studying a variety of human diseases.[51]

2.2.1 Damage to male gonads. Studies have shown that various PFASs can cause changes in the weight of organs such as the testes. Cui *et al.*[52] reported that oral exposure to PFOS and PFOA increased the gonadosomatic index (GSI) in rats; however, the study did not provide data on changes in absolute gonadal weight. Li *et al.*[53] found that gavage exposure to PFOS at 10 mg kg^{-1} for 21 days led to a reduction in testicular weight, while both 5 mg kg^{-1} and 10 mg kg^{-1} doses significantly decreased the weight of the seminal vesicles. They also conducted *in vitro* exposure experiments on immature Leydig cells from rats and found that PFOS significantly inhibited androgen synthesis, presumably by downregulating the expression of HSD17B3. Research on perfluorododecanoic acid (PFDoA)[54] found that oral exposure to 10 mg per kg per day of PFDoA for 14 days resulted in a decrease in absolute testicular weight, while at doses of 5 and 10 mg per kg per day, the relative testicular weight significantly increased. These results are similar to those observed in previous studies on PFOS. Another study[55] found that oral exposure to 5 and 10 mg kg^{-1} of perfluoroundecanoic acid (PFUnA) for 21 days also significantly reduced the weight of the testes and epididymis.

PFOS has been the most extensively studied in terms of changes in sperm count and quality, as well as testicular damage. Two studies[12,53] showed that PFOS exposure leads to a decrease in epididymal sperm count and disrupts spermatogenesis in rats. Umar Ijaz *et al.*[12] also reported that exposure to 20 mg kg^{-1} of PFOS significantly reduces the number of spermatogonia and primary and secondary spermatocytes in rats and results in a high degree of abnormalities in the sperm head, midpiece, and tail. Moreover, PFOS exposure causes testicular lesions, including a significant reduction in the seminiferous tubule diameter, epithelial height, and basement membrane thickness, along with an increase in the lumen diameter of the tubules. Another study[56] showed that oral exposure to 1, 3, and 6 mg kg^{-1} of PFOS for 28 days induces gonadal cell degeneration, sperm loss and degradation, as well as abnormalities in the seminiferous tubules, accompanied by surrounding edema.

Other PFASs have been shown to produce similar effects. In an earlier study, Feng *et al.*[57] found that oral exposure to 3 and 5 mg kg^{-1} of PFNA for 14 days resulted in disorganization and atrophy of the spermatogenic epithelium in rats, causing testicular cell apoptosis and subsequently disrupting spermatogenesis. Another study[58] indicated that oral exposure to 0.5 mg per kg per day of PFDoA caused cell shedding in some seminiferous tubules of rat testes. Later, Yan *et al.*[55] found that after 21 days of oral exposure to 5 and 10 mg kg^{-1} of PFUnA, sperm counts were significantly reduced in the epididymal tubules of the head, body, and tail sections of rats.

Damage to interstitial cells in male rats is also a significant indicator of reproductive toxicity. According to the research timeline, Shi *et al.*[54] observed that after 14 days of oral exposure to 5 and 10 mg per kg per day of PFDoA, interstitial cells, support cells, and spermatogenic cells in rats exhibited apoptotic features, including irregularly shaped and densely packed nuclei, chromatin condensation, unclear nuclear membranes, and mitochondrial abnormalities. Feng *et al.*[57] found that oral exposure to PFNA for 14 days caused the formation of large vacuoles between supporting cells and spermatogonia in rats. However, a subsequent study[53] found that exposure to 5 and 10 mg per kg per day of PFOS for 21 days did not affect the number of interstitial cells, suggesting that PFOS does not influence the proliferation of immature interstitial cells. This highlights that different PFASs may have distinct effects on interstitial cells. PFUnA has been shown to reduce the number of interstitial cells in rats,[55] whereas perfluorotridecanoic acid (PFTrDA) has been found to inhibit interstitial cell proliferation.[59]

2.2.2 Transgenerational toxicity. The impact on offspring is one of the toxic effects of PFASs. After 9 days of oral exposure to 20 mg kg^{-1} of PFOS in pregnant rats, the male offspring exhibited a reduction in testicular weight and a decrease in anogenital distance (AGD).[60] These effects may be attributed to a decline in T levels and endocrine disruption in the male rats of the F_1 generation. Later, Tian *et al.*[61] reduced the dosage and extended the exposure duration, finding that 60 days of 5 mg per kg PFOS exposure resulted in various toxic effects. The F_0 generation female rats exhibited lower pregnancy rates and higher fetal mortality. Additionally, the F_1 female rats became infertile, while the male rats showed testicular swelling and congestion, with some presenting hemorrhagic spots. Compared to the control group, the PFOS-exposed group displayed a significant reduction in the number of spermatogenic cell layers, along with a notable increase in the proportion of abnormal seminiferous tubules and tubules with disorganized or sparse epithelium. Another study[62] showed that exposure of pregnant female mice to 30 mg per kg HFPO-DA results in reduced birth weight in offspring, while a dose of 62.5 mg kg^{-1} significantly decreases neonatal survival rates. In a separate study,[63] NBP2 was likewise found to reduce pup survival.

2.2.3 Sex hormone imbalance. Two studies,[12,56] both using oral administration, found that exposure to PFOS resulted in changes in various reproductive-related hormone levels in rats, including gonadotropins, androgens, LH, FSH, and plasma T. Both studies concluded that PFOS disrupted the hypothalamic–pituitary–gonadal (HPG) axis in rats. Li *et al.*[53] found that oral exposure to PFOS for 21 days reduced serum T levels, but did not affect the levels of LH or FSH. In a separate low-dose study,[64] rats orally administered PFOS at 0.015 and 0.15 mg per kg per day for 60 days showed significant increases in progesterone and testosterone levels, in contrast to the results reported in earlier high-dose studies.

Table 2 Summary of reproductive toxicity of eight PFASs in rats

Toxicity	Male reproductive organ lesions		Interstitial cell abnormalities		Transgenerational toxicity		Hormone level changes	
Compounds	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose
PFOS	Increased GSI	5 and 20 mg kg^{-1} d^{-1}, 28 days[52]	No effect	5 and 10 mg kg^{-1} d^{-1}, 21 days[53]	Reduced offspring testicular weight and AGD	20 mg kg^{-1} d^{-1}, 9 days[60]	Decreased T	20 mg kg^{-1} d^{-1}, 56 days[12]
	Decreased testis weight	5 and 10 mg kg^{-1} d^{-1}, 21 days[53]			Increased fetal death rate, F_1 female infertility, and male testicular damage	5 mg kg^{-1} d^{-1}, 60 days[61]		5 and 10 mg kg^{-1} d^{-1}, 21 days[53]
	Decreased sperm count	20 mg kg^{-1} d^{-1}, 56 days[12]			Decreased offspring survival	10 and 30 mg kg^{-1} d^{-1}, 18 days[63]		1, 3, and 6 mg kg^{-1} d^{-1}, 28 days[56]
		5 and 10 mg kg^{-1} d^{-1}, 21 days[53]					Increased T and P	0.015 and 0.15 mg kg^{-1} d^{-1}, 60 days[64]
	Testicular tubule damage	1, 3, and 6 mg kg^{-1} d^{-1}, 28 days[56]					Decreased LH	20 mg kg^{-1} d^{-1}, 56 days[12]
							Increased FSH	1, 3, and 6 mg kg^{-1} d^{-1}, 28 days[56]
								1, 3, and 6 mg kg^{-1} d^{-1}, 28 days[56]
							Decreased FSH	20 mg kg^{-1} d^{-1}, 56 days[12]
							Disruption of GnRH levels	1, 3, and 6 mg kg^{-1} d^{-1}, 28 days[56]
PFOA	Increased GSI	5 and 20 mg kg^{-1} d^{-1}, 28 days[52]					Increased T, P, and E2	0.015 and 0.15 mg kg^{-1} d^{-1}, 60 days[66]
PFDoA	Decreased testis weight	5 and 10 mg kg^{-1} d^{-1}, 14 days[54]	Abnormalities and apoptosis	5 and 10 mg kg^{-1} d^{-1}, 14 days[54]			Decreased male T	5 and 10 mg kg^{-1} d^{-1}, 14 days[54]
								0.5 mg kg^{-1} d^{-1}, 110 days[58]
	Testicular tubule damage	0.5 mg kg^{-1} d^{-1}, 110 days[58]					Decreased LH	5 and 10 mg kg^{-1} d^{-1}, 14 days[54]
							Decreased female E2	3 mg kg^{-1} d^{-1}, 28 days[65]
PFUnA	Decreased testis weight Reduced sperm count	5 and 10 mg kg^{-1} d^{-1}, 21 days[55]	Reduced cell count	5 and 10 mg kg^{-1} d^{-1}, 21 days[55]			Decreased T, LH, and FSH	5 and 10 mg kg^{-1} d^{-1}, 21 days[55]
PFNA	Spermatogenesis disruption	3 and 5 mg kg^{-1} d^{-1}, 14 days[57]	Vacuolation	3 and 5 mg kg^{-1} d^{-1}, 14 days[57]			Decreased T	3 and 5 mg kg^{-1} d^{-1}, 14 days[57]
PFTrDA			Reduced proliferation	10 mg kg^{-1} d^{-1}, 21 days[59]			Decreased T and LH	10 mg kg^{-1} d^{-1}, 21 days[59]
							Unchanged E2 levels	

Table 2 (*Contd.*)

Toxicity	Male reproductive organ lesions		Interstitial cell abnormalities		Transgenerational toxicity		Hormone level changes	
Compounds	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose
HFPO-DA					Reduced offspring survival and pup weight	30 mg kg^{-1} d^{-1}, 18 days[62]		
NBP2					Reduced offspring survival rate	10 and 30 mg kg^{-1} d^{-1}, 18 days[63]		

Research on other PFASs is relatively limited. Earlier, Shi *et al.*[54] investigated male rats and found that oral exposure to 10 mg per kg per day of PFDoA for 14 days significantly reduced LH levels, while exposure to 5 and 10 mg per kg per day of PFDoA resulted in a significant decrease in T levels. Subsequently, the team reduced the exposure dose and extended the exposure duration.[58] They found that oral exposure to 0.2 and 0.5 mg PFDoA per kg per day for 110 days also resulted in a decrease in serum T levels. In a separate study by the same team on female rats,[65] oral administration of PFDoA at 3 mg per kg per day for 28 days significantly decreased serum estradiol (E2) levels. Yan *et al.*[55] found that PFUnA significantly reduced serum T, luteinizing and serum follicle-stimulating hormone levels. Furthermore, oral exposure to 5 mg per kg per day of PFNA was also found to significantly reduce serum T levels in rats.[57] PFTrDA has been shown to significantly decrease serum T and LH levels, while leaving serum E2 and FSH levels unaffected.[59] However, Han *et al.*[66] found that exposure to 0.015 and 0.15 mg kg^{-1} of PFOA for 2 months significantly increased serum P, T, and E2 levels. They suggested that low-dose PFOA exposure may stimulate the production of steroid hormones in the testes of rats (Table 2).

2.2.4 Summary. In studies on reproductive toxicity in rats, eight PFASs have been investigated: PFOS, PFOA, PFNA, PFUnA, PFDoA, PFTrDA, HFPO-DA, and NBP2. Similar to studies in mice, PFOS and PFOA are the most extensively researched compounds. PFASs exert reproductive toxicity in rats, primarily affecting the male reproductive organs. Macroscopically, they cause a reduction in absolute testicular weight, while the relative testicular weight (GSI) increases, which contrasts with the results observed in mice. At the tissue and cellular levels, PFASs induce histopathological damage to the seminiferous tubules and epididymis, a decline in sperm count and quality, and damage to interstitial cells. However, research on female reproductive organs is scarce. Only one study has reported that PFOS can reduce pregnancy rates in female mice. At the molecular level, T levels in rats are generally decreased following exposure to most PFASs, with the exception of PFOA, which causes an increase in T levels. This is consistent with the findings in mice. Other sex hormones, such as E2 and P, exhibit varying degrees of alteration. Regarding transgenerational toxicity, PFASs cause a decrease in offspring survival rates, body weight, and testicular weight in rats.

Regarding the dose and exposure duration that induce reproductive toxicity, the dose range for PFOS is 0.015–20 mg per kg per day, with exposure durations ranging from 14 to 60 days. For PFOA, the dose range is also 0.015–20 mg per kg per day, with exposure durations ranging from 7 to 28 days. As for other PFASs, the dose range for PFDoA varies from 0.2 to 10 mg per kg per day, with the longest exposure duration reaching up to 110 days. Several other PFASs also show effective concentrations in the mg per kg per day range. Since rats and mice both belong to the rodent family, their exposure methods (gavage and oral administration) and dose selection are generally similar (Fig. 1).

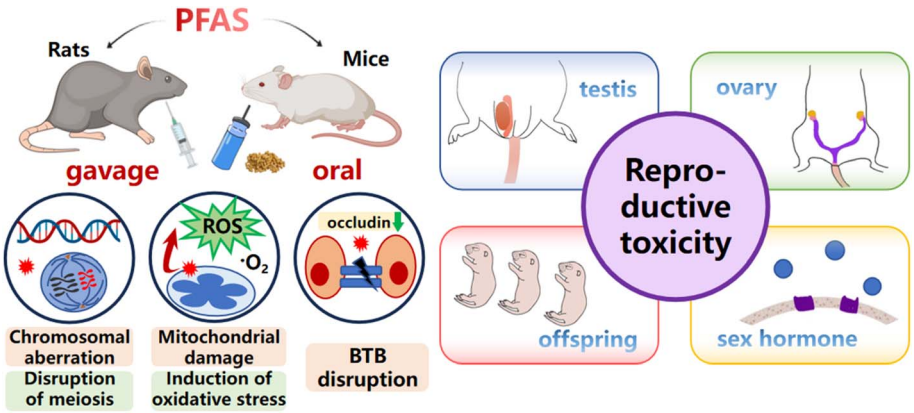

Fig. 1 Schematic diagram of the reproductive toxicity of PFASs in rats and mice.

2.3 Reproductive effects in zebrafish

Zebrafish are a tropical freshwater species with several distinct advantages, including external fertilization, high fecundity, a short developmental cycle, small size, the ability for *in vivo* imaging, and a wide range of transgenic strains. Their biological structure and physiological functions are highly similar to those of mammals, with over 85% of their genes homologous to human genes.[67] As a result, zebrafish are extensively used in research fields such as environmental toxicology. The National Institutes of Health (NIH) in the United States has recognized zebrafish as the third major vertebrate model organism, following mice and rats.

2.3.1 Damage to the female gonads. PFOS has been shown to impair reproductive capacity and reduce the gonadosomatic index (GSI) in female fish.[68] Chen *et al.*[69] exposed zebrafish larvae, 8 hours post-fertilization (hpf), to 250 μg L^{-1} of PFOS for 5 months. They observed morphological changes in the female gonads. TEM analysis revealed that the oocytes exhibited an increase in vesicles, disorganized endoplasmic reticulum, reduced mitochondrial cristae, increased cytoplasmic vacuolization, elevated heterochromatin, and nuclear condensation.

Long-term exposure to PFOA at environmental concentrations (2 nM initially, followed by 8 pM) for 5 months is considered to reduce egg production in zebrafish.[70] Zhang *et al.*,[71] based on histological analysis, found that short-term exposure to PFOA induces ovarian damage characterized by vacuolization of ovarian somatic tissue and disrupted contact between oocytes and follicular cells, with the incidence of abnormalities increasing in a dose-dependent manner. Furthermore, there was an increase in the number of oocytes at the primary growth stage and a decrease in the proportion of mature oocytes. Additionally, there was an increase in oocytes at the primary growth stage, while the percentage of mature oocytes decreased. Furthermore, the GSI of the female fish was significantly reduced. The study also revealed that PFOA exposure decreased the fertilization and hatching rates of the fish in a dose-dependent manner. A recent finding by Lu *et al.*[72] demonstrates that a 28-day exposure to 200 μg L^{-1} of PFOA can induce the formation of cortical alveolar oocytes (CAOs).

Besides the two aforementioned PFAS, a study[73] has shown that exposure to PFNA significantly reduced egg production in female fish. Similar effects were observed with 6:2 chlorinated polyfluorinated ether sulfonate (F-53B),[74] where exposure to F-53B for 180 days also led to a decrease in egg production, a reduction in GSI, and alterations in the structure of gonadal tissue. However, Hu *et al.*[75] observed the opposite effect after exposure to PFBS, with an increase in egg production in female fish. In another study on PFBS,[76] exposure to 0.14 mg L^{-1} for 7 days caused significant changes in the initial deposition and utilization of yolk sacs in embryos, leading to a marked reduction in yolk sac area. Two recent studies on HFPOs reported that HFPO-TA significantly increases the number of Stage I oocytes while decreasing those at stages III-IV, thereby impairing oocyte maturation and causing pathological lesions in ovarian tissue. The extent of damage caused by HFPO-TA was found to be greater than that induced by PFOA.[77] Both HFPO-DA and PFOA have been shown to induce the formation of CAO, while HFPO-TA at concentrations as low as 1 μg L^{-1} can stimulate the formation of early vitellogenic oocytes (EVO). These findings suggest that environmental levels of HFPO-TA can significantly promote ovarian development in female zebrafish.[72]

2.3.2 Damage to the testis. PFOS has been shown to affect testicular development in zebrafish. Du *et al.*[68] reported that after 70 days of exposure to 250 μg per L PFOS, the testes of treated fish exhibited a high abundance of early-stage germ cells, including spermatogonia and primary spermatocytes, indicating delayed testicular maturation. Wang *et al.*[78] demonstrated that PFOS exposure adversely affected male gonadal development in a dose-dependent manner, resulting in decreased sperm motility and quality. One study,[69] using the same concentrations as Du *et al.* and the same exposure duration as Wang *et al.*, found a decline in sperm quality in male fish. Additionally, morphological changes in the male gonads were observed, including incomplete structure of the testicular lobules, ectopic clusters of spermatogonia in the lobule centers, and rupture of the basement membrane. TEM examination revealed cytoplasmic vacuolization, reduced mitochondrial cristae, and axon fragmentation. Bao *et al.*[79] observed that PFOS exposure significantly reduced the frequency of sexual

behaviors in male zebrafish, including chasing, nipping, and tail-touching. They proposed that PFOS may alter the reproductive system of zebrafish by disrupting endocrine function and suppressing mating behavior.

For other PFASs, Zhang *et al.*[73] conducted a 180-day exposure study on zebrafish and found that PFNA at three concentrations (0.01, 0.1, and 1 mg L^{-1}) significantly reduced the GSI in male zebrafish. F-53B has been found to enlarge the testicular lumen in zebrafish, decrease the number of mature spermatocytes, and impair spermatogenesis.[74] Lu *et al.*[72] investigated PFOA and its alternatives, HFPO-DA and HFPO-TA, and found that exposure to high concentrations (200 μg L^{-1}) of HFPOs and PFOA for 28 days induced severe testicular abnormalities, including disorganization of spermatogonia and spermatocytes, incomplete formation of spermatocyte clusters, prominent interstitial cavities within the testes, and numerous undifferentiated gonadal cells. These findings suggest delayed testicular development. This delay mirrors the effects observed with exposure to flutamide (an AR antagonist), indicating that these three PFASs may disrupt sex differentiation in zebrafish larvae through an AR-mediated mechanism.

2.3.3 Transgenerational toxicity. PFOS exposure at concentrations of 50 and 250 μg L^{-1} for 70 days was found to significantly increase the deformity rate in F_1 generation zebrafish, with all deformed larvae dying within 96 hours.[68] Two additional studies also observed similar effects following exposure to 250 μg L^{-1}[78] and 300 μg L^{-1}[80] PFOS for 5 months. Chen *et al.*[69] found that exposure to PFOS from the embryonic stage resulted in a female-biased sex ratio. In adult fish, there were more mature oocytes than spermatogonia. Jantzen *et al.*[70] reported that even low doses of PFOA were sufficient to reduce embryo survival rates.

Besides PFOS and PFOA, several other PFAS compounds have been shown to exert reproductive toxicity in zebrafish offspring. Specifically, PFNA exposure has been found to significantly reduce embryo hatching rates.[73] After 180 days of F0 generation exposure to F-53B, significant reproductive effects were observed in F_1 adult zebrafish. In males, both T levels and the T/E2 ratio were significantly decreased, while in females, the GSI was markedly reduced.[74] Elevated malformation rates and reduced survival in F_1 and F_2 embryos suggest that parental exposure to F-53B disrupts embryonic development and inhibits growth and reproduction in subsequent zebrafish generations. F-53B accumulates in the F_0 gonads and is maternally transferred to the F_1 generation *via* eggs, remaining detectable in both adult F_1 fish and their F_2 offspring's eggs even after 180 days. In another study by the same team,[81] a mixture of 6:2 FTAB and 6:2 FTAA was found to reduce the average number of eggs laid by the offspring, while increasing deformity rates and mortality. Research on maternal preconception exposure to PFBS[76] has shown that exposure to 0.25 mg per L PFBS for just one week can transfer from the mother to the mature oocytes, resulting in a slight impact on the nutritional status and growth of the offspring.

2.3.4 Disruption of sex hormone levels. PFOS has been shown to significantly increase vitellogenin (VTG) and E2 levels[68] while reducing T levels in zebrafish.[69] Conversely, Chen *et al.*[77] reported that exposure to 10 μg per L PFOA for 28 days resulted in decreased serum E2 levels in zebrafish. Lu *et al.*[72] demonstrated that exposure to PFOA at an environmental concentration (2 μg L^{-1}) for 28 days significantly reduced 11-ketotestosterone (11-KT) levels while substantially elevating E2 levels.

Besides the two PFASs mentioned above, 6:2 FTOH has been found to significantly elevate plasma E2 and T levels in both male and female mice. Notably, the T/E2 ratio decreased in females while increasing in males.[82] PFNA has been found to produce similar effects.[73] Shi *et al.*[74] revealed that exposure to 5 μg L^{-1} of F-53B for 180 days significantly increased serum E2 and VTG levels in adult male zebrafish. In females, T levels were significantly elevated, E2 levels slightly increased, and the T/E2 ratio underwent significant changes. In another study[81] by the same team, adult female zebrafish exposed to a 6:2 FTAB and 6:2 FTAA mixture for 180 days showed elevated levels of E2 and T, while males exposed to 500 μg L^{-1} exhibited a decrease in T levels. Additionally, exposure to HFPO-TA for 28 days was found to induce an increase in serum T levels in female zebrafish.[77] Lu *et al.*[72] also studied HFPO-TA and found that exposure to environmental concentrations (1 μg L^{-1} for 28 days) significantly increased E2 levels and decreased 11-KT levels in male fish. The team further observed that HFPO-DA exhibited similar effects to HFPO-TA, although at higher concentrations (20 and 200 μg L^{-1}) (Table 3).

2.3.5 Summary. This summary identifies nine PFASs that induce reproductive toxicity in zebrafish: PFOS, PFOA, PFBS, PFNA, HFPO-DA/TA, F-53B, 6:2 FTOH, and the 6:2 FTAA/FTAB mixture. First, from an intuitive standpoint, given that female zebrafish produce a large number of offspring, the impact of PFASs on their reproductive capacity can be assessed by directly measuring the quantity and quality of the offspring. The results showed that, except for PFBS, which increased egg production in female zebrafish, PFOS, PFOA, PFNA, and F-53B all reduced reproductive capacity. Examination of the reproductive organs revealed that PFAS exposure led to a decrease in the GSI of zebrafish, aligning with findings from studies on mice and rats. Additionally, female zebrafish exhibited reduced yolk sac area, vacuolization of ovarian somatic cells, impaired oocyte maturation, and decreased fertilization and hatching abilities. Male zebrafish showed delayed testicular development, incomplete structures of seminiferous lobules, disorganized spermatogenesis, and reduced sperm motility. Additionally, PFNA accumulation in male gonads was nearly twice that in female gonads, indicating that male zebrafish may bear a greater gonadal burden of PFAAs.

The observed phenomena may be linked to changes in hormonal levels in zebrafish, such as VTG, T, and 11-KT. Specifically, PFOS, HFPOs, PFNA, and 6:2 FTOH were found to increase E2 levels, while PFOA had the opposite effect. Even for the same compound, different studies may yield conflicting results. For instance, Lu *et al.*[72] reported that PFOA elevated E2 levels in fish, while Chen *et al.*[77] observed a decrease. Furthermore, PFASs have been found to increase offspring mortality, deformity rates, and developmental delays and induce a female-biased sex ratio.

Table 3 Summary of reproductive toxicity of the eight PFASs on zebrafish

Toxicity	Female reproductive system damage		Male reproductive system damage		Transgenerational toxicity		Hormonal level changes	
Compounds	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose
PFOS	Reduced reproductive capacity	50 and 250 μg L^{-1}, 70 days[68]	Delayed testicular development	50 and 250 μg L^{-1}, 70 days[68]	Increased F_1 mortality rate	50 and 250 μg L^{-1}, 70 days[68]	Upregulated VTG in female fish	50 and 250 μg L^{-1}, 70 days[68]
			Gonadal histological injury	250 μg L^{-1}, 5 months[69]		5, 50, and 250 μg L^{-1}, 5 months[78]	Increased E2 and decreased T in juvenile fish	250 μg L^{-1}, 5 months[69]
	Decreased GSI	50 and 250 μg L^{-1}, 70 days[68]	Decreased sperm quality	250 μg L^{-1}, 5 months[69]		300 μg L^{-1}, 5 months[80]	Increased E2 in adult fish	
	Gonadal histological damage	250 μg L^{-1}, 5 months[69]		5, 50, and 250 μg L^{-1}, 5 months[78]	Female bias	5, 50, and 250 μg L^{-1}, 5 months[78]		
			Reduced frequency of sexual activity	200 μg L^{-1}, 21 days[79]	F1 embryo malformations	250 μg L^{-1}, 5 months[69]		
PFOA	Decreased GSI	100 mg L^{-1}, 15 days[71]	Testicular abnormalities and delayed development	200 μg L^{-1}, 28 days[72]	Reduced embryo survival rate	2 nM initially, followed by 8 pM, 5 months[70]	Reduced 11-KT	2 μg L^{-1}, 28 days[72]
	Reduced fertilization and hatching ability						Increased E2	2 μg L^{-1}, 28 days[72]
	Ovarian histological damage	100 mg L^{-1}, 15 days[71] 200 μg L^{-1}, 28 days[72]					Decreased E2	10 μg L^{-1}, 28 days[77]
	Decreased egg production	2 nM initially, followed by 8 pM, 5 months[70]						
F-53B	Decreased GSI and egg quantity Gonadal structural changes	5 and 50 μg L^{-1}, 180 days[74]	Disruption of spermatogenesis	5 and 50 μg L^{-1}, 180 days[74]	Reduced offspring GSI Changes in offspring hormone levels Offspring malformations	5 and 50 μg L^{-1}, 180 days[74]	Elevated E2 and VTG in males Increased E2	5 and μg L^{-1}, 180 days[74]
PFNA	Decreased egg quantity	0.1 mg L^{-1}, 180 days[73]	Decreased GSI	0.01 and 0.1, 1 mg L^{-1}, 180 days[73]	Reduced embryo hatching rate	0.01, 0.1, and 1 mg L^{-1}, 180 days[73]	Elevated T and E2 in both sexes	0.1 mg L^{-1}, 180 days[73]

Table 3 (*Contd.*)

Toxicity	Female reproductive system damage		Male reproductive system damage		Transgenerational toxicity		Hormonal level changes	
Compounds	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose
PFBS	Decreased yolk sac area	0.14 mg L^{-1}, 7 days[76]			Offspring growth affected	0.14 mg L^{-1}, 7 days[76]		
	Increased egg quantity	0.1 mg L^{-1}, 180 days[73]						
HFPO-DA\TA	Promoted ovarian development	1000 μg L^{-1}, 28 days[72]	Testicular abnormalities and delayed development	200 μg L^{-1}, 28 days[72]			Decreased 11-KT in female fish	1 μg L^{-1}, 28 days[72]
	Ovarian damage	1000 μg L^{-1}, 28 days[77]					Increased E2 in females	
							Elevated T in males	10 and 100 μg L^{-1}, 28 days[77]
Mixture of 6:2 FTAA & FTAB					Decreased offspring fecundity	50 and 500 μg L^{-1}, 180 days[81]	Increased E2 and T in females	50 and 500 μg L^{-1}, 180 days[81]
					Increased rates of deformities and mortality		Decreased T in males	
6:2 FTOH							Elevated T and E2 in both sexes	0.3 and 3 mg L^{-1}, 7 days[82]
							Increased T/E2 in females and decreased T/E2 in males	

Exposure methods in fish studies have been relatively limited. While Jantzen *et al.*[70] innovatively combined early-stage water exposure with later-stage dietary exposure, most studies have relied on waterborne exposure, as it closely mimics the way fish encounter pollutants in the environment. Regarding dosage selection, the concentrations used in fish studies are generally lower than those in rodent studies, more closely reflecting environmental levels. The PFOS concentration range was 50–300 μg L^{-1}, with exposure durations ranging from a minimum of 21 days to a maximum of 5 months. The concentration range for PFOA spanned widely, from 3.31 ng L^{-1} (8 pM) to 100 mg L^{-1}, with exposure durations ranging from 15 days to 5 months. For other PFASs, concentration ranges were typically within the μg L^{-1} level.

2.4 Reproductive effects in *Oryzias*

The commonly used model organism *Oryzias* includes two species: the freshwater medaka (*Oryzias latipes*) and the marine medaka (*Oryzias melastigma*). These species offer research advantages similar to those of the model organism zebrafish, such as a short generation time (3–4 months), daily egg production, small adult size (2.5–3.5 cm), transparent embryos, sexual dimorphism, and the ease of large-scale cultivation in the laboratory.[83] Given the growing concerns over marine pollution, the unique advantages of *Oryzias melastigma* are making it an increasingly prominent model organism in the field of marine ecotoxicology research.

2.4.1 Gonadal damage. PFASs can impair the gonads and reproductive capacity of medaka. Ji *et al.*[84] found over a decade ago that exposure to PFOS increased the GSI in female medaka, while the number of eggs laid decreased in a concentration-dependent manner, and hatching rates significantly declined. Kang *et al.*[85] later found that PFOA and PFOS significantly reduced the reproductive capacity of medaka. They proposed that PFAS exposure altered vtg1 gene transcription, leading to changes in VTG levels and subsequent reproductive impairments. A study on PFBS[86] exposure found that at environmental concentrations (1.0, 2.9, and 9.5 μg L^{-1}), female marine medaka exhibited severe reproductive impairment, including a reduced GSI and impaired oocyte development. In the 9.5 μg L^{-1} group, vitellogenin pre-oocytes increased and ovarian tissues showed significant hypermethylation. No histological abnormalities were observed in the testes of male fish.

2.4.2 Disruption of sex hormone levels. PFOA has been shown to significantly induce VTG levels in male medaka over time, whereas PFOS did not exhibit a comparable effect.[85] Mechanistically, PFOA exposure led to an increase in vtg1 expression, while PFOS exposure resulted in a decrease in vtg1 expression. Another study[86] on long-term PFBS exposure (6 months) reported that PFBS induced endocrine disruption of the hypothalamic–pituitary–gonadal axis. Exposure to 1.0 μg L^{-1} and 2.9 μg per L PFBS significantly elevated plasma FSH levels in bisexual marine medaka. In the 2.9 and 9.5 μg L^{-1} groups, T levels in males were significantly reduced, while plasma levels of E2 and 11-KT in females also showed a significant decrease. The trend of the E2/T ratio for both sexes was reversed. PFBS displayed anti-estrogenic activity in females, while exhibiting estrogenic activity in males. In subsequent studies on marine medaka embryos,[87] PFBS has been shown to significantly reduce plasma E2 levels in juvenile fish, leading to a decreased E2/T ratio and exhibiting anti-estrogenic activity. In a 21-day exposure experiment on adult fish,[88] it was found that 10 μg per L PFBS increased E2 levels in male fish, decreased 11-KT levels in female fish, and elevated the E2/T and E2/11-KT ratios in female fish. Furthermore, T levels were reduced in both sexes.

2.4.3 Transgenerational toxicity. An early study[84] found that after 14 days of exposure to 0.01–1 mg per L PFOS and 0.1–10 mg per L PFOA, the survival rate of medaka F_1 offspring was significantly reduced. Lee *et al.*[89] exposed medaka to 30 mg per L PFOA for 259 days and found that it suppressed the reproductive capacity of F_0 medaka, with continued inhibition in F_1 and F_2 generations and reduced survival rates in F_2. Additionally, gonadal development accelerated in F_1 and F_2 medaka. The team also investigated the reproductive toxicity of a mixture of four prevalent PFAAs—PFOS, PFOA, PFBS, and PFNA—in medaka.[90] Long-term exposure (238 days) to a total of 20 μg L^{-1} of PFAAs reduced reproductive rates from F_0 to F_2 generations and lowered the GSI in F_1 females, indicating that the estrogenic effects of PFAAs can be passed on to offspring. One study[86] exposed marine medaka to PFBS for 6 months, revealing a male-biased sex ratio. Parental exposure to PFBS significantly increased F1 offspring mortality, while F_1 eggs showed reduced weight. In contrast, F_2 eggs increased in weight, indicating that PFBS-induced changes are persistent (Table 4).

2.4.4 Summary. Research on *Oryzias* is relatively limited compared to the other three model organisms, with studies focusing on only four types of PFASs: PFOS, PFOA, PFBS, and a mixture of PFOS, PFOA, PFBS, and PFNA. PFASs can cause changes in the GSI, reduce fertility, lower hatching rates, and prolong hatching times. Additionally, these substances can affect offspring in varying degrees, leading to toxic effects such as decreased survival rates, reduced fertility, altered sex ratios, and lower GSI. The changes in hormone levels are similar to those observed in zebrafish, mainly involving alterations in E2, T, FSH, and VTG.

The exposure methods and dosage selection for *Oryzias* are similar to those used for zebrafish, with direct exposure in water. The dosage range for PFOS is 10–1000 μg L^{-1}, with exposure durations typically shorter than those for zebrafish, lasting two to three weeks. In contrast, the dosage range for PFOA is an order of magnitude higher, ranging from 100–30,000 μg L^{-1}. The exposure duration ranged from 21 to 259 days. For PFBS, environmental concentrations were used, with exposure lasting up to 6 months. In the case of the mixture, the total concentration was 20 μg L^{-1} and exposure lasted up to 238 days. At the given concentrations and under the given exposure durations, PFASs caused varying levels of reproductive toxicity in *Oryzias* (Fig. 2).

2.5 Reproductive effects in *Caenorhabditis elegans*

Caenorhabditis elegans is commonly found in soil and offers numerous advantages for research, including ease of laboratory

Table 4 Summary of the reproductive toxicity of PFASs on *Oryzias*

Toxicity	Gonadal damage		Hormone level changes		Transgenerational toxicity	
Compounds	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose
PFOS	Increased female GSI Reduced fertility	0.01, 0.1, and 1 mg L^{-1}, 14 days[84] 0.01, 0.1, and 1 mg L^{-1}, 14 days[84] 1 mg L^{-1}, 21 days[85]			Decreased offspring survival rate	0.01, 0.1, and 1 mg L^{-1}, 14 days[84]
PFOA	Reduced fertility	10 mg L^{-1}, 21 days[85]	Induces male VTG levels	10 mg L^{-1}, 21 days[85]	Reduced offspring fertility Reduced offspring survival rate Accelerated gonadal development in offspring	30 mg L^{-1}, 180 days[89]
PFBS	Reduced female GSI Oocyte development hindered	1.0, 2.9, and 9.5 μg L^{-1}, 180 days[86]	Decreased T and increased E2	1.0, 2.9, and 9.5 μg L^{-1}, 180 days[86] 10 μg L^{-1}, 21 days[88]	Reduced egg quality in offspring Male bias	1.0, 2.9, and 9.5 μg L^{-1}, 180 days[86]
			Decreased E2 in juvenile fish Decreased 11-KT in female fish Elevated FSH	1, 3.3, and 10 mg L^{-1}, 15 days[87] 10 μg L^{-1}, 21 days[88] 1.0, 2.9, and 9.5 μg L^{-1}, 180 days[86]	Reduced offspring survival rate[81]	
Mixture of PFOS, PFOA, PFBS, and PFNA					Decreased F_1 GSI Reduced F_1 and F_2 fertility rates	20 μg L^{-1}, 238 days[90]

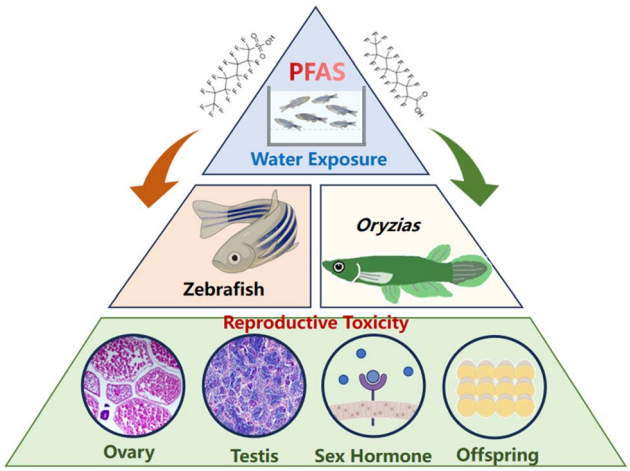

Fig. 2 Schematic diagram of the reproductive toxicity of PFASs in zebrafish and *Oryzias*.

maintenance, small size, transparency, a short lifecycle, high reproductive output, a fully sequenced genome, and a wide range of mutations and transgenic strains.[91] *Caenorhabditis elegans* is hermaphroditic, with males constituting only about 0.2% of the population. It is capable of self-fertilization or sexual reproduction. This nematode is highly sensitive to environmental stressors, with exposure to chemicals leading to changes in growth, reproduction, and lifespan.[92] Since its introduction as a model organism in developmental biology research in the 1960s, *C. elegans* has been widely used in a range of studies, including those focused on reproductive toxicity.

2.5.1 Transgenerational toxicity. PFASs can induce transgenerational toxicity in *Caenorhabditis elegans*. Preconceptional exposure to PFOS (≥10 μM) or PFBS (≥1000 μM) appears to disrupt embryonic nutrient allocation and composition, potentially contributing to altered growth dynamics, morphological abnormalities, and diminished locomotor activity in the F_1 generation.[93] Another study[94] found that exposure to PFOS and PFOA resulted in a reduced number of larvae. Chowdhury *et al.*[95] exposed parents to three concentrations of PFOS (0.01, 0.1, and 1.0 μM) for 48 hours, followed by continuous exposure of both parents and offspring to 0.001 μM PFOS. They monitored behavioral endpoints, including locomotion, offspring number, and lifespan. The results showed that PFOS (≥0.01 μM) impaired locomotion, with transgenerational effects that persisted until the F_3 generation. HFPO-DA exposure has been associated with significant delays in reproductive development in the offspring.[96]

2.5.2 Fertility impairment. PFOS is considered to delay gonadal development in *C. elegans*, as evidenced by an increased number of L_1-stage larvae observed after 72 hours of exposure.[97] Chen *et al.*[98] found that exposure to 0.1 μM PFOS and 1000–1500 μM PFBS significantly reduced egg production and egg count in *Caenorhabditis elegans*. Additionally, significant increases in germ cell apoptosis and reactive oxygen species (ROS) production have been observed, which are likely to impair reproductive capacity through damage to germ cells. Another study[91] found that PFOS (≥10 μM) and PFBS (≥1000 μM) exposure significantly reduced total egg count and offspring number in *Caenorhabditis elegans*, without affecting the hatching rate. Exposure to PFOS and PFOA for 48 hours reduced reproductive cell count, sperm size, and motility, while increasing sperm deformity rates.[92] Chowdhury *et al.*[99] reported that parental exposure to ≥0.1 mM PFBS significantly reduced larval numbers, though these effects did not persist in subsequent generations. In another study,[95] they also observed that PFOS (≥0.01 μM, 48 h) significantly inhibited larval hatching in a dose-dependent manner (Table 5).

2.5.3 Summary. As a hermaphroditic model organism, *Caenorhabditis elegans* exhibits distinct reproductive toxicity responses to PFASs compared to the other four model

Table 5 Summary of the reproductive toxicities of four PFASs in *Caenorhabditis elegans*

Toxicity	Transgenerational toxicity		Reproductive capacity	
Compounds	Toxicity	Exposure duration and effective dose	Toxicity	Exposure duration and effective dose
PFOS	Altered embryo nutritional load and composition and F_1 abnormalities	10, 20, and 40 μM, 48 h[93]	Reduced egg production	10, 20, and 40 μM, 48 h[93] 0.1 μM, 48 h[98]
	Decreased larval quantity	0.001, 0.01, and 0.1 mM, 48 h[94]	Delayed gonadal development	0.25, 2.5, and 25.0 μM, 72 h[97]
	Affects nematode motility and can be inherited by offspring	0.1 and 1.0 μM, 48 h, 0.001 μM continue[95]	Damaged germ cell count and sperm	0.001, 0.01, and 0.1 mM, 48 h[94]
			Decreased hatchability	0.1 and 1.0 μM, 48 h, 0.001 μM continue[95]
PFOA	Decreased larval quantity[89]	0.001, 0.01, and 0.1 mM, 48 h[94]	Damaged germ cell count and sperm	0.001, 0.01, and 0.1 mM, 48 h[94]
HFPO-DA	Delayed offspring production	6–12 mM, 48 h[96]		
PFBS	Altered embryo nutritional load and composition and F_1 abnormalities	1000 and 2000 M, 48 h[93]	Decreased egg production	1000 and 1500 μM, 48 h[98] 10, 20, and 40 μM, 48 h[93]
			Reduced larval quantity	≥0.01 mM, 48 h[99]

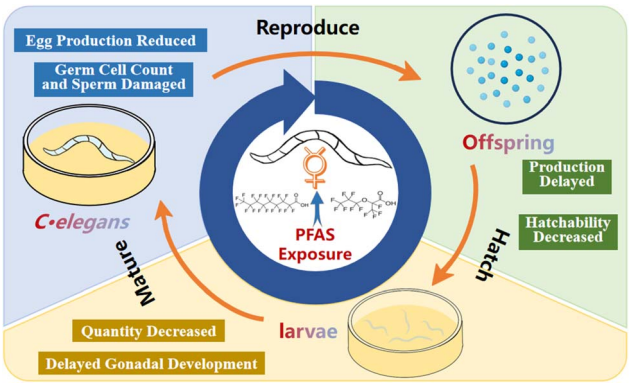

Fig. 3 Schematic diagram of the reproductive toxicity of PFASs in *Caenorhabditis elegans*.

organisms. The primary effects focus on transgenerational toxicity (increased larval numbers, impaired offspring motility, and extended reproductive timelines in progeny) and reproductive system damage (reduced germ cell counts, sperm impairment, and delayed gonadal development).

Caenorhabditis elegans is typically cultured in agar plates, with PFASs being directly added to the culture medium at molar concentrations. Due to its short generation time, the exposure duration is also relatively brief. Overall, four types of PFASs—PFOS, PFOA, PFBS, and HFPO-DA—induce reproductive toxicity in *Caenorhabditis elegans*. The concentration range of PFOS varies widely, from 0.01 to 1000 μM, with exposure durations ranging from 48 to 72 hours. PFOA was investigated in only one study, with concentrations ranging from 1 to 100 μM and an exposure duration of 48 hours. Among the other PFASs, PFBS was tested at higher concentrations, ≥1000 μM, while HFPO-DA was administered at concentrations of 6–12 mM for 48 hours (Fig. 3).

2.6 Reproductive effects in other model organisms

Some researchers have explored less commonly used model organisms to gain a more comprehensive understanding of the reproductive toxicity induced by PFASs.

2.6.1 The reproductive toxicity of PFASs in aquatic organisms

2.6.1.1 The reproductive toxicity of PFASs in fish (excluding zebrafish and Oryzias). In an early study,[100] researchers investigated the blackhead minnow and found that after 21 days of exposure to 1 mg per L PFOS, the cumulative fertility of female blackhead minnows decreased. Additionally, the percentage of pre-ovulatory atretic follicles and late-stage follicles increased, suggesting a delay in the final maturation and release of oocytes. In terms of hormones, both T and 11-KT concentrations were found to increase. Suski *et al.*[101] also conducted a study on blackhead minnows and found that exposure to 44 μg per L PFOS significantly reduced male GSI and decreased fertility in females. Han *et al.*[102] reported contrasting findings, though their study focused on viviparous swordtail fish. They found that exposure to 0.5 mg per L PFOS for three weeks led to an increase in the GSI in both female adults and juvenile fish.

Benninghoff *et al.*[103] conducted exposure experiments on rainbow trout using various PFAAs through oral administration. They found that medium- and long-chain perfluoroalkyl carboxylic acids, containing 8–14 fluorinated carbon atoms, significantly induced the expression of the estrogenic biomarker Vtg in rainbow trout. Among them, PFOA, PFNA, PFDA, and perfluoroundecanoic acid (PFUnDA) were the most potent inducers of Vtg. Rotondo *et al.*[104] investigated the effects of PFOA on carp, and the results revealed that exposure to both environmental (200 ng L^{-1}) and experimental (2 mg L^{-1}) concentrations of PFOA led to changes in the expression levels of the CYP19A gene (which encodes the enzyme responsible for estrogen conversion) in the gonadal tissues of both male and female carp. Specifically, expression was elevated in male gonads and decreased in female gonads. Yang *et al.*[105] selected the rare minnow, a native Chinese species, for their study. They found that exposure to chlorinated polyfluoroalkyl ether sulfonate (Cl-PFESA) resulted in a concentration-dependent decrease in the female GSI, an increase in degenerated and perinuclear oocytes, and a reduction in the number of mature yolk cells. In males, there was an enlargement of the testicular interstitial spaces.

2.6.1.2 The reproductive toxicity of PFASs in Daphniidae. *Daphniidae* are invertebrate crustacean zooplankton widely distributed in various freshwater ecosystems, where they play a crucial role. Due to their sensitivity, *Daphniidae* are commonly used as model organisms in aquatic toxicology research.[106] Several studies have exposed *Daphniidae* to PFASs to investigate their reproductive toxicity.

Ji *et al.*[84] simultaneously studied two model organisms, *Daphnia magna* and Moina macrocopa, and found that for *Moina macrocopa*, exposure to PFOS (>0.31 mg L^{-1}) and PFOA (>6.25 mg L^{-1}) resulted in a decrease in offspring numbers. For *D. magna*, exposure to high concentrations of PFOS (5 mg L^{-1}) and PFOA (>12.5 mg L^{-1}) delayed the time required for the first batch of offspring to be produced and reduced the number of offspring. The toxic effects of PFOS were observed at lower concentrations in both species compared to PFOA. Liang *et al.*[107] selected *D. magna* for their study and found that exposure to 8 mg per L PFOS for 21 days significantly inhibited several reproductive endpoints, including the time to produce the first batch of offspring, the number of offspring in the first brood, and the intrinsic natural growth rate. These effects threaten the survival and reproduction of the *Daphnia* population. This is consistent with the findings of Ji *et al.* Another study[108] on *D. magna* found that exposure to 10 and 25 μM PFOS, as well as 25 μM PFOA, significantly reduced reproductive capacity. The toxicity of PFOS during this life stage was greater than that of PFOA. Additionally, the downregulation of genes involved in development and reproduction, such as *vtg2*, *vasa*, *EcRA*, and *EcRB*, offers mechanistic insights into the potential causes of the observed decline in fertility.

2.6.2 The reproductive toxicity of PFASs in other terrestrial organisms. Fruit flies are one of the most common model organisms in the field of biology. A study by Kim *et al.*,[109] using *Drosophila*, found that, compared to the control group treated with acetone, the average egg-laying number of females exposed

to 2 ng PFOS was significantly reduced, with a 57% decrease observed 5 days after exposure, indicating a significant decline in fertility. Furthermore, female adults that laid eggs early following PFOS exposure showed a notable reduction in body weight. A study by Narizzano *et al.*[110] on white-footed mice found that prenatal exposure to high doses of PFOS (5 mg kg^{-1} d^{-1} for 7 days) resulted in neonatal mortality.

3. Reparative effects of certain substances on PFAS-induced reproductive toxicity

In addition to the reproductive toxicity of PFASs on model organisms mentioned above, some studies have also investigated the potential role of other substances in mitigating PFAS toxicity. For mice, Zhang *et al.*[35] found that icariin effectively alleviated PFOS-induced testicular toxicity by reducing Sertoli cell damage and downregulating the p38MAPK/MMP9 pathway. Liang *et al.*[36] found that 1α,25-dihydroxyvitamin D3 intervention could mitigate PFOS-induced reproductive damage in male mice through the Nrf2-mediated oxidative stress pathway, significantly improving the decline in sperm quality and testicular damage caused by PFOS exposure.

As for rats, Tian *et al.*[61] found that, compared to the PFOS-only exposure group, the PFOS + SAM group exhibited a significant increase in the pregnancy rate of F_0 females and the survival rate of F_1 offspring. Combined exposure also enhanced the proliferation of spermatogonia and stem cells, alleviated structural damage to testicular tissue, and improved intergenerational growth retardation and infertility induced by chronic PFOS stress. Since SAM is a methyl donor, it is suggested that regulating DNA methylation levels could be a potential strategy for preventing and treating the epigenetic toxicity of PFOS. Umar Ijaz *et al.*[12] identified a substance called pachypodol, which can scavenge free radicals and exhibit antioxidant properties. In rats exposed to PFOS alone, the numbers of spermatogonia, spermatocytes, and both primary and secondary spermatocytes were significantly reduced. In the co-exposure group, supplementation with pachypodol nearly completely restored the abnormalities in the testicular structure and the number of reproductive cells. Moreover, pachypodol reversed the decrease in CAT, SOD, GPx, and GSR activities induced by PFOS, significantly reducing the elevated ROS and TBARS levels associated with PFOS exposure. This suggests that pachypodol exhibits free radical scavenging activity, which helps mitigate testicular dysfunction induced by PFOS. These findings offer new insights into the mechanisms by which PFASs impact the reproductive system and provide theoretical support for the development of treatments for the toxicity of perfluorinated compounds in the future.

4. Discussion

Some epidemiological studies suggest that exposure to PFASs may be linked to reduced testicular volume,[111] decreased sperm quality and lower T levels in men,[112,113] while in women, it has been associated with hormonal imbalances, an increased risk of infertility, and irregular menstrual cycles.[114,115] PFAS exposure during pregnancy may also be associated with an increased risk of preterm birth.[116] In a study conducted among Taiwanese adolescents, a significant inverse dose–response relationship was observed between PFAS concentrations and levels of FSH and testosterone in females aged 12 to 17 years.[117] These toxic effects closely align with the results observed in experiments on model organisms. Since direct toxicity testing on humans is not possible, the findings from model organism studies will play a crucial role in guiding further research on potential toxic effects in humans.

According to the CRED guidelines,[118] the exposure methods employed in these studies are appropriate. While some studies used relatively high doses—such as PFOA at 100 mg per kg per day—these concentrations remain well below the solubility limit of PFOA (1.37×10^{-2} mol L^{-1}). Moreover, no studies reported exposure gradients exceeding a 10-fold range. We compiled and reviewed all the studies identified in this article and found that a total of 16 PFASs have been shown to exhibit reproductive toxicity in model organisms. As shown in Fig. 4, the two PFASs, PFOS and PFOA, have been the most widely studied, with researchers examining these substances in all model organisms. The reproductive toxicity associated with these compounds is observed in each of the model organisms. PFOS can induce histological damage to ovaries in female mice, resulting in reduced ovulation and impaired oocyte development. In male mice and rats, it can lead to changes in the GSI, reduced sperm quality and quantity, and disruption of the BTB. Similarly, PFOS can cause gonadal damage in fish, with females exhibiting histological damage to gonads, changes in the GSI, and reduced fertility, while males experience delayed gonadal development and poor sperm quality. From a hormonal perspective, exposure to PFOS leads to varying degrees of changes in sex hormone levels in both rodents and fish. As for the effects on offspring, the transgenerational toxicity of PFOS spans multiple model organisms, resulting in reduced fertility, increased mortality and deformity rates in offspring, as well as alterations in offspring sex ratios.

Excluding PFOS and PFOA, research on PFNA, PFBS, and HFPOs is also relatively abundant. The reproductive toxicity they induce does not have a specific focal point, but is observed at the tissue, cellular, hormonal, and offspring levels. Research on other PFASs is relatively limited. Some, such as PFUnA and F-53B, exhibit a broad range of effects, while others, like PFDoA and PFTrDA, focus on one or two aspects of reproductive toxicity. Notably, these substances do not appear to affect sex hormones, despite their studies being conducted on rats, which are a suitable model for investigating hormone changes. There is no data on the reproductive organ toxicity of model organisms for 6:2 FTOH, 6:2 FTAA, and 6:2 FTAB. In contrast, PFHxS and Cl-PFESA primarily exhibit toxicity to the gonads of model organisms, while PFDA and NBP2 primarily affect the sex hormone levels in these organisms. PFHpA, on the other hand, has only been studied for its toxicity in offspring. However, these findings may be related to the structure and functional

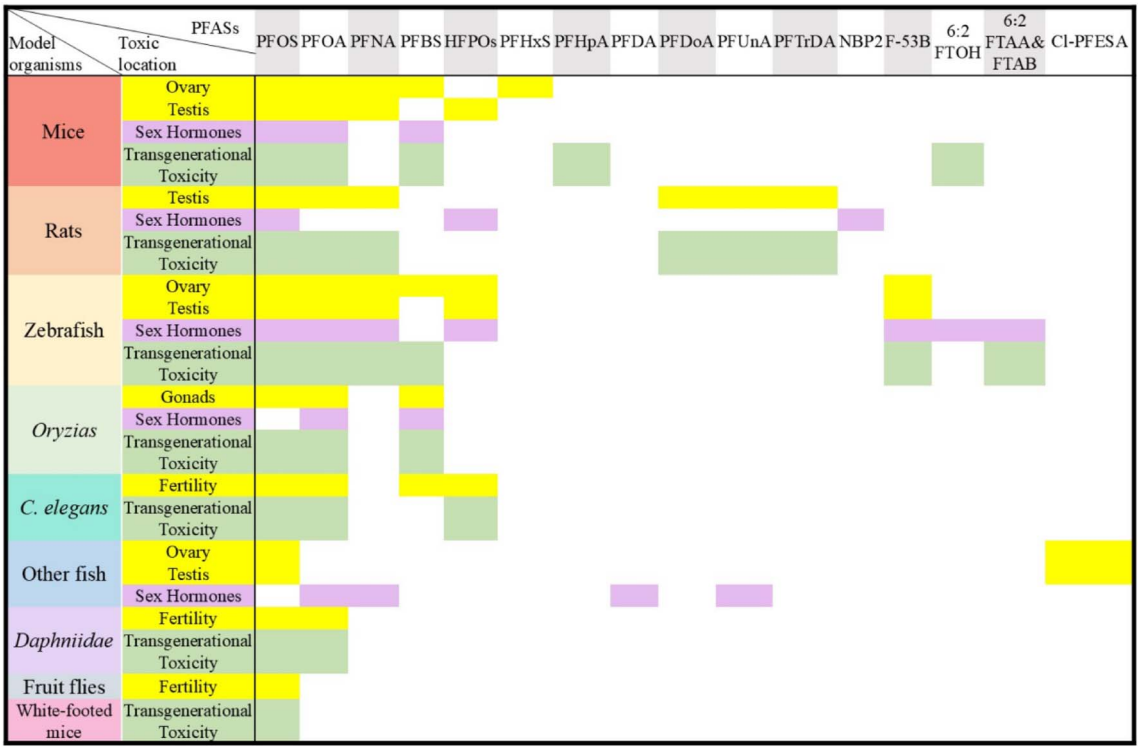

Fig. 4 Schematic summary of reproductive toxicity induced by PFASs in all model organisms.

groups of different PFASs or could reflect data gaps due to insufficient research.

From the dose–response perspective, long-chain PFASs within the same class generally exhibit greater toxic potency, typically reflected by lower LOAEL values. Studies on mice show that the LOAEL values of perfluoroalkyl carboxylic acids (PFCAs) with carbon chain lengths of 4, 6, and 8 are 200, 62.5, and 0.1 mg per kg per day, respectively, indicating a progressive decrease with increasing chain length. Functional groups are also key determinants of PFAS toxicity at equivalent carbon chain lengths. Taking the most common PFASs, PFOS and PFOA, as examples: both have the same carbon chain length, but due to the presence of a sulfonic acid group, PFOS exhibits greater toxic effects than PFOA, which contains a carboxylic acid group. Based on experimental evidence of gonadal damage in female mice, the LOAEL of PFOS (0.1 mg per kg per day) is lower than that of PFOA (1 mg per kg per day), indicating higher reproductive toxicity potential. At equivalent doses, the toxic potency of PFOA is generally lower than that of PFOS, a pattern observed in both *Oryzias* and *Daphnia magna*. Moreover, although all three compounds reduce the gonadosomatic index (GSI) in female zebrafish, their lowest observed adverse effect levels (LOAELs) differ significantly: 100 mg L^{-1} for PFOA, 50 μg L^{-1} for PFOS, and only 5 μg L^{-1} for F-53B. These results demonstrate that PFAS toxicity is jointly influenced by both carbon chain length and functional group characteristics. Clarifying their dose–response relationships is essential for effectively prioritizing the regulation of high-risk PFASs.

In terms of model organism selection, for commonly used mammalian model organisms, such as mice and rats, exposure is typically administered through controlled methods like gavage or oral feeding. Given the relatively short exposure duration, the doses are usually set at laboratory concentrations, often in the milligram range, which are much higher than the concentrations animals would typically encounter through food or water. As a result, there is a lack of experimental data at realistic environmental concentrations. However, since both species are mammals, their evolutionary relationship to humans is closer than that of fish or *Caenorhabditis elegans.* The reproductive cycles of both rodents and humans are regulated by sex hormones, with several signaling pathways conserved between the two, making them ideal models for studying various diseases or metabolic pathways.

Although the two fish model organisms are distantly related to humans, the experimental approach involves direct exposure in aquatic environments, making it more representative of natural conditions. Moreover, due to their low cost and ease of maintenance, many researchers opt for long-term exposures lasting five to six months. In these studies, the concentrations are typically much lower, usually in the microgram range and sometimes even in the nanogram or picogram ranges. In addition, due to the short reproductive cycles and the large number of offspring produced by fish, which facilitate statistical analysis, some researchers have observed changes in offspring numbers and sex ratios caused by PFAS exposure. Therefore, zebrafish and *Oryzias* serve as effective platforms for assessing sex bias, environmental dose evaluations, and sub-chronic to chronic toxicity tests. Additionally, their high-throughput screening capabilities enable low-cost monitoring of transgenerational toxic effects.

Caenorhabditis elegans, a hermaphroditic model organism, is widely used in reproductive toxicity studies, with a primary focus on fertility and the impact on offspring. As a representative model for soil organisms, *Caenorhabditis elegans* offers the advantages of a short life cycle and simple exposure procedures. Many researchers opt for a 48-hour exposure period in their studies, which makes it an ideal model for short-term experiments. However, due to its relatively simple physiological structure, it is not an ideal platform for studying the changes in reproductive hormone levels and metabolism.

Therefore, in subsequent studies, different model organisms can be selected based on specific toxicity endpoints or types of pollutants. For instance, fish species may be chosen for research on waterborne dosages, offspring quantity, and sex ratios, or for chronic toxicity studies. To investigate the repair effects of gonadal damage, reproductive toxicity, and metabolic mechanisms and to assess hormonal homeostasis, rodent models can be selected. For studies on soil contamination or acute toxicity testing, where sex differences are not a primary concern, *Caenorhabditis elegans*, a hermaphroditic organism with a short life cycle, may be chosen. The above summary offers theoretical guidance for future researchers studying reproductive toxicity, assisting them in selecting appropriate model organisms based on the specific toxicity type or effect endpoints they intend to investigate.

However, in summarizing the current research, several limitations were identified. First, existing research has predominantly focused on two legacy PFASs—PFOS and PFOA—which were widely used but have now been phased out. In comparison, their substitutes, emerging PFASs, and other fluorinated alternatives with undefined classifications have received considerably less attention. Moreover, considering the actual exposure levels of PFASs in human environments, as well as human-specific metabolic pathways, it is inappropriate to directly extrapolate toxicological findings from model organisms to humans. How to reasonably and effectively extrapolate toxicological effects observed in model organisms to epidemiological outcomes remains a key area for future investigation. Researchers have predominantly focused on commonly used laboratory model organisms, while studies involving primates and other large mammals—species more closely related to humans—remain notably scarce. Finally, most existing studies have focused on the reproductive toxicity of individual PFASs. However, real-world environmental exposures are far more complex, involving potential combined toxicities or even antagonistic effects. Traditional statistical approaches are insufficient to address the complexity and uncertainty of exposure patterns, high intercorrelations, and the intricate interactions among multiple compounds.

Based on the shortcomings mentioned above, future research on the reproductive toxicity of PFASs in model organisms should place greater emphasis on the following areas: (1) placing greater emphasis on emerging PFASs and novel PFAS alternatives; (2) focusing on female model organisms, as research in this area is relatively scarce and their effects on offspring are more direct; (3) expanding the selection of model organisms beyond existing models, and opting for species more closely related to humans, would provide valuable insights into the toxicity and metabolic pathways of PFASs in the human body; (4) focusing on the realistic concentrations to which humans may be exposed, as well as exploring the potential for complex interactions involving combined toxicity or remediation effects in the environment.

5. Conclusion

This review provides a comprehensive overview of recent studies investigating the reproductive toxicity of PFASs, with model organisms serving as experimental platforms. By categorizing the literature according to five commonly used classes of model organisms, it was found that in higher-order mammals such as mice and rats, as well as in zebrafish and *Oryzias*, PFAS exposure primarily induces histopathological damage to reproductive organs, disruption of sex hormone levels, and developmental abnormalities in offspring. For the lower-order model organism *Caenorhabditis elegans*, PFAS exposure has been shown to affect reproductive capacity and offspring health. In subsequent studies, the selection of an appropriate model organism should be determined by the specific toxicological endpoints of interest, along with considerations of exposure dose, duration, and overall experimental design.

In conclusion, this review provides a comprehensive synthesis of the reproductive toxicity of PFASs across various model organisms. It offers practical guidance for selecting suitable models in future toxicological research, theoretical support for developing potential therapeutic strategies, and a scientific basis to inform future regulatory policies.

Author contributions

Ran Tao: investigation, writing – original draft. Mingliang Sun: writing – review and editing. Jiateng Ma: writing – review and editing. Jiali Li: writing – review and editing. Xinni Yao: writing – review and editing. Minjie Li: conceptualization, supervision, writing – review & editing. Lianghong Guo: conceptualization, supervision, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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