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Review

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Small fish making a big difference: beloved star of environmental toxicology research in the current era

Yang JIANG[1,2], Zhen SU[2], Jing ZHENG[1], Chih-Hung HSU[1]✉, Ye CHEN[1,2]✉

[1]*Department of Genetic and Metabolic Disease, The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, Hangzhou 310003, China*
[2]*College of Life Sciences, Key Laboratory for Cell and Gene Engineering of Zhejiang Province, Zhejiang University, Hangzhou 310058, China*

Abstract: The zebrafish has emerged as a powerful model organism in life science owing to its remarkable biological characteristics and wide-ranging applications. This review provides a comprehensive overview of the recent advancements in research on zebrafish within the field of environmental toxicology, highlighting specific studies where this species was used to investigate various pollutants to elucidate their impacts and underlying mechanisms. The findings of these studies underscore the significant potential of zebrafish as a model to gain crucial insights into the ecological consequences of environmental contamination and toxicity pathways. By incorporating cutting-edge technologies such as artificial intelligence (AI), high-throughput screening, and omics approaches, the use of zebrafish as a model organism is poised to significantly accelerate toxicological investigations, promote environmental conservation efforts, contribute to safeguarding human health, and advance sustainable development objectives.

Key words: Zebrafish model; Environmental toxicant; Environmental toxicology

1 Introduction

In recent decades, environmental science has become increasingly important for addressing global environmental issues and now plays a leading role in scientific research. The primary objective of this discipline is to promote the sustainable use of Earth's resources and maintain ecosystem stability over time, fostering the harmonious coexistence of humans and nature (Ma R et al., 2023). However, the introduction of novel organic pollutants into aquatic ecosystems presents unprecedented challenges, exacerbating water pollution and posing significant threats to both natural ecosystems and human well-being (Tozer, 2023). To address these issues, research has turned to the zebrafish (*Danio rerio*), which is considered a highly promising model organism due to its unique biological characteristics, genetic similarity to humans, and suitability for studying the effects of environmental contaminants on overall organismal health. Given its extensive adoption across various research fields and growing importance in environmental science, the zebrafish model plays a crucial role in assessments of the toxicological impact of chemical pollutants on aquatic organisms (Jin MQ et al., 2018). In this review, we provide a concise overview of toxicological studies using zebrafish conducted by diverse research teams since the beginning of the 21st century. The aim was to provide an insightful understanding of this rapidly evolving field while also suggesting potential avenues for future advancements.

2 Zebrafish as an indispensable tool for contemporary scientific inquiry

2.1 Advantages of zebrafish models

During recent decades, the use of zebrafish in life science research has significantly increased due to its numerous advantages. Firstly, zebrafish share a high degree of genetic and physiological similarity with mammals, with most of their approximately 26 000

✉ Ye CHEN, yechency@zju.edu.cn
Chih-Hung HSU, ch_hsu@zju.edu.cn
Ye CHEN, https://orcid.org/0000-0003-3671-2504
Chih-Hung HSU, https://orcid.org/0000-0001-8071-0122

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genes having human counterparts, making this fish an ideal model organism for studying pathophysiological and toxicological mechanisms that may be conserved across species. Secondly, zebrafish embryos are transparent, enabling real-time observations of processes such as development, organ formation, and drug distribution. Thirdly, this species is characterized by a rapid reproduction rate, with a short generation time and a large number of offspring produced, which facilitates high-throughput screening of chemical compounds and the study of toxicological responses within a relatively short timeframe (Lin et al., 2016). Fourthly, embryos develop quickly and are well-suited for studying the effects of toxins at various stages of development. This allows for the assessment of how exposure to chemicals at specific time points can impact organogenesis, neurodevelopment, and other critical processes (Bugel et al., 2014). Finally, by leveraging their diverse behaviors, zebrafish can be used as an excellent platform for studying the effects of chemicals or gene mutations and elucidating their underlying mechanisms at the same time (Spence et al., 2008). Moreover, zebrafish are easily subjected to genetic manipulation techniques such as gene knockdown or knock-in, allowing the creation of specific mutant lines for investigating the role of individual genes in toxicological responses (Jia et al., 2024). Overall, owing to these advantages, the zebrafish model offers valuable insights into complex pathophysiological and toxicological mechanisms (He et al., 2023). It also assists in identifying critical pathways involved in various physiological and pathological phenotypes, providing knowledge that may be relevant for understanding human health impacts.

2.2 Experimental techniques and methods frequently used in zebrafish models

The versatility and robustness of zebrafish as a model organism in studies of environmental pollution derive from the diversity of experimental techniques and methodologies that can be applied to it, facilitating the comprehensive analysis of biological and toxicological processes.

2.2.1 Gene editing techniques

Gene editing techniques, such as the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system, have revolutionized genetic research using zebrafish as a model organism (Jiang et al., 2024). This state-of-the-art technology enables precise and efficient modifications of the zebrafish genome, allowing investigations into specific gene functions and related disease models with unprecedented accuracy. Moreover, by using homologous recombination or other innovative approaches, it is possible to introduce foreign genes or markers into targeted locations within the zebrafish genome. These techniques facilitate studies on protein expression patterns, cell fate tracking, and functional analyses (Thisse and Thisse, 2008).

2.2.2 Real-time imaging

The transparency that characterizes zebrafish embryos and larvae provides an ideal environment for real-time imaging, a fundamental technique used in zebrafish research. Advanced live imaging methods, such as confocal and two-photon microscopy, offer valuable insights into the dynamic processes occurring at the cellular and tissue levels within transparent zebrafish tissues. When combined with time-lapse photography, these techniques allow continuous documentation of developmental processes from the early embryonic stages to adulthood, facilitating the meticulous tracking of specific cell or tissue developmental pathways (Tomer et al., 2012). For instance, in studies on vascular development, the formation of new blood vessels and alterations in blood flow can be observed by labeling vascular cells with fluorescent proteins and using time-lapse imaging techniques (Lawson and Weinstein, 2002).

2.2.3 Behavioral analysis

The compact size of zebrafish larvae makes them highly suitable for chemical screening, as they can be conveniently placed in microplates containing a diverse range of drugs or chemicals, and impacts on behavior can be subsequently evaluated using automated analysis techniques (MacRae and Peterson, 2015). The implementation of high-throughput screening has facilitated the development of automated behavior-tracking systems that quantify responses to environmental changes or drug treatments, including startle responses, social preferences, memory retention, learning ability (Spence et al., 2008), and exploration tendencies (Kalueff et al., 2014).

3 Zebrafish as a model organism for investigating the effects of environmental pollutants

As a prominent model organism in environmental toxicology, the zebrafish has been used in numerous studies in this field over recent decades. Fig. 1 illustrates a comprehensive literature search conducted on the Web of Science platform using keywords such as "zebrafish" and "environmental toxicology" or "environmental pollutants." A total of 4125 articles published between 2001 and 2023 were retrieved and, throughout this period, a consistent year-on-year increase was observed in both the variety and quantity of pollutants examined (Fig. 1). A comprehensive analysis of these research papers resulted in the identification of six distinct categories into which pollutants can be broadly classified: endocrine disruptors, organic pollutants, heavy metals, pesticides, pharmaceuticals and personal care products (PPCPs), and nanomaterials. Among these, the most extensively studied were organic pollutants, followed by PPCPs and endocrine disruptors. Overall, efforts to investigate all six pollutant categories noticeably increased during 2001–2023. Among the endocrine disruptors (Fig. 2), bisphenol compounds and brominated flame retardants were the most extensively studied, which reflects their significant impacts on the environment and health. Additionally, research on antibiotics was substantial. Emerging pollutants such as perfluorinated compounds and nanomaterials, including nanoplastics (NPs) and metal nanoparticles, also attracted considerable attention, along with heavy metals and organophosphorus compounds, albeit to a lesser extent. These data revealed the broad interest and concern of the scientific community about the physiological and ecological impacts of pollutants, underscoring the need for further research on these chemical substances.

3.1 Studying different types of pollutants using the zebrafish model

The zebrafish model offers a versatile and powerful tool for studying the complex effects of environmental pollutants (Fig. 3). Ranging from impacts on development to molecular mechanisms, research on zebrafish is advancing our understanding of how different pollutants influence aquatic life and human health.

3.1.1 Endocrine disruptors

The zebrafish has emerged as a valuable tool for investigating the impacts of endocrine-disrupting chemicals (EDCs) on various biological systems. Recent studies have focused mainly on several pivotal EDCs, including bisphenol A (BPA) and its analogs, such as bisphenol S (BPS), bisphenol F (BPF), and other compounds like tebuconazole and dydrogesterone (refer to Table S1 for further details). BPA and its analogs have been found to exert significant effects on both zebrafish embryos and adults through multiple mechanisms, including alterations in cytotoxicity, modulation of gene expression, developmental toxicity, regulation of the gut–brain axis, and modifications in epigenetic patterns. The presence of BPA compounds has been shown to induce substantial vitellogenin expression (Tarafdar et al., 2022) and influence genes associated with neurogenesis and heart development, as well as contributing to inflammatory responses in intestinal epithelial cells (Zhao et al., 2024). Moreover, exposure

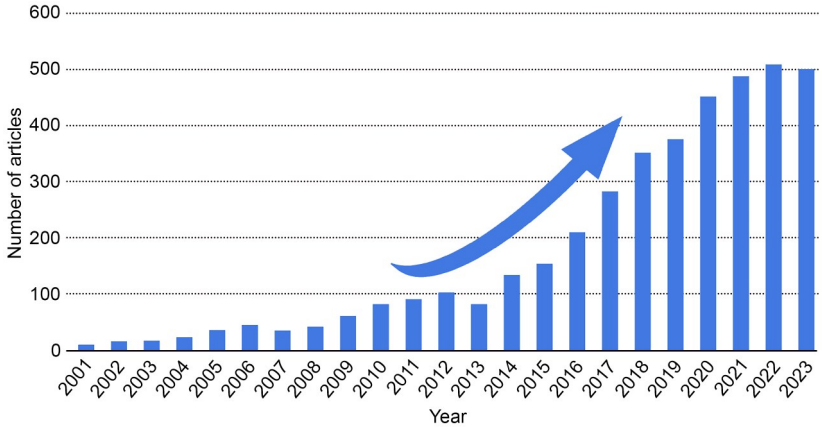

Fig. 1 Rapid increase in environmental toxicology research using zebrafish models.

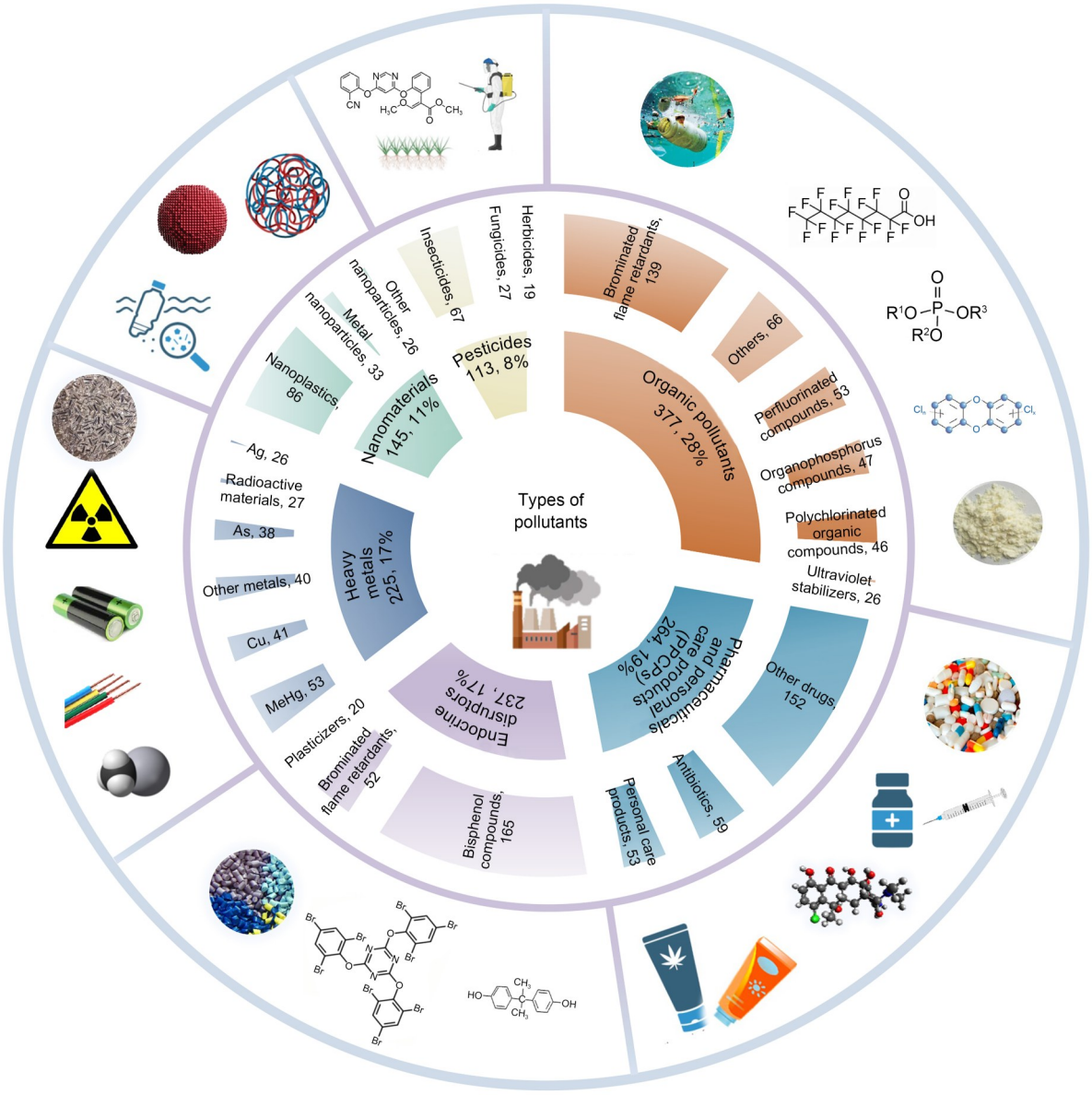

Fig. 2 Proportions of different types of contaminants in zebrafish models for aquatic ecotoxicology. Proportional distribution of six pollutant categories in zebrafish studies (2001–2023) is shown, with publication counts labeled per category.

to BPA has been implicated in persistent abnormalities in otolith development independent of estrogen receptor activity, suggesting the involvement of non-estrogenic pathways (Qiu et al., 2021). BPS and similar compounds exhibit a spectrum of toxic responses that include effects on estrogen levels, cellular toxicity, genetic damage, nerve impairment, and disruption of the immune system. They also interfere with thyroid hormone signaling and affect neurological behaviors. Exposure to low levels of BPS affects the endocrine system and reproductive function in zebrafish, impairing offspring development (Qiu et al., 2018). Moreover, due to their estrogenic and anti-androgenic properties, these compounds have been associated with abnormal cardiovascular structure, reduced heart rates, and alterations in organ weights and sex hormone levels (Song et al., 2020). Exposure to BPF has been linked to significant neurotoxicity in zebrafish embryos by impairing the development of peripheral motor neurons without directly affecting neurotransmission (Qiu et al., 2019). Furthermore, such exposure can induce cognitive dysfunction through complex mechanisms involving modifications in specific types of neural cells and inflammatory responses (Mu et al., 2022). Other EDCs

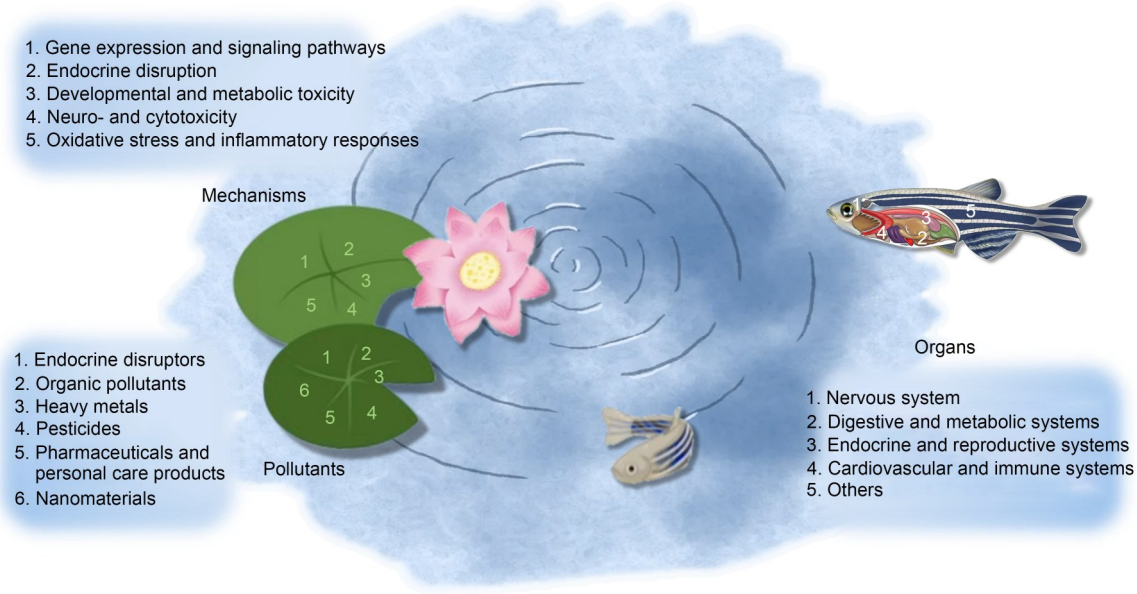

Fig. 3 Zebrafish model as a versatile and robust tool for investigating the intricate impacts of environmental pollutants.

investigated using the zebrafish model include tetrabromobisphenol A (TBBPA), tebuconazole, and dydrogesterone. Exposure to environmentally relevant concentrations of TBBPA has been shown to affect the mitochondrial function of neuroendothelial cells mediated by mitochondrial reactive oxygen species (mitoROS) signaling, thereby impairing cerebral and ocular angiogenesis (Chen et al., 2016; Zhu BR et al., 2022). Tebuconazole, which is known for its capacity to induce male-biased sex differentiation, inhibits aromatase activity and reduces estrogen production, consequently affecting reproductive outcomes (Qiao et al., 2023). Moreover, compounds such as boron dipyrromethene (BDP), 2,4-dichlorophenol (2,4-DCP), polychlorinated biphenyls (PCBs), propylthiouracil (PTU), and synthetic progestins have also shown disruptive effects on physiology and endocrine axes in zebrafish by influencing key developmental processes and metabolic pathways. For instance, dydrogesterone, a synthetic progestin, has been shown to skew the gender ratio toward males within populations while simultaneously accelerating sperm maturation, thus posing potential ecological risks (Shi et al., 2022).

3.1.2 Organic pollutants

Organic pollutants comprise diverse chemical compounds, including pesticides, industrial chemicals, and by-products generated during industrial processes. These pollutants can pose significant risks to human health and the natural environment. Persistent organic pollutants (POPs) are a particularly concerning subset of organic pollutants that are widely used in agriculture, industry, and manufacturing and are released into the environment. Due to their persistent nature and capacity for bioaccumulation within living organisms, they can exert long-lasting and extensive impacts (refer to Table S2 for further details).

Polybrominated diphenyl ethers (PBDEs), such as 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) and 2,2',4,4',5-pentabromodiphenyl ether (BDE-99), are also widely found in the environment due to their application as flame retardants (Huang et al., 2023). Studies have indicated that exposure to PBDEs has negative effects on neurodevelopment and thyroid function in zebrafish. For instance, even at low concentrations, these pollutants can disrupt thyroid hormone levels in parental zebrafish and impact offspring growth through maternal transfer, thereby demonstrating transgenerational toxicity (Wang et al., 2023). Moreover, PBDEs interfere with thyroid hormone signaling pathways, leading to impaired visual development. Specifically, compounds like BDE-99 affect the expression of genes crucial for visual function (Zhuang et al., 2020). Polycyclic aromatic hydrocarbons (PAHs), commonly found in crude oil, are potent aryl hydrocarbon receptor (AHR) activators that induce significant developmental toxicity in zebrafish embryos. Exposure to PAHs disrupts genes involved in phototransduction

pathways, which are crucial for visual signal transduction, consequently impairing visual function in zebrafish larvae (Wilson et al., 2023). Prolonged exposure to these pollutants leads to morphological and behavioral abnormalities, which highlights their profound impact on aquatic life (Philibert et al., 2021). PCBs are persistent environmental pollutants known for their neurotoxicity, ability to induce apoptosis, and inflammatory properties. In zebrafish, exposure to PCBs triggers intestinal inflammation, oxidative stress, and disruptions in lipid metabolism. Specifically, studies have shown that these pollutants suppress microbial activities associated with the metabolism of primary bile acids in the intestine, increasing their concentrations and causing subsequent metabolic disturbances (Xu et al., 2018). The effects of tetrabutylphosphonium hydroxide (TBPH), which is commonly detected in environmental matrices due to its presence in plastics and electronic materials, are due to chronic exposure and include enhanced visceral adiposity and potential hepatic dysfunction. Studies on zebrafish have revealed that TBPH induces regional DNA demethylation (Liu et al., 2022), exacerbates the progression of nonalcoholic fatty liver disease, and disrupts lipid metabolism (Zhou et al., 2021), possibly exerting transgenerational effects (Li JY et al., 2024). Benzotriazole ultraviolet (UV) stabilizers and decabromodiphenyl ethane (DBDPE) also have significant impacts, as they cause embryonic mortality and substantial disruptions in hepatic tissue regeneration (Liang et al., 2019; Hua et al., 2022; Li G et al., 2024). Furthermore, short-chain chlorinated paraffins have been shown to interfere with the genetic material and disrupt energy metabolism in zebrafish embryos, which highlights their potential for bioaccumulation and toxicity (Liu LH et al., 2016). Moreover, per- and polyfluoroalkyl substances (PFASs) have been implicated in the alteration of immune cells and disruption of thyroid hormone homeostasis, indicating broader environmental and health concerns (Huang et al., 2022). Finally, exposure to di-2-ethylhexyl phthalate (DEHP) has been shown to alter microbial metabolites that modulate intestinal and immune responses, significantly upregulating gene networks associated with helper T cell pathways in zebrafish (Santangeli et al., 2017).

3.1.3 Heavy metals

The zebrafish has emerged as a key organism for understanding the impacts of heavy metal pollution on aquatic organisms and the environment. Specifically, extensive insights have been gained regarding the effects of arsenic, copper, cadmium, selenium, and mercury, as well as the biological impacts of radiation (refer to Table S3 for further details). In zebrafish, exposure to copper has been shown to significantly increase its accumulation in the gills, liver, and intestines, leading to oxidative damage (Hsiao et al., 2020), while arsenic contamination has been shown to substantially decrease reproductive output by affecting spawning, hatching, and egg production (Chen L et al., 2022). Studies have also revealed the profound impacts of cadmium and selenium on neurodevelopment. Exposure to cadmium induces structural and functional alterations in the olfactory epithelium, including reduced numbers of olfactory receptor cells and abnormal morphology (Zhang R et al., 2020), while elevated selenium levels trigger oxidative stress and disrupt dopaminergic neurotransmission, resulting in cognitive impairment (Zhu et al., 2023). Zebrafish exhibit heightened sensitivity to selenium-induced developmental toxicity, which leads to enduring impairments in swimming performance, increased oxygen consumption, and altered metabolic rates (Thomas and Janz, 2015). Exposure to methylmercury has transgenerational effects, as lifelong parental exposure to environmentally relevant concentrations can lead to hyperactivity and increased foraging efficiency in offspring (Debofsky et al., 2018; Guo SJ et al., 2023). This pollutant disrupts the functions of the central nervous system by perturbing cellular homeostasis and altering gene expression in various tissues, including the brain and skeletal muscles (Zhang QL et al., 2020).

In addition to heavy metals, studies have explored the implications of radiation exposure in zebrafish. For instance, cesium-137, a radioactive isotope with a prolonged half-life, raises significant environmental concerns. Embryos exposed to this isotope show notable chromosomal abnormalities and cellular death, highlighting the suitability of the zebrafish model in investigations of the effects of radiation on cellular and genetic structures during early development (Asana Marican and Shen, 2024). Similarly, other rare metals, such as indium and indium tin oxide, have been shown to induce endoplasmic reticulum stress and oxidative stress responses in zebrafish, leading to apoptosis and inflammatory reactions (Brun et al., 2014).

3.1.4 Pesticides

Pesticides are extensively used in agriculture, yet they can exert substantial adverse effects on environmental and biological health. Recent studies using zebrafish as a model organism have yielded invaluable insights into the impacts of various pesticides on aquatic life and ecosystems (refer to Table S4 for further details). For instance, tebuconazole, a widely used fungicide, has been shown to affect thyroid and liver functionality in embryos (Li et al., 2020) and alter developmental gene expression, highlighting the potential long-term consequences of exposure even during early life stages (Liu N et al., 2016). Studies have also revealed that this pesticide induces male-biased sex differentiation in zebrafish by inhibiting aromatase activity, reducing the levels of estrogen production, and modulating gene expression associated with sex differentiation (Qiao et al., 2023). Moreover, in aquatic environments, neonicotinoid insecticides, such as imidacloprid and thiamethoxam, exhibit high water-solubility and persistence. Research on zebrafish has provided evidence for the potential impact of these chemicals on fecundity, sex hormone levels, and gonadal development. Furthermore, it has been shown that long-term exposure to environmentally relevant concentrations of indoxacarb, another neonicotinoid, leads to significant endocrine disruption and altered reproductive capabilities (Ma LL et al., 2023). Another study reported that exposure to environmentally relevant concentrations of azoxystrobin (AZO), a strobilurin fungicide, resulted in long-term effects in zebrafish, including delayed sexual differentiation, disrupted gonadal development, and impaired reproductive health. Importantly, AZO exposure has potential transgenerational effects that can impact the health and development of subsequent generations (Guo XJ et al., 2023). The herbicide glyphosate has been shown to decrease egg production, potentially by inhibiting oogenesis, suppressing ovulation, or increasing oocyte apoptosis. Glyphosate disrupts the pathways regulating steroidogenesis and induces oxidative stress, thereby affecting the reproductive ability (Lu et al., 2022), while cholinergic pesticides can induce excessive stimulation of nerve cells, resulting in paralysis and mortality. Nevertheless, most zebrafish larvae exhibit rapid recovery as exposure ceases, indicating a certain degree of resilience to short-term exposure (Zhang et al., 2021). Moreover, the emergence of nanopesticides such as copper hydroxide poses novel challenges to aquatic ecosystems. Investigations on zebrafish have revealed that this pollutant can alter larval behavior, for example causing hypoactivity and modified responses to light stimuli, which suggests neurotoxic effects (Aksakal and Sisman, 2020). It has also been shown to lead to elevated mortality rates and deformities in zebrafish embryos compared to control groups while increasing copper accumulation at the same time (Wang XH et al., 2021).

3.1.5 Pharmaceuticals and personal care products

Growing concern regarding the impact of PPCPs on aquatic ecosystems has generated significant interest in understanding their diverse effects. Various studies have elucidated the profound influence of PPCPs on zebrafish, providing insights into their effects on neurological and reproductive systems as well as on development in aquatic organisms (refer to Table S5 for further details). Antidepressants and antibiotics have been shown to negatively affect aquatic animals through neurotoxicity, cytotoxicity, and disruption of the gut microbiota. Diazepam has been shown to disrupt behavior and nervous system function in zebrafish (Zhao et al., 2022). Moreover, even environmentally irrelevant concentrations of antibiotics can impair reproductive and developmental outcomes in this species by reducing survival rates, hatching success, and developmental parameters. These effects may persist across generations, as evidenced by observations made of the F2 generation, suggesting potential epigenetic influences or other enduring consequences caused by initial exposure (Xu et al., 2024). Similarly, studies of corticosteroids, which are present in aquatic environments as a result of natural excretion and medical usage, have revealed disruptive effects on glucose metabolism, immune response, and circadian rhythms in zebrafish, thereby emphasizing their ecological implications (Willi et al., 2019). Reports on environmental chemicals that exhibit endogenous estrogen-like properties have raised concerns regarding abnormalities in the male reproductive system, including testicular and gonadal anomalies. Synthetic progestins and antiprogestins have been shown to significantly modulate gene expression during early developmental stages in zebrafish, potentially impeding brain and gonadal differentiation (Coe et al., 2010), while exposure to 17α-ethinylestradiol has been reported to disrupt male sexual development and alter adult population

dynamics (Coe et al., 2008; Lin et al., 2023). Drospirenone and progesterone in particular interfere with the endocrine system, influencing gene transcription and physiology even at environmentally irrelevant concentrations (Schmid et al., 2020). UV filters, which are commonly present in personal care products, have shown adverse effects on embryo development, specifically on the heart rate, hatching rate, and metabolic enzyme activity. Certain UV filters, such as UV-328, can impede crucial signaling pathways for cell cycle regulation and the DNA damage response (Zhang L et al., 2023). Additionally, the accumulation of the antidiabetic drug metformin in zebrafish tissues has been documented to significantly influence growth, reproductive capabilities, and survival rates while also altering DNA methylation patterns, which is indicative of epigenetic toxicity (de Oliveira et al., 2019). Other pharmaceuticals investigated in zebrafish include: citalopram, which elicits neurotoxic effects, impacting motor function, learning ability, and memory performance (Zindler et al., 2020); oxazepam, whose persistence in aquatic environments induces behavioral tolerance to its anxiolytic properties (Vossen et al., 2020); niclosamide, a chemical compound used for medical and agricultural purposes that accumulates in the liver and brain, suppressing body growth and influencing lipid metabolism (Chen et al., 2024); and boscalid, a fungicide found in surface waters that causes reproductive toxicity in individuals with low fertility as well as alterations in steroidogenesis (Qian et al., 2019). Finally, exposure to carbamazepine in male adults can significantly affect offspring reproduction and endocrine function (Rodrigues et al., 2023).

3.1.6 Nanomaterials

Research on zebrafish has yielded essential insights into the potential hazards of various nanomaterials found in aquatic environments (refer to Table S6 for further details). Notably, carbon nanotubes can be effectively traced within distinct organs and tissues, which facilitates the analysis of their distribution patterns and underlying mechanisms of toxicity (Audira et al., 2024). These studies have contributed significantly to identifying the potential sites of nanomaterial toxicity, enhancing our understanding of the ecological implications of these pollutants. Investigations of metal oxide (MO) nanoparticles, such as titanium dioxide (TiO_2), have revealed significant ecotoxicological effects even at environmentally irrelevant concentrations, resulting in elevated mortality rates and developmental disorders in zebrafish embryos (Shih et al., 2016). Moreover, MO nanoparticles have shown the potential to induce significant molecular and genetic disruption in aquatic organisms, affecting crucial pathways associated with cell cycle regulation, DNA repair, and homologous recombination. The zebrafish model has also played a pivotal role in investigating the interactions between nanomaterials and other environmental pollutants. For instance, nano-TiO_2 has been found to augment the bioaccumulation and toxicity of BPA in this species, leading to adverse reproductive outcomes (Fang et al., 2016). These results highlight the significance of considering complex environmental scenarios when evaluating risks associated with nanomaterials (Fu et al., 2024).

Additionally, the combined impact of microplastics (MPs) and NPs, both emerging pollutants causing growing concern, has been intensively investigated in zebrafish (Lu et al., 2024). These plastic particles can be ingested by aquatic organisms and, in zebrafish, their effects can be observed throughout different life stages. Research has shown that NPs significantly influence the expression of genes associated with antioxidant enzymes, aromatase, and DNA methyltransferases during early embryonic development (Torres-Ruiz et al., 2021), while MPs induce developmental toxicity and disrupt the immune system (Qiao et al., 2019). The liver and intestines have been identified as the major target organs in which the toxicity of plastic particles is manifested. Exposure to polystyrene MPs has been shown to induce hepatic inflammation, lipid accumulation, and necrosis (Yu et al., 2023). In the intestinal tract, these pollutants disrupt immune cell function and alter microbial communities, potentially leading to an increased abundance of pathogenic bacteria (Xu et al., 2021). Studies have also revealed that NPs serve as carriers of other contaminants, influencing their bioavailability and toxicity. Interestingly, diurnal fluctuations in the retention of MPs have been observed in zebrafish, highlighting the intricate dynamics of MP exposure (Liu et al., 2024). These findings underscore the complex interactions between nanomaterials and other pollutants in aquatic environments.

3.2 Effects of pollutants on different organ systems in zebrafish

The zebrafish is an efficient model organism for assessing the toxicological effects of pollutants on

various organ systems owing to its genetic resemblance to humans, rapid developmental rate, and ease of observation and handling. Valuable insights into the broader implications of pollutants can be gained by studying their effects on different organ systems, including the nervous, gastrointestinal, endocrine, reproductive, cardiovascular, and immune systems, which are the main ones under scrutiny. The systematic analysis conducted in this review revealed that the main organ systems regarding the effects of pollution are the liver, central nervous system, and gonads, followed by the thyroid and cardiovascular systems. In contrast, studies on the kidneys, eyes, and gills are relatively scarce (Fig. 4). This indicates a significant focus on understanding the response to pollutant exposure in certain organ systems, while highlighting potential gaps in research on other systems. These findings provide directions for future studies, thereby facilitating a more comprehensive understanding of the overall impact of environmental pollutants on organisms.

3.2.1 Nervous system

Recent research has extensively explored the neurotoxic effects of various types of environmental pollutants, including MPs, heavy metals, and organic compounds, in zebrafish, yielding significant results. Notably, MPs and NPs have been shown to be highly neurotoxic. These particles can accumulate in the brain, leading to physical damage, disrupted neuronal connections, and impaired synaptic transmission. The leaching of neurotoxic chemicals from plastic particles induces oxidative stress and neuroinflammation, damaging cells and impairing the nervous system function (Teng et al., 2022). Behavioral studies have

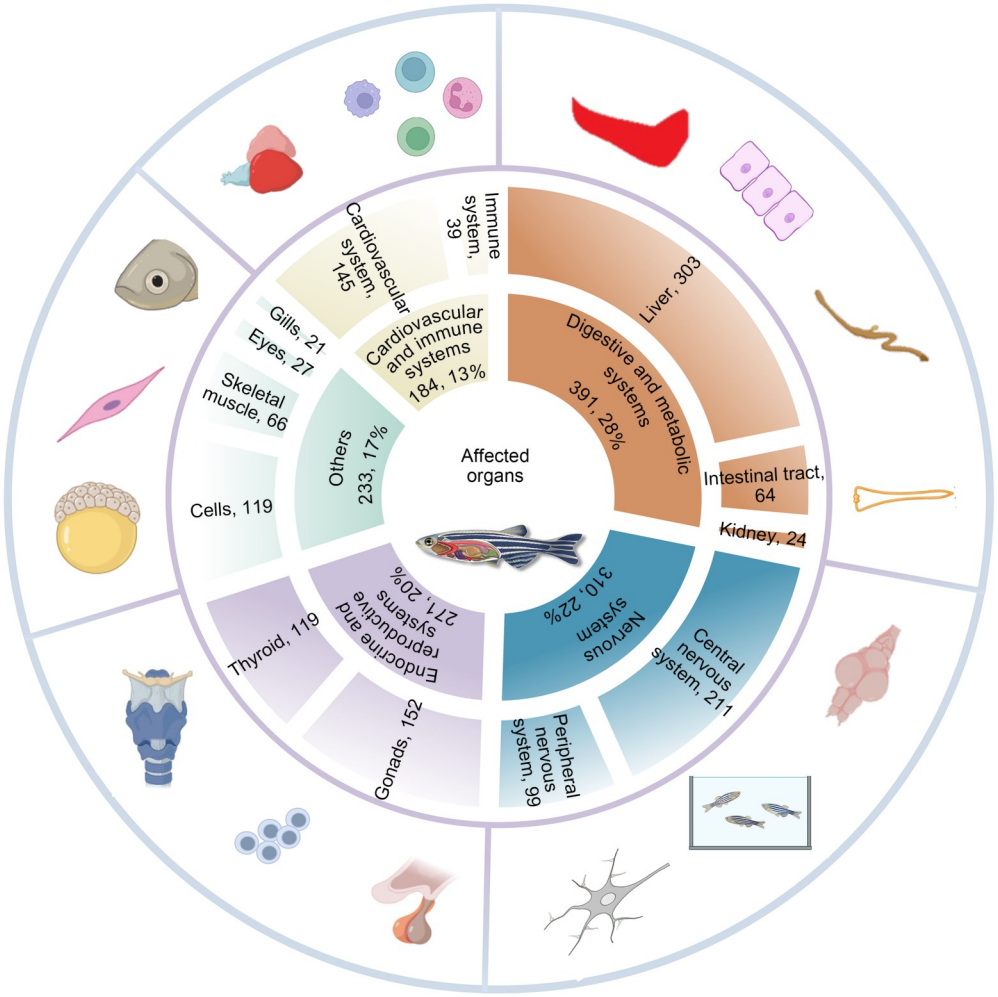

Fig. 4 Proportions of target organs in zebrafish models for aquatic ecotoxicology. Proportional distribution of target organs in zebrafish studies (2001–2023) is shown, with publication counts labeled per category.

shown that exposure to MPs can alter locomotor activity, learning, memory, social behavior, and predator avoidance responses in zebrafish (di Domenico et al., 2024). The neurotoxic effects of heavy metals such as lead, mercury, and cadmium, along with those of organic pollutants like pesticides and flame retardants, have also been investigated. The results showed that these substances can induce oxidative stress, leading to the production of reactive oxygen species (ROS) and subsequent neuroinflammation (Yuan et al., 2023). Oxidative damage can disrupt neuronal signaling pathways and cause neurobehavioral abnormalities. For instance, exposure to the pesticide fipronil has been associated with increased levels of superoxide dismutase, lipid peroxidation, and malondialdehyde, indicating oxidative stress and potential neurotoxicity (Wang et al., 2016). Moreover, BPA compounds significantly affect the development of zebrafish embryos, leading to abnormal development of the nervous system. BPS induces alterations in gene expression, thereby affecting neural function (Gyimah et al., 2021), while BPF triggers neuroinflammation and apoptosis, thereby impairing brain function (Gu et al., 2022). Furthermore, antidepressants such as diazepam exert direct toxic effects on the nervous system (Zhao et al., 2022).

3.2.2 Gastrointestinal system

Recent research has significantly advanced our understanding of the effects of environmental pollutants on the gastrointestinal system in zebrafish, providing critical insights into potential human health risks and environmental impacts. Exposure to MPs and NPs can cause substantial damage to the zebrafish gut. These particles have been shown to accumulate in the gut, physically damaging the intestinal lining, causing inflammation, and disrupting the gut barrier function (Jin YX et al., 2018). Studies have reported changes in gut morphology, including villus atrophy and increased goblet cell numbers, which indicate an inflammatory response (Marana et al., 2022). Numerous environmental pollutants have been shown to induce oxidative stress in the gut, leading to increased ROS production and inflammatory responses. This oxidative damage can result in cell death, tissue damage, and impaired gut function. Furthermore, environmental pollutants, including MPs, heavy metals, and organic pollutants, have been shown to significantly alter the gut microbiome composition, potentially leading to dysbiosis and thereby affecting nutrient absorption, immune function, and overall gut health (Yin et al., 2021). Exposure to certain pollutants has been linked to a reduction in the abundance of beneficial bacteria and an increase in potentially pathogenic species. In zebrafish, pollutants have a strong impact on the development of the digestive system. Delayed gut development, malformed digestive organs, and impaired functionality have been observed in embryos and larvae exposed to various pollutants, including perfluorooctanesulfonate (PFOS), glyphosate, and PCBs (Huang et al., 2010).

3.2.3 Endocrine and reproductive systems

The endocrine and reproductive systems are among the organs most affected by pollutant exposure, displaying developmental disorders and changes in gene expression. This underscores the potential risks posed by environmental pollution to reproductive health. EDCs are the main pollutants affecting these two systems in zebrafish, with BPA, PFOS, and tributyltin specifically impacting the endocrine system. These compounds have been found to alter hormone levels, affect gene expression in the hypothalamic–pituitary–gonadal (HPG) axis, and impact reproductive behavior. For instance, BPA exposure has been linked to changes in estrogen receptor activity as well as in the expression of genes involved in steroidogenesis (le Fol et al., 2017). Other pollutants, including heavy metals, PAHs, MPs, and NPs, have also been proven to interfere with the endocrine system, leading to hormonal imbalances. Copper exposure has been found to cause reductions in body weight, affect gonadal development, and alter the levels of hormones such as estradiol, testosterone, and 11-ketotestosterone as well as gene expression in steroidogenesis and the HPG axis (Zhao et al., 2020). Benzo(a)pyrene (BaP), a common PAH, has been shown to affect thyroid development and function in zebrafish. Specifically, short-term exposure to BaP can result in decreased expression of markers for thyroid hormone synthesis and alter the hypothalamus–pituitary–thyroid (HPT) axis, leading to central hypothyroidism (Vignet et al., 2014). Studies have found that polystyrene MPs disrupt female reproductive health and fertility, potentially via modulation of the Sirtuin-1 (SIRT1) pathway (Feng et al., 2022). Exposure to NPs and other pollutants,

such as diethylstilbestrol, exacerbates damage to the liver and gonads, inhibits sex hormone and vitellogenin secretion, and reduces fecundity and embryo viability (Han et al., 2022). Furthermore, it has been shown that hormone pollutants influence gene expression within the reproductive system, thereby affecting reproductive function, while other pollutants induce apoptosis in reproductive cells, with consequences for overall reproductive health.

3.2.4 Cardiovascular system

Advanced imaging techniques and transgenic zebrafish lines have enabled detailed analyses of cardiac structure and function in response to toxicant exposure in zebrafish embryos. For example, pesticides such as mancozeb have been associated with cardiac developmental toxicity, and exposure to environmentally relevant concentrations may potentially lead to pericardial edema, myocardial fibrosis, and congestion in the heart area (Wang YF et al., 2021). Transcriptome analyses have also revealed the activation of pathways related to cardiac development and apoptosis. Exposure to particulate matter has been associated with arrhythmia-like cardiotoxicity, which potentially occurs via oxidative stress induction, disruption of the expression of genes related to cardiac function, and alteration of the heart rate and rhythm. The accumulation of MPs and NPs has been shown to result in physical damage to the heart, disruption of cardiac function, and alteration of the expression of genes associated with cardiovascular development (Park et al., 2023). Furthermore, studies have indicated that heavy metals such as cadmium and thallium can induce significant cardiotoxic effects. These effects include impaired cardiac looping, reduced heart rate, and increased incidences of cardiac edema (Chang et al., 2023). Generally, PAHs, which are commonly found environmental pollutants, have been linked to adverse cardiovascular effects in zebrafish. Exposure to these compounds disrupts the expression of genes critical for heart development and function, leading to structural defects and compromised cardiac output (Gao et al., 2018). Another significant finding is the impact of EDCs on cardiovascular health. Chemicals such as BPA have been shown to alter cardiac morphology and function. These chemicals can interfere with hormone signaling pathways, leading to changes in the heart rate, rhythm, and overall cardiac performance (Lombó et al., 2015). Overall, as a model organism, the zebrafish continues to provide valuable insights into the impacts of various environmental pollutants on the cardiovascular system at both the molecular and physiological levels, facilitating the identification of potential mechanisms of toxicity and informing risk assessment and policy making.

3.2.5 Immune system

Pollutant-induced immune dysfunction has been linked to potential risks for both aquatic ecosystems and human health. Research has focused on various pollutants, including endocrine disruptors, fluorinated compounds, MPs, and NPs. Specifically, in recent years, the cumulative toxic effects of environmental endocrine disruptors on the immune system in zebrafish have been highlighted, with exposure to substances like BPA being shown to potentially trigger immune and inflammatory responses in larvae. Studies have revealed that BPS disrupts normal immune function by promoting inflammation, oxidative stress, and the activation of immune cells, which can lead to an overall heightened immune response and immune dysfunction (Dong et al., 2018). Exposure to fluorotelomer sulfonic acid has been reported to cause oxidative stress and inflammatory responses, with significant changes observed in immune-related biochemical indexes and proteins (Zhang J et al., 2023). Additionally, exposure to DEHP disrupts intestinal immune signaling pathways, inducing adaptive immune dysregulation (Santangeli et al., 2017), while exposure to mercuric chloride induces histological changes as well as alterations in antioxidant status and immune-related gene expression in the liver of adult zebrafish. These findings suggest that heavy metals can compromise immune function, making organisms more susceptible to infections and diseases (Chen et al., 2017). Recent studies have also focused on the immunotoxic effects of MPs and NPs. These pollutants can cause physical damage to immune tissues, induce oxidative stress, and alter the distribution and functionality of immune cells. For example, exposure to polystyrene MPs has been shown to disrupt the immune system, leading to increased susceptibility to infections and other health issues. Notably, these MPs can specifically affect ROS generation by phagocytes, increase mucus secretion by secretory cells, and alter lysosomal functions in macrophages (Jin YX et al., 2018). Exposure to pesticides

such as pymetrozine has been found to induce significant immunotoxic effects in zebrafish, including physical, hematological, and histopathological abnormalities characterized by alterations in immune cell distribution and function (Gonçalves et al., 2020). Moreover, antibiotics disrupt immune responses by altering the structure and quantity of gut microbiota in zebrafish. For example, studies have shown that exposure to enrofloxacin leads to a significant reduction in microbial diversity, richness, and evenness, resulting in immunosuppression (Qiu et al., 2022b), while exposure to chlortetracycline not only affects the immune system of parents but also suppresses both innate and adaptive immune responses in their offspring. These disruptions, which manifest as reduced numbers of macrophages and neutrophils, decreased expression of immune-related genes, and weakened immune function (Qiu et al., 2022a), can impair the ability of zebrafish to mount effective immune responses, increasing their vulnerability to pathogens.

3.2.6 Other organs

The skin and gills are the primary organs through which zebrafish interface with the aquatic environment and are therefore particularly vulnerable to pollutants. Studies have shown that exposure to toxicants such as heavy metals, pesticides, and MPs can cause significant damage to these organs. For instance, exposure to heavy metals like cadmium and copper can lead to histopathological changes in the gills, including hyperplasia, lamellar fusion, and necrosis, thereby impairing respiratory function and osmoregulation (Santos et al., 2022). Similarly, MPs can cause physical abrasions and inflammatory responses in the skin and gills, disrupting their protective and respiratory roles (Amorim et al., 2022). Research has shown that nephrotoxic compounds such as aristolochic acid (AA) and microcystin-LR can induce renal damage. Specifically, prolonged exposure to low concentrations of AA causes renal fibrosis, a condition characterized by disorganized glomeruli and tubular damage (Lim et al., 2022), while exposure to microcystin-LR causes histopathological lesions, including eosinophilic casts and abnormal renal tubules (Ge et al., 2024). These findings highlight the vulnerability of the zebrafish kidney to chronic pollutant exposure and its potential as a model for human renal diseases. Exposure to environmental pollutants such as pesticides and heavy metals also affects the development and function of skeletal muscles in zebrafish. For example, the pesticide pymetrozine has been linked to muscle atrophy and impaired locomotion (Hu et al., 2022), while heavy metals like mercury can induce oxidative stress and apoptosis in muscle tissues, leading to structural and functional deficits (Zhang QL et al., 2020).

3.3 Elucidating toxicological mechanisms using the zebrafish model

The zebrafish model has provided valuable insights into mechanisms of toxicity that are often translatable to human health. The classification of these studies based on the mechanisms examined reveals specific pathways through which impacts occur. Common mechanisms include cytotoxicity, endocrine disruption, developmental and metabolic toxicity, gene expression and signaling pathway regulation, oxidative stress and inflammatory responses, and epigenetic effects.

RNA sequencing has been widely adopted in this kind of research, revealing numerous changes in gene expression associated with exposure to environmental pollutants. These studies have shown that heavy metals, endocrine disruptors, and organic pollutants can significantly affect gene expression profiles and signaling pathways in zebrafish. In particular, Kyoto Encyclopedia of Genes and Genomes (KEGG) and Ingenuity Pathway Analysis (IPA) have revealed the effects of endocrine disruptors on multiple signaling pathways, including nitric oxide synthase 2 (NOS2), Toll-like receptor 4 (TLR4), cytochrome b-245 β chain (CYBB), nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK), colony-stimulating factor (CSF), Janus kinase-signal transducer and activator of transcription (JAK-STAT), and phosphatidylinositol 3-kinase (PI3K), which play crucial roles in inflammatory responses. Exposure to environmental endocrine disruptors also leads to the activation of estrogen receptor and calcium signaling pathways as well as changes in their expression, resulting in endocrine disruption and organ toxicity. For example, studies have shown that exposure to 2,4-DCP increases the messenger RNA (mRNA) level of estrogen receptor 2 α (*ESR2a*) and activates the homonymous signaling pathway, potentially interfering with

normal sex differentiation in zebrafish (Hu et al., 2022). BPA exposure has also been associated with alterations in the Notch signaling pathway, which is significantly involved in the progression of calcific aortic valve disease (CAVD) (Qiu et al., 2021; Wang et al., 2022). In addition, studies have shown that exposure to the emerging contaminant perfluorooctane sulfonamide activates the AHR pathway, resulting in significant upregulation of AHR-targeted genes such as *ahrra* and *cyp1a1*, thereby leading to cardiotoxicity, while exposure to perfluorooctane sulfonates significantly affects the bone morphogenetic protein (BMP), transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), and wingless-type MMTV integration site family (WNT) signaling pathways during cardiac development, leading to aberrations in heart morphology and function (Chen HH et al., 2022). The antibiotic chlortetracycline, which exhibits binding affinity toward NF-κB1, NF-κB2, and NF-κB3, can activate the NF-κB signaling pathway and thereby induce transgenerational immunosuppression in parental zebrafish, leading to a significant reduction in the immune defense capabilities of their offspring (Qiu et al., 2022a). Furthermore, heavy metals have been shown to affect various key signaling pathways in zebrafish, including calcium ion channels, olfactory signal transduction pathways, and G-protein-coupled receptor signaling pathways. Notably, exposure to low copper concentrations has been found to significantly influence the expression of genes related to calcium ion channels and ion transport, as well as disrupting normal cellular functions by inducing oxidative stress signaling pathways (Gao et al., 2020). Certain rare elements, such as lanthanum and praseodymium, can also affect the Notch signaling pathway, leading to disruptions in cell differentiation and development (Zhao et al., 2021).

Epigenetic modifications are also a matter of significant concern. According to the literature, the main epigenetic mechanisms investigated using zebrafish models include DNA methylation, histone modifications, and chromatin remodeling. Several studies have reported alterations in DNA methylation in zebrafish following exposure to contaminants such as arsenic, BaP, and tris(1,3-dichloro-2-propyl)phosphate (TDCIPP), with effects being observed, for example, in histone modification patterns. Exposure to TBPH and tetrabutyl mono(2-ethylhexyl)phthalate (TBMEHP) has been shown to significantly reduce DNA methylation levels within the peroxisome proliferator-activated receptor γ (PPARγ) promoter region in zebrafish larvae at 72 h post-hatching, leading to the upregulated expression of this gene. These findings suggest that TBPH and TBMEHP regulate gene expression in this species by influencing DNA methylation, ultimately affecting lipid metabolism and energy balance (Liu et al., 2022). Additionally, exposure to metformin significantly decreases global methylation levels in the gills, intestines, and liver in a dose-dependent manner, which suggests that this drug disrupts the process of DNA methylation, thereby affecting gene stability and expression (Zhou et al., 2024). Moreover, exposure to the organic pollutant BDP induces significant alterations in N^6-methyladenosine (m^6A) RNA methylation levels in zebrafish offspring, thereby impacting numerous genes associated with vascular development and function, and also alters 5-methylcytosine (m^5C) DNA methylation levels at gene loci related to vascular formation in both parental gonads and offspring, consequently disrupting normal development (Zhu Z et al., 2022). Another study on zebrafish revealed that DBDPE exposure leads to changes in global DNA methylation levels in the brain, with sex-specific responses. The epigenetic toxicity of DBDPE is characterized by its capacity to induce transgenerational alterations in DNA methylation, oxidative DNA damage, and gene expression, which disrupt neurodevelopment and function (Sun et al., 2024).

Recent studies have highlighted the complex interactions among environmental toxicants, the gut microbiome, and various physiological systems, providing valuable insights into the mechanisms through which pollutants disrupt crucial processes mediated by the gut microbiome, for example, within the gut–brain and liver–gut axes. Several studies have elucidated the impact of toxicants on the liver–gut axis in zebrafish. Organic pollutants such as dioxin-like compounds have been shown to modulate the activity of gut microbiota via the AHR signaling pathway, disrupting the metabolism of primary bile acids and vitamins and further altering the gut function as well as the metabolic cross-talk between the gut and liver (Xu et al., 2018). Exposure to niclosamide has also been found to disturb this bidirectional cross-talk, as evidenced by the significant inhibition of glycogen and triglyceride synthesis in the liver, along with the induction of hepatic inflammation and mitochondrial damage.

Moreover, niclosamide exposure induces damage to the intestinal barrier, leading to intestinal inflammation and oxidative stress responses. Extensive research has shown the interactions occurring within the gut–brain axis in zebrafish (Chen et al., 2024). Exposure to 6:6 perfluoroalkyl phosphinic acids (PFPiAs) significantly alters the composition of gut microbiota, resulting in elevated levels of lipopolysaccharides in the gastrointestinal tract, which then cross the blood–brain barrier and activate brain inflammation pathways, thereby affecting the gut–brain axis and causing neurotoxicity as well as behavioral abnormalities (Zhang TX et al., 2023). Notably, exposure to BPA analogs has been shown to significantly reduce both the number and distribution of enteroendocrine cells in the intestine. These cells play a crucial role in signal transmission between the gut and brain; hence, a reduction in their number directly affects neurotransmitter levels and behavioral activities. These findings suggest that the gut microbiome may mediate developmental neurotoxicity. Additionally, the HPG axis, which is the primary endocrine pathway regulating reproductive functions and the secretion of sex hormones, is also susceptible to environmental pollutants. Studies have shown that BPS disrupts the balance of sex hormones by affecting the feedback regulation loop of the HPG axis, resulting in impacts on reproductive function and offspring development in zebrafish (Qiu et al., 2018). Another study has reported that BDE-47 affects the HPT axis through different mechanisms, leading to various developmental toxicities and changes in gene expression. Specifically, this compound significantly downregulates the mRNA expression of the thyroid-stimulating hormone receptor, thyroid hormone receptor, sodium/iodide symporter, and transthyretin, while upregulating the expression of thyroglobulin and thyrotropin-releasing hormone (TRH) (Zhuang et al., 2020). Notably, tris(2-chloroethyl)phosphate (TCEP) can induce hepatotoxicity in zebrafish by disrupting the HPT and liver–gut axes, leading to liver inflammation and oxidative stress. TCEP exposure also significantly reduces the levels of TRH and thyroid-stimulating hormone (TSH) in the brain, causing a significant decrease in the T3/T4 ratio, which indicates impairment of the HPT axis function. At the same time, it causes dysbiosis of the gut microbiota, increases lipopolysaccharide levels in the plasma, activates immune pathways, and induces liver inflammation (Tian et al., 2023).

4 Conclusions and perspectives

The zebrafish model has emerged as an invaluable tool in the field of environmental toxicology, offering unique insights into the impacts of various environmental pollutants and their mechanisms of action (Liu et al., 2017). The versatility of this model has enabled research into the effects of different toxicants on multiple organ systems, including the nervous, digestive, endocrine, reproductive, cardiovascular, and immune systems. Studies have revealed that many pollutants can induce oxidative stress, disrupt gene expression, alter epigenetic patterns, and interfere with important developmental processes. The zebrafish model has also been instrumental in elucidating the potential transgenerational effects of certain pollutants, highlighting the long-term consequences of environmental contamination.

When considering the future of toxicological research using zebrafish models, several key areas are particularly promising and require further investigation:

(1) Metabolomics and biomarker discovery: There is increasing demand for the zebrafish model in research on metabolism. The identification of distinctive metabolites that are altered by environmental toxicants in zebrafish could offer valuable biomarkers for environmental assessments and human health monitoring, thereby bridging the gap between animal and human models. This approach can provide diagnostic tools and therapeutic targets for pollution-related diseases.

(2) Research on radioactive pollutants: Given the increasing concern over nuclear waste pollution, there is an urgent need to strengthen research on radioactive pollutants using the zebrafish model. As these contaminants can spread globally through ocean currents, evaporation, and precipitation, it is essential to understand their impacts on aquatic life. Studies on zebrafish could provide valuable insights into the biological effects of low-dose, chronic exposure to radioactive materials, informing risk assessment and mitigation strategies.

(3) Integrated multi-organ research: Future investigations should aim to combine different research methods and simultaneously examine the effects of pollutants across multiple organ systems. This holistic approach would provide a more comprehensive understanding of impacts, allowing researchers to move beyond single-organ studies and explore systemic effects and inter-organ interactions. Advanced imaging

techniques and multi-omics approaches could be particularly useful in this field.

(4) Combined exposure studies: Given that environmental pollution at low doses typically involves multiple factors, there is a pressing need to increase research on combined exposures. Examining the mixed effects of different pollutants could reveal synergistic or antagonistic interactions that are not apparent in studies focusing on single toxicants. This approach would more accurately reflect real-world exposure scenarios and provide valuable insights for risk assessment.

(5) Microbiome interactions: Considering the growing recognition of the importance of the microbiome in human health, future research should explore the mechanisms through which environmental toxicants affect the zebrafish microbiome and how, in turn, these changes impact host physiology. This could provide valuable insights into the broader ecological impacts of pollution and strengthen our understanding of the interactions between different organs, as in the case of the liver–gut and brain–gut axes, which represent an important frontier in environmental toxicology. This research avenue could reveal how pollutants disrupt homeostasis across multiple systems and provide a more nuanced understanding of the effects of pollutants.

(6) Epigenetic and transgenerational effects: Further investigations into the epigenetic and transgenerational effects of environmental toxicants are warranted. The short generation time characterizing the zebrafish model makes it ideal for studying these long-term impacts, allowing researchers to elucidate how pollution may affect ecosystems and human health across generations.

(7) Artificial intelligence (AI) applications: Integrating AI within zebrafish models in environmental toxicology holds great potential for advancing our understanding of how pollutants affect biological systems. AI can enhance high-throughput screening, predictive toxicology, multi-omics integration, real-time monitoring, systemic studies, behavioral analysis, regulatory frameworks, and combined exposure studies.

In conclusion, the zebrafish model has proven to be a powerful tool in the field of environmental toxicology, offering unique advantages for studying the impacts of various pollutants. Integrating advanced technologies, adopting more holistic research approaches, and focusing on real-world exposure scenarios will be key to providing new insights as research advances. These efforts will enhance our understanding of environmental toxicants and contribute to developing more effective strategies to protect both the environment and human health. The continued refinement and expansion of zebrafish models hold promise for addressing the complex challenges posed by environmental pollution, offering a comprehensive and nuanced understanding of the impact of toxicants across biological systems.

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Author contributions

Yang JIANG conceived the review topic and supervised the overall project. Zhen SU performed the literature search and drafted the initial manuscript. Jing ZHENG contributed to data analysis and interpretation of the reviewed studies. Chih-Hung HSU critically revised the manuscript for important intellectual content. Ye CHEN contributed to the discussion and provided significant edits and final approval of the manuscript. All authors have read and approved the final version of the manuscript.

Compliance with ethics guidelines

Yang JIANG, Zhen SU, Jing ZHENG, Chih-Hung HSU, and Ye CHEN declare that they have no conflicts of interest.

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Supplementary information

Tables S1–S6