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Toxicological Studies of Fish and Fish Cells in Vitro and in Vivo

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Abstract With the rapid development of industrialization, urbanization, and agriculture, various pollutants pose significant threats to fish health and may harm human health through the food chain. This study comprehensively explores advancements in in vitro and in vivo toxicological studies of fish and fish cells, revealing the mechanisms by which various pollutants impact the immune system, nervous system, and overall physiological functions of fish. In vitro models, such as the rainbow trout cell lines (RTgill-W1 and RTgutGC), demonstrate high efficiency in predicting toxicity, while whole fish experiments provide a realistic ecological context for assessing the comprehensive effects of pollutants. The review focuses on the synergistic toxicity of emerging contaminants and their potential for bioaccumulation, emphasizing future directions based on omics technologies and high-throughput methods to optimize toxicological research approaches and enhance pollution monitoring capabilities.
Keywords Aquatic toxicology; Fish bioindicators; Pollutant bioaccumulation; In vitro cell models; Emerging contaminants

1 Introduction

As industrialization, urbanization and agricultural activities continue to increase, pollution levels are also rising. This makes toxicological research in aquatic ecosystems increasingly important. Many pollutants, such as heavy metals, nanoparticles, pesticides and persistent organic pollutants (POPs), threaten not only aquatic animals, but also may affect human health (Bawa-Allah et al., 2021). Moreover, new pollutants continue to emerge, making the problem of water pollution more complicated. Therefore, we must have a deeper understanding of their toxic mechanisms and expand the scope of toxicological research.

Because fish are located at the top of the aquatic food chain, they have become an important indicator of environmental pollution. They easily accumulate pollutants through the food chain and are affected by biological amplification effects (Grimard et al., 2020). The health status and physiological responses of fish can reflect changes in the entire aquatic ecosystem. Fish can accumulate a large amount of metal from the water, which not only harms the fish itself, but may also affect humans who eat fish. Fish are now often used for ecotoxicology research to detect the impact of various pollutants such as heavy metals, pesticides and drugs on the water environment (Wang et al., 2022).

This study mainly studies the specific impact of different pollutants on fish health. The focus is on analyzing the immunotoxicity, neurotoxicity and overall physiological changes caused by pollutants. We used fish as a model organism to systematically explore the toxicological reactions triggered by various pollutants. The research content includes different types of pollutants, such as heavy metals, nanoparticles and emerging pollutants, and their impact on fish is comprehensively analyzed from the cell, tissue to the entire individual level.

2 In vitro Toxicology Research

2.1 Fish cell lines as a model for toxicology research

Fish cell lines have now become an important tool in toxicology research. They provide a good alternative to traditional in vivo testing. For example, the rainbow trout gill cell line RTgill-W1 is widely used to evaluate the toxicity of chemicals because of its stability and accuracy (Schug et al., 2019; Scott et al., 2022). Also, RTgutGC cell lines extracted from the intestine of rainbow trout have been shown to effectively predict fish responses to acute toxicity, especially against hydrophobic chemicals entering fish through food. Other cell lines, such as RTLfrom the liver and SAF-1 from the gilthead bream, are also often used to study the toxicity of different types of pollutants such as heavy metals and complex mixed pollutants (Panneier et al., 2018).

2.2 Methods for chemical toxicity detection using fish cells

There are many test methods now available to evaluate chemical toxicity using fish cell lines. The RTgill-W1 cell line is often used in cell viability tests to measure the concentration of poisons that deactivate half of the cells (EC50). This result is usually very close to the lethal concentration (LC50) measured with live fish (Scott et al., 2020). RTgutGC cell lines are more suitable for detecting hydrophobic and toxicity of volatile chemicals, and test results in vitro and in vivo often correspond well (Schug et al., 2019). In different cell lines, researchers use MTT tests, neutral red staining (NR tests), comet experiments to measure multiple endpoints such as cytotoxicity, reactive oxygen species (ROS) production and DNA damage (Langan et al., 2017).

2.3 Advantages and limitations of in vitro methods

Compared with traditional in vivo methods, in vitro methods have many advantages. First of all, they use fewer live fish, which not only reduces ethical issues but also saves costs. These methods also operate faster and are easier to standardize between laboratories. For example, the detection results of the RTgill-W1 cell line have good repetition and reliability (Fischer et al., 2019). However, in vitro methods also have some limitations. For example, they cannot fully simulate the complex responses of the fish's complete physiological system. Also, cell lines may differ from real fish. Nevertheless, in vitro methods are still a very valuable step in toxicity research. They can be used for initial screening, supplementing in vivo research, and helping us to gain a more comprehensive understanding of the toxicity of chemicals (Scott et al., 2023).

3 In Vivo Toxicology Research

3.1 The role of whole fish in evaluating environmental toxins

The use of whole fish is very important when evaluating environmental pollutants. Because fish can provide complete information on the impact of pollutants in real environments. Fish are often used in environmental risk assessments to detect the impact of chemicals on aquatic ecosystems (Liu and Huang, 2024). In particular, young fish are very sensitive to environmental toxins. Their growth, survival and reproduction are all susceptible to chemical contamination (Scott et al., 2023). Using whole fish can see complex interactions between fish and the environment, which are difficult to fully observe with cellular experiments alone. This holistic research approach can help us better understand all the effects of pollutants, including changes in fish behavior and threats to long-term survival of the population (Zurita et al., 2019).

3.2 Key endpoints of fish toxicology: survival, reproduction and behavior

In fish toxicology research, there are several particularly important observation points: survival, reproduction, and behavior. Survival rate is the most intuitive indicator, which can directly reflect the acute toxicity of chemicals, that is, the ability to die quickly. Reproduction-related data, such as egg spawning and hatching rates, can tell us the effect of pollutants on population continuity, even if fish do not die in the short term (Langan et al., 2017). Changes in behavior, such as changes in swimming styles or reduced eating, are also important. They often early warnings of neurotoxicity or other subfatal effects, which are usually undetectable by looking at mortality alone (Schug et al., 2019). Combining these indicators can give a more comprehensive understanding of the toxic effects of pollutants on fish.

3.3 Moral considerations and alternative methods

When doing fish toxicology research, there are many moral issues involved. According to the 3R principle (replacement, reduction and optimization), we hope to minimize the use of live animals. Testing directly with the whole fish will cause painful problems for fish. Therefore, scientists are working to develop alternative methods, such as using fish cell lines or fish embryos for in vitro testing (Panneier et al., 2018). These methods not only meet ethical requirements, but also save a lot of costs and can also obtain reliable toxic data. For example, RTgill-W1 cell line and fat-headed minnow embryos have shown high predictive power in many studies and can be used to assess the toxicity of overall pollutant emissions (Scott et al., 2022). In addition, advances in modeling and simulation technologies can also predict in vivo responses through data from in vitro experiments, further reducing the use of live fish (Shaliutina et al., 2021).

4 Common Contaminants Assessed in Fish Toxicology

4.1 Heavy metals (such as mercury, lead, cadmium)

Mercury (Hg), lead (Pb) and cadmium (Cd) are very important pollutants in the water environment. These heavy metals are highly toxic, can exist for a long time, and can continue to accumulate and amplify in the food chain (Wang and Xu, 2024). Research has found that they can cause serious harm to fish, such as cytotoxicity, oxidative stress response, and changes in gene expression. For example, when fish red blood cells are exposed to Hg, Pb and Cd, cell viability will decrease with the increase in exposure, and at the same time, genes related to cell protection, stress and cell death also undergo significant changes. Further tissue section analysis showed that important organs of fish, such as kidneys and gills, showed obvious morphological changes after exposure.

Images stained with H&E can be seen that in the severely contaminated Milazzo East Coast samples, the centers of melanin macrophages in the kidneys increased significantly (Figures B, D, F). PAS staining and Van Gieson staining further confirmed that these changes were also accompanied by tubular degeneration (dt) and tissue fibrosis, which are typical biomarkers of heavy metal toxicity under environmental stress (Figure 1) (Alesci et al., 2022). In addition, heavy metal accumulation has also been found in the kidneys and gills of fish in different fish species and regions, with significant differences in metal concentrations (Tolkou et al., 2023).

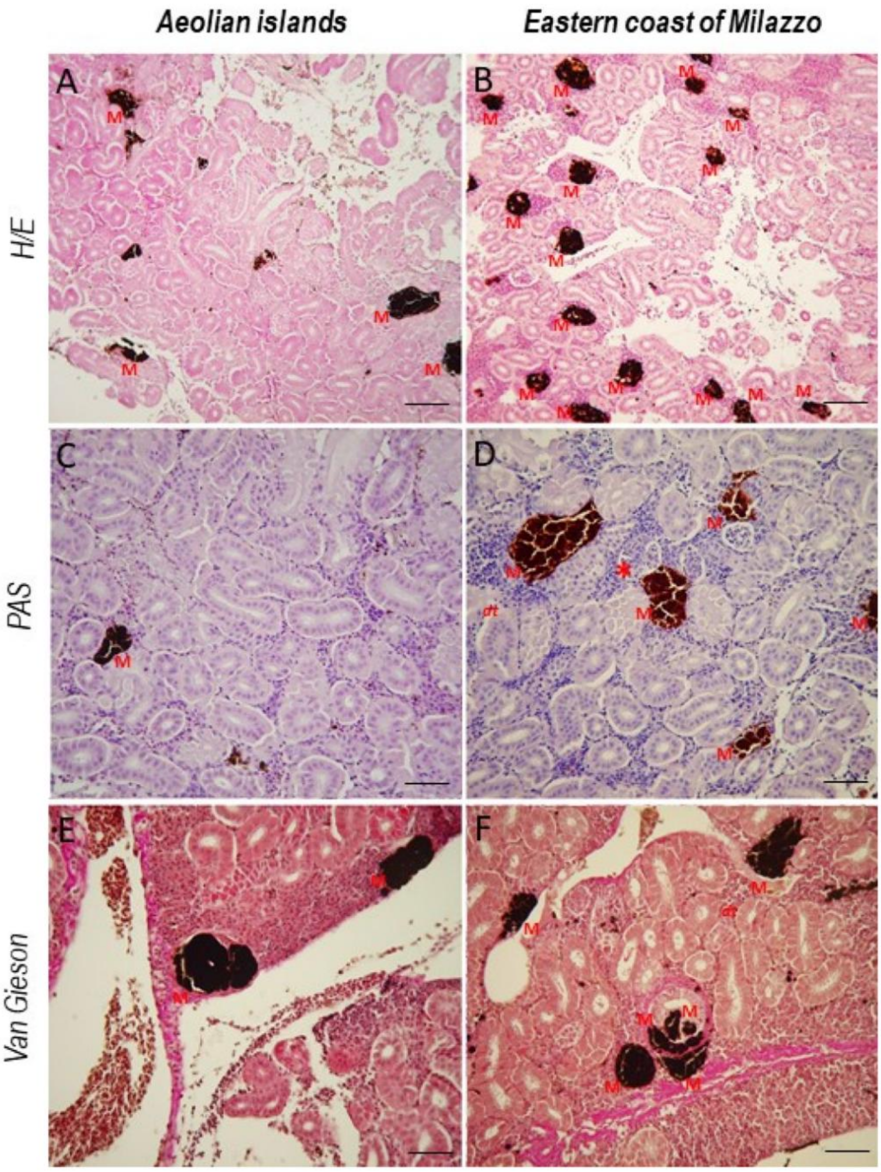

Figure 1 *Boops boops* kidney section (Addopted from Alesci et al., 2022)

4.2 Organic pollutants (such as pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls)

Another major class of pollutants that affect fish are organic pollutants such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). These pollutants can also be adsorbed on microplastics (MPs) in the environment and enter aquatic animals with water flow and biofeeding. Toxicological tests with fish cell lines found that microplastics with PAH and PCB can cause DNA damage and alter the enzyme activity in the cells, indicating that their potential toxicity is very strong (Panneier et al., 2019). The study also found that higher concentrations of pesticide residues, such as p-p'-DDE and endosulfan, are potential health threats to aquatic and human eaters (Topal and Onaç, 2020).

4.3 Newly emerging contaminants (e.g., microplastics, drugs)

Microplastics and drugs are considered to be important pollutants emerging in the aquatic environment in recent years. These pollutants are harmful in themselves and can also interact with other pollutants (such as heavy metals) to change their toxicity. Studies have found that when discus fish are exposed to microplastics and cadmium environments at the same time, although microplastics alone have little impact on growth and survival, when combined with cadmium, it will lead to a decrease in metal accumulation in the body, while increasing the oxidative stress and immune response of the fish body (Wen et al., 2018).

5 Comparative Toxicology: in vitro and in Vivo

5.1 Correlation between in vitro and in vivo results

Recent studies have focused on the relationship between in vitro and in vivo toxicological results. For example, the test results of the RTgill-W1 cell line are very similar to the in vivo toxicity results of the whole fish, especially for fragrance chemicals, the in vitro and in vivo results are almost 1:1. Similarly, RTgutGC cell lines have been shown to be well predictive of acute toxicity in fish, which is very consistent with the data from in vivo experiments (Langan et al., 2017). There are also studies that have found that there is a high correlation between the in vitro EC50 values of RTgill-W1 cells and many poisons (except low water-soluble and volatile compounds) in the LC50 values in vivo (Scott et al., 2020). These findings suggest that in vitro models have great potential to accurately predict in vivo conditions, thereby reducing dependence on live animal experiments.

5.2 Understand the mechanism of toxic effects

In vitro models can also help us understand how toxicity occurs. For example, the RTgutGC cell line was used to study the transformation and genotoxicity of benzopyrene, and the activation and metabolic processes involved were discovered (Shaliutina et al., 2021). In vitro studies also show that enzymes such as cytochrome P450 enzyme and glutathione transferase play an important role in toxic metabolism. This gives us a clearer understanding of the biochemical process of toxic effects. The RTgill-W1 cell line is also very helpful in this regard, which can simulate the toxic mechanism of some chemicals in vivo (Natsch et al., 2018).

5.3 Limitations and gaps in integrating survey results

Although there are good results in vitro and in vivo, there are still some gaps and problems between the two. A major problem is that there are differences in sensitivity between different cell lines and real fish. For example, the RTG-2 cell line responds to poisons weaker than real fish and crustaceans (Kolářová et al., 2021), indicating that in vitro models cannot completely replicate the real situation in the body. Moreover, factors such as osmotic pressure will also affect the absorption and toxicity of chemicals in the organisms, making it more complicated to apply external results directly to the body. Although useful in predicting acute toxicity, in vitro methods may not capture the chronic effects and complex interactions of organisms after long-term exposure (Scott et al., 2022). These problems suggest that more research is needed to improve in vitro models so that they can more accurately reflect the real in vivo situations.

6 Application of Toxicology Research

6.1 Regulatory framework and environmental risk assessment

Toxicological research on fish and fish cells is very important in formulating regulatory rules and conducting environmental risk assessments. Traditional methods usually use live fish to test the toxicity of chemicals, but this method is not only costly, but also creates moral controversy. Now, in vitro methods, such as using fish cell lines, are becoming new options. They show great potential in predicting chemical toxicity and can reduce dependence on living animals. For example, the RTgill-W1 cell line has been used to effectively predict acute fish toxicity, showing a strong correspondence between in vitro and in vivo test results, especially for fragrance chemicals (Natsch et al., 2018). The application of RTgutGC cell lines can also be expanded to assess chemicals in the intake pathway, providing a more comprehensive approach to environmental risk assessment (Langan et al., 2017). These new approaches are not only reliable, but are more cost-effective and ethical, and also help regulators more accept new standards.

6.2 Application of toxicological data in aquaculture management

In aquaculture, toxicological data are very important to ensure the health and production of farmed fish. In vitro toxicological research can provide important information on the health effects of pollutants on fish and help improve breeding methods. Research has found that fish sperm can be used as a model for detecting genotoxicity, can predict the quality of embryos, and can also help select healthy parent fish (Shaliutina et al., 2021). Understanding the metabolic processes of harmful substances such as polycyclic aromatic hydrocarbons (PAH) through in vitro experiments can also provide reference for measures to reduce the impact of these pollutants (Franco and Lavado, 2019). These applications not only make aquaculture more sustainable, but also ensure that the fish raised are safer and higher quality.

6.3 Impact on public health and food safety

Toxicological research on fish and fish cells also has a great impact on public health and food safety. Pollutants in the water environment accumulate in the fish, and people may face health risks when eating these fish. In vitro toxicological testing can identify and measure the harm of these contaminants, helping to develop public health policies and food safety standards. Studies have shown that in vitro models can effectively predict the toxicity of chemicals such as pesticides and polycyclic aromatic hydrocarbons to fish, and can also be used to infer their risks to humans (Rodrigues et al., 2019). Combining in vitro and in vivo detection can form a monitoring framework based on actual effects and more comprehensively evaluate the impact of pollutants on fish health (Mehinto et al., 2020). Doing so will ensure that fish entering the food market will not contain harmful substances that exceed the standard, thereby improving the safety of the overall food supply.

7 Case Studies

7.1 Assessing heavy metal toxicity in freshwater fish species

Heavy metals such as chromium (Cr), cadmium (Cd) and copper (Cu) can accumulate in freshwater fish, especially in different tissues, causing obvious toxicological damage. A study on carp (Cyprinus carpio) found that when carp are exposed to a mixture of these metals, oxidative stress, tissue damage, and changes in the gut microbiota occur (Kakade et al., 2020). There are also studies that heavy metals such as cadmium, mercury and lead can cause oxidative stress and cell death in the SAF-1 cell line of marine bony fish, further demonstrating the cytotoxic effect of these metals. In addition, heavy metal exposure also leads to obvious changes in biochemical reactions in carp, such as decreased antioxidant enzyme activity and weakened immune function (Rajeshkumar et al., 2017).

7.2 The impact of pesticide runoff on fish behavior and survival

Pesticides flow from farmland into water will also affect fish. For example, model fish such as zebrafish and cyanobacteria will show anxiety-like behaviors, changes in social preferences, and decreased spatial learning and memory ability after exposure to pesticides such as imidacloprid and chlorpyrifos (Hong and Zha, 2019). These behavioral changes can serve as early warning signals for water pollution, helping scientists evaluate the ecological risks posed by pesticides.

7.3 Study on long-term exposure of drugs in fish populations

7.3.1 The chronic effect of antibiotics on fish reproduction and growth

Antibiotics exposed to water for a long time will affect the reproductive ability and growth rate of fish. Research shows that antibiotics can interfere with the endocrine system of fish, reduce the number of egg laying in the fish and slow down the development of fish, and also cause the accumulation of drugs in the body and aggravate the toxicity problem. In addition, antibiotics can also affect the normal secretion of fish hormones, resulting in an imbalance in gender ratios. The immune function of fish individuals may also decrease due to long-term exposure to antibiotics, thereby increasing the risk of infection.

7.3.2 The effect of endocrine interference chemicals on fish physiology

Some heavy metals and pesticides are endocrine disruptors (EDCs), which can destroy fish's hormone systems. For example, cadmium (Cd) and mercury (Hg) have been shown to trigger oxidative stress and cell death in fish white blood cells, thereby affecting the fish's immune system and overall health (Rehman, 2021). These injuries have long-term consequences, such as reducing fertility and increasing the risk of illness.

7.3.3 Potential bioaccumulation and transfer of drugs in food webs

In an aquatic environment, drugs can accumulate in fish and can also be passed along the food chain to animals of higher trophic levels, including humans. Studies have shown that heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As) can destroy the normal physiological functions of fish, leading to abnormal development and decreased fertility in young fish. These pollutants are not only a sign of environmental pollution, but also affect the health of predators through food webs, ultimately threatening human consumption safety (Figure 2) (Choudhary et al., 2023). Therefore, it is important to monitor and manage drug and heavy metal contamination in water bodies to reduce their risks to biodiversity and food safety.

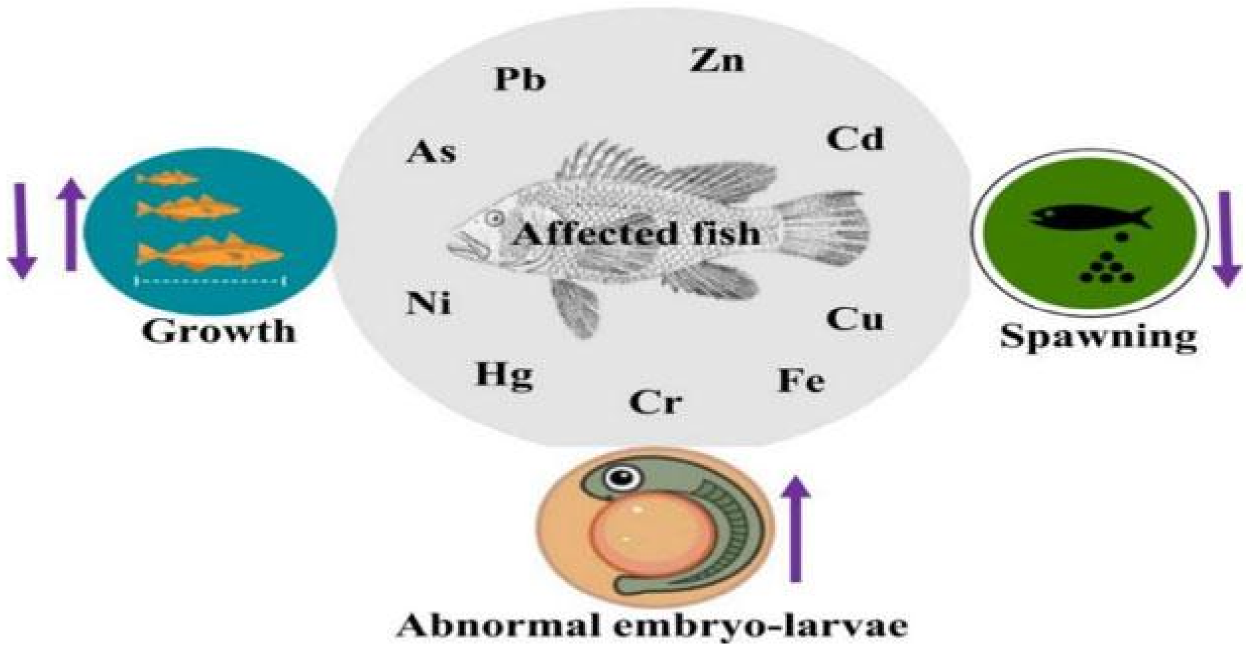

Figure 2 Effects of different heavy metal accumulation in fish on growth, spawning, and embryo development (Adopted from Choudhary et al., 2023)

8 Future Directions of Fish Toxicology

8.1 Advances in molecular and cytotoxicology technology

Recently, there have been great advances in molecular and cytotoxicology technologies, which have greatly improved our understanding of fish toxicity responses. High-throughput fish embryo toxicity testing (FET) has become a promising alternative to traditional animal testing. It can assess chemical risks and water quality faster and more ethically. Although the FET method is increasingly accepted by regulators, it also has problems, such as the testing process requires a lot of manual labor and some poisons have limited testing effects under static or semi-static conditions. However, advances in mechatronics, fluid control and digital imaging technologies are driving the development of FET testing automation. This means that high-throughput detection can be achieved in the future, just like in vitro drug screening (Wlodkowic and Campana, 2021).

8.2 Integration of omics technology in fish toxicology

Omics technologies such as genomics, proteomics, metabolomics and transcriptomics are now increasingly applied to fish toxicology. These technologies can analyze thousands of molecular changes simultaneously, helping us to fully understand what happens to fish when they are affected by pollutants. For example, there are studies using multiomics methods to study the effects of low-dose pollutants on zebrafish larvae, and many changes related to dose and chemical species have been found (Huang et al., 2017). In addition, combining the omics data also helped scientists better explain the response of fat-headed minnows under early exposure, demonstrating commonalities between toxic mechanisms at different biological levels (Alcaraz et al., 2021). The application of omics not only allows us to understand the molecular action process of poisons more clearly, but also helps find biomarkers that can be used for environmental monitoring (Marie, 2020).

8.3 Develop alternatives to reduce animal tests

Now more and more people are paying attention to how to reduce their dependence on living animals. Developing new alternative methods has become an important direction for the development of fish toxicology. One new approach is to use mechanotoxicology models, such as the fat-headed minnow model in the embryo-young phase, instead of traditional adult fish tests. This model combines transcriptome, proteome, histological and behavioral data to more comprehensively analyze the toxic effects of chemicals. High-throughput, automated detection methods like FETs are also increasingly considered to be a viable alternative to traditional animal experiments. These new approaches not only reduce ethical controversy, but also save costs and improve testing efficiency (Lai et al., 2021). Continuing to develop and refine these alternative technologies is important to advance the advancement of fish toxicology and can also minimize the moral and practical problems brought about by animal experiments.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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