

## Zebrafish larvae: a modern window into developmental toxicity

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Have you ever imagined that the secrets of human development could be hidden inside a creature barely a few millimetres long? Picture a tiny, transparent fish drifting gently in a glass tank, its beating heart visible, its brain forming right before your eyes. It may look delicate, but this little larva is transforming the future of toxicology.

Development begins as a beautifully coordinated process, where cells divide, organs form, and life takes shape. But what happens when a developing embryo encounters harmful chemicals in its environment? This question lies at the heart of developmental toxicity. The study of how substances such as pollutants, drugs, plastics, or heavy metals interfere with normal growth. These disturbances can lead to birth defects, impaired organ formation, behavioural abnormalities, or long-term health problems.

For decades, scientists relied mainly on mammalian models to answer these questions. While accurate, these models were time-consuming, expensive, and raised ethical concerns. The search for a faster, more humane, and equally reliable method led researchers to an unexpected hero: the **zebrafish larva**.

With their transparent bodies, rapid development, and surprising genetic similarity to humans, zebrafish larvae have emerged as a modern, ethical, and highly efficient model for studying developmental toxicity. Today, they allow scientists to observe development in real time and uncover how chemicals affect living organisms, while reducing the use of higher animals in research.

At first glance, zebrafish may appear to be just ordinary aquarium pets a small, striped, and charming. But beneath their simple appearance lies a biological treasure that has captivated scientists around the world. The real magic begins at the larval stage, where their tiny bodies reveal everything happening inside them.

One of the most remarkable facts is that zebrafish share nearly 70% of their genes with humans.

Even more fascinating, almost 80% of human disease-related genes have a counterpart in zebrafish. This means that many processes involved in heart formation, brain development, metabolism, and organ repair happen in similar ways in both species. In other words, studying a zebrafish larva often gives us valuable clues about what might happen in humans.

Another incredible feature is their transparent embryos. Unlike mammals, zebrafish embryos develop outside the mother's body, floating freely in the water. This natural gift lets scientists literally watch life develop. The beating heart, the branching blood vessels, the forming eyes, the expanding brain all become visible without a single incision or instrument. This transparency makes zebrafish a living window into developmental biology.

And everything happens fast. Very fast. Within just five days, most major organs including the heart, liver, kidney, eyes, and nervous system are already formed and functioning. What takes weeks in mammals can be observed in days in zebrafish. This rapid growth is a huge advantage for developmental toxicity research, where scientists need to detect subtle changes in development over short periods.

Beyond their biology, zebrafish offer practical benefits too. They are easy to maintain, require very little space, and produce hundreds of eggs at a time. This makes them extremely cost-effective compared to rodent or larger animal models. Their small size and high embryo numbers allow researchers to test many chemicals at once, enabling efficient large-scale screening for safety testing, drug evaluation, and environmental monitoring.

Together, these qualities, genetic similarity, transparency, rapid development, and practicality make zebrafish larvae one of the most powerful and versatile models in modern toxicology research.

Once zebrafish embryos are collected, they become tiny test subjects that reveal how chemicals can affect developing life. The process is surprisingly

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simple yet scientifically powerful. Researchers place freshly fertilized embryos into small wells or dishes containing water mixed with different concentrations of a chemical it could be a phthalate from plastics, a pesticide from agriculture, a pharmaceutical drug, a heavy metal, or even a pollutant suspected to be harmful.

Because zebrafish embryos absorb chemicals directly from the surrounding water, the exposure is both natural and controlled. Scientists can adjust the dose drop by drop, allowing them to observe how even very low concentrations influence development.

### **Studying Physical Malformations**

As the embryos grow, researchers watch closely for structural changes. Under a microscope, they can easily detect a curved or bent spine, indicating developmental disruption; pericardial edema, where fluid collects around the heart; abnormal brain or eye formation; and yolk sac or tail deformities. Since their bodies are transparent, any change in shape, size, or organ structure becomes instantly visible.

### **Delayed Hatching and Slower Growth**

Healthy zebrafish embryos typically hatch within 48–72 hours. Toxic chemicals can slow down this process. Scientists measure hatching time, body length, and growth rate over the first few days. A delayed or stunted larva often signals developmental toxicity, even when no obvious malformation is present.

### **Changes in Movement and Behavior**

Zebrafish larvae are active swimmers, making behavior an important indicator of toxicity. Researchers track hyperactivity or excessive movements, reduced swimming or sluggishness, abnormal responses to light or touch, and erratic or circular swimming patterns.

Such behavioural changes often point to neurotoxic effects on the brain and nervous system.

The strength of zebrafish research lies in its reliability and biological relevance. Many chemicals that cause defects in zebrafish larvae also cause similar problems in mammals. This means zebrafish studies act as an early-warning system, helping scientists predict risks to humans, wildlife, and the environment before moving to mammalian testing. Their ability to reveal toxic effects rapidly, ethically, and accurately makes zebrafish a crucial bridge between laboratory research and real-world safety assessments.

One of the biggest reasons zebrafish have become so popular in developmental toxicology is their remarkable ability to combine scientific power with ethical responsibility. In modern research, scientists are guided by the 3Rs principle: Replacement, Reduction, and Refinement, which encourages the use of methods that minimize harm to animals. Zebrafish larvae fit perfectly into this vision.

### **Supporting the 3Rs: Fewer Mammals, More Humane Research**

Before zebrafish became widely accepted, developmental toxicity studies relied heavily on mammals such as rats and rabbits. These tests were expensive, time-consuming, and raised significant ethical concerns. Zebrafish larvae offer a humane alternative.

Because early-stage zebrafish embryos (up to 5 days post-fertilization) are not classified as protected animals in many guidelines, they can be studied without the ethical issues associated with higher vertebrates. This means fewer mammals are needed for early screening, only the most promising or concerning chemicals move to higher animal testing, and overall suffering and animal usage are greatly reduced. Zebrafish help replace and reduce mammalian use while still providing reliable scientific data.

### **Easy to Maintain and Scientifically Efficient**

Zebrafish are not just ethical; they are also incredibly practical. They require small tanks, minimal space, simple feeding, and low maintenance cost.

A single breeding pair can produce **hundreds of embryos every week**, making them ideal for continuous research. Their embryos develop in water, making exposure experiments straightforward, clean, and easy to standardise.

### **Perfect for High-Throughput Screening**

In developmental toxicology, researchers often need to test many chemicals at different doses. This is where zebrafish truly shine. Because embryos are tiny, transparent, and plentiful, they can be placed in 24-well plates, 48-well plates, or 96-well plates. This allows scientists to test hundreds of embryos at the same time, dramatically increasing the speed of data generation. Automated imaging systems can scan plates, measure heart rate, capture movements, or detect malformations all in just minutes.

High-throughput capability means faster results, lower costs, more reliable comparisons, and the ability to test dozens of chemicals simultaneously. This efficiency makes zebrafish larvae one of the most powerful tools in toxicology today.

One of the reasons zebrafish have become so valuable in developmental toxicology is how accurately they reflect the effects of real-world chemicals. Many substances that harm human or animal embryos leave similar marks on zebrafish larvae making them excellent predictors of developmental risk. Here are a few impactful examples:

### **Plasticizers and Phthalates: Hidden Threats in Everyday Items**

Phthalates, such as BBP (butyl benzyl phthalate) and DBP (dibutyl phthalate), are widely used to soften plastics in toys, food packaging, cosmetics, and household items. When zebrafish embryos are exposed to these chemicals, scientists often observe a

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curved body axis or bent spinal column, heart swelling (pericardial edema), delayed hatching, and reduced body length.

These deformities reflect how phthalates may interfere with hormonal and developmental pathways in higher organisms as well.

### **Heavy Metals: Lead and Mercury Damage Developing Brains**

Environmental contamination with heavy metals like lead, mercury, and cadmium remains a serious global concern. Zebrafish studies show that even low doses of these metals can cause impaired brain and eye development, reduced neural activity, and abnormal swimming patterns.

Such effects mirror the neurodevelopmental problems seen in mammals and humans exposed to heavy metals, especially during early life stages.

### **Pharmaceuticals: Detecting Drug-Induced Birth Defects**

Some prescription drugs, while life-saving, may pose risks during pregnancy. A classic example is valproic acid, an anti-epileptic medication known to cause birth defects in humans. When tested in zebrafish, researchers observe facial and brain malformations, growth delays, and heart defects.

These effects closely resemble human congenital abnormalities associated with the drug, demonstrating the predictive power of the zebrafish model.

Together, these examples highlight how zebrafish larvae not only reveal how chemicals interfere with developing life but also help scientists understand risks long before they affect humans or wildlife. Their responses often align with observations in mammals, making zebrafish a reliable early-warning system in toxicology.

As science continues to advance, zebrafish are gaining an even more influential role in shaping the future of toxicology and biomedical research. Their simplicity, transparency, and genetic similarity to humans make them ideal partners for cutting-edge scientific tools and emerging technologies.

### **Merging Zebrafish with Omics Technologies**

Modern biology is shifting toward deeper, more holistic approaches such as genomics, transcriptomics, proteomics, and metabolomics. When these technologies are applied to zebrafish larvae, researchers can explore toxicity at an unprecedented depth. For example, genomics reveals how genetic variations influence susceptibility to toxins, transcriptomics uncovers which genes are turned on or off when embryos encounter harmful chemicals, and metabolomics helps identify biochemical changes, stress markers, and metabolic disruptions.

Together, these tools allow scientists not only to detect toxicity but also to understand why and how it occurs at the molecular level.

### **Expanding into Drug Discovery and Nanotoxicology**

Zebrafish are rapidly becoming a favourite model in drug discovery. Their rapid development, transparent bodies, and high-throughput suitability allow researchers to screen thousands of potential drug molecules in just days. This speeds up the search for safe and effective medicines.

They are also proving invaluable in nanotoxicology, a field focused on how nanoparticles behave in living organisms. Because nanoparticles can enter cells and organs easily, zebrafish provide a clear, real-time view of their distribution and effects—something difficult to achieve in mammals.

### **Powerful Tool for Environmental Monitoring**

With increasing concerns about water pollution, microplastics, and industrial waste, zebrafish larvae offer a sensitive, cost-effective system for detecting environmental toxins. By observing developmental changes, locomotion, and oxidative stress markers, scientists can monitor water quality and identify harmful contaminants before they affect ecosystems or human health.

### **Reducing the Cost and Time of Safety Assessments**

Traditional toxicity testing often takes months or years and requires significant financial investment. Zebrafish help overcome these limitations by offering faster results within days, lower maintenance costs, high-throughput capabilities, and early prediction of toxic effects before moving to mammalian studies.

This efficiency means safer chemicals, faster drug development, and reduced reliance on expensive animal testing.

As technology evolves, zebrafish will continue to bridge the gap between basic science and real-world applications. Their combination of biological relevance, ethical advantages, and compatibility with modern research tools positions them as one of the most promising models in the future of developmental toxicology.

In the world of developmental toxicology, zebrafish larvae have emerged as one of the most remarkable and versatile models. Their transparent bodies, rapid growth, and genetic similarity to humans allow scientists to observe the earliest stages of life with unmatched clarity. From revealing how chemicals disrupt organ formation to predicting long-term health risks, these tiny organisms play a critical role in protecting both human health and the environment.

By reducing the need for higher animal testing, supporting modern high-throughput methods, and integrating effortlessly with advanced omics technologies, zebrafish continue to reshape the landscape of safety research. Their impact stretches

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across drug discovery, environmental monitoring, and the global effort to understand how pollutants affect developing life.

Zebrafish larvae may be small, but their contribution to science and safety is enormous helping us build a healthier and more responsible future.

### **Conclusion**

Zebrafish embryos and larvae have emerged as a powerful model for developmental toxicology due to their transparency, rapid development, genetic similarity to humans, and suitability for high-throughput screening. They allow researchers to easily

observe structural, behavioral, and physiological changes caused by toxic substances. From environmental pollutants such as heavy metals and phthalates to pharmaceutical compounds, zebrafish studies provide valuable insights into how chemicals affect early development. Additionally, their use supports ethical research by reducing reliance on higher vertebrates while delivering reliable scientific data. As modern technologies like genomics, transcriptomics, and metabolomics continue to advance, zebrafish models will play an even greater role in understanding toxicity mechanisms and improving chemical safety assessment in the future.

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