Mixtures, Metabolites, and Mechanisms: Understanding Toxicology Using Zebrafish

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Abstract

For more than 60 years, zebrafish have been used in toxicological studies. Due to their transparency, genetic tractability, and compatibility with high-throughput screens, zebrafish embryos are uniquely suited to study the effects of pharmaceuticals and environmental insults on embryonic development, organ formation and function, and reproductive success. This special issue of Zebrafish highlights the ways zebrafish are used to investigate the toxic effects of endocrine disruptors, pesticides, and heavy metals.

BIOMEDICAL RESEARCHERS CONSIDER zebrafish a premier model system for studying vertebrate embryonic development and organ formation. Its status was announced to the world in 1996, upon the simultaneous publication of 37 articles describing the results of two mutagenesis screens that identified ~1500 mutations in >400 genes regulating development and organ formation.^{1,2} Since then, thousands of publications have reported the results of experiments using zebrafish to study embryonic development.

Many researchers do not realize that zebrafish have been employed in toxicological studies for >60 years, well before their adoption by developmental biologists. Initially used to identify teratogens and chemicals that perturb cell division,^{3,4} today zebrafish are also used to study the effects of pharmaceuticals and environmental insults (such as endocrine disruptors, pesticides, and heavy metals) on embryonic development, organ formation and function, and reproductive success.

Zebrafish possess numerous advantages for toxicology. Since fertilization and development occur externally, outside of the mother, it is simple to administer and/or withdraw toxicants to the embryo with tight temporal resolution. The embryos are transparent, allowing for comprehensive observation of cell movement and organ formation. Their small size and high fecundity make zebrafish easy and inexpensive to maintain and enable high-throughput screens of chemical libraries for hazard assessment,^{5,6} an approach that would be prohibitively expensive using rodent models. Moreover, there are established techniques for generating transgenic and mutant zebrafish and for observing and tracking cell populations of interest noninvasively in live embryos and larvae. Due to their transparency, genetic tractability, and compatibility with high-throughput screens, zebrafish have advantages

over other test systems when investigating the molecular and cellular mechanisms of toxicant exposure.

Three articles in this issue give insight into the mechanisms of how common toxicants can cause subtle physiological effects. Saley *et al.* investigate the effects of triclosan, an antibacterial agent found in consumer products such as soaps and detergents, on heart development in zebrafish.⁷ Hallauer *et al.* report how chronic arsenic exposure leads to neurological dysfunction and reduced body weight in progeny from exposed adults.⁸ Finally, Baker *et al.* examine the molecular and cellular changes in the testes after exposure to the persistent organic pollutant 2,3,7,8-tetrachlorodibenzodioxin, an endocrine disruptor that exerts transgenerational effects through the male germline.⁹

It is easy to expose animal models to a single toxicant and study its effects, but the real world is far more complex, where organisms are exposed to mixtures of multiple toxicants simultaneously. Fortunately, the ability of zebrafish to absorb agents administered in the water allows scientists to expose zebrafish to toxicant cocktails, mimicking what occurs in the environment. For example, naturally occurring dissolved organic carbon (DOC) can influence the bioavailability of some types of toxicants; Carmosini *et al.* demonstrate that the presence of DOC partially mitigates triclosan toxicity in zebrafish embryos.¹⁰ Velasques *et al.* examine the effects of the popular herbicide Roundup, a mixture of glyphosate and various adjuvants, on the antioxidant capacity of zebrafish liver and gills,¹¹ whereas Ku-Centurion *et al.* report that the commercial fungicide Monceren 250 SC, containing pencycuron with a mixture of carriers, causes DNA damage in zebrafish embryos.¹² Finally, wood ash, in widespread use as a forest fertilizer, can also contain many heavy metals. Consigli *et al.* correlate the metal content of

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ashes from various tree sources with effects on embryonic development.¹³

Understanding how toxicants are metabolized by the body is important for monitoring exposure and for mitigating exposure effects. Two articles in this issue advance the utility of the zebrafish model system by examining the metabolic effects of two environmentally relevant compounds. Berry *et al.* use high-resolution magic angle spin NMR to quantify metabolite changes after exposure to polymethoxy-1-alkenes, teratogenic compounds produced by freshwater algae.¹⁴ Makarova *et al.* compare the toxicity of the endocrine disruptor and estrogen receptor ligand bisphenol A (BPA) to intermediate products of its degradation and model how degradation products of BPA differentially bind to estrogen receptors.¹⁵

Zebrafish are also a valuable model for studying the side effects of pharmaceuticals and drugs of abuse, which are clinically studied in human adults. Results from nonpregnant adults are usually extrapolated to pregnant women and their fetuses, because compounds are not tested in this population for ethical reasons. Testing of pharmaceuticals on children is increasing, thanks to new regulatory agency requirements, but a large backlog remains. These issues create potential problems, as all of these groups are exposed to drugs, but the effects on an adult are often different from their effects on a fetus or child. Zebrafish can serve as a practical and powerful vertebrate model to explore how pharmaceuticals and drugs of abuse influence embryonic development and organ formation. Two articles in this issue review the use of zebrafish to investigate the effects of two widely used drugs, hallucinogens and alcohol.^{16,17} Of course, the degree to which various drugs' pharmacology in zebrafish is conserved in humans remains an open question.

Zebrafish are becoming more widely used for *in vivo* toxicology studies. They combine some of the high-throughput and molecular biology advantages of *in vitro* systems (e.g., cell culture) but add the relevance of whole-organism studies. It is unlikely that zebrafish will replace the traditional model organisms of toxicology such as the rat; rather, the zebrafish can extend the reach of the toxicologist to ascertain previously impossible or impractical data sets. Such progress, as in many fields, will be fastest at the borders between disciplines. Therefore, it will be important for researchers in developmental biology and toxicology to reach out to one another and share ideas, tools, and techniques. We hope that efforts such as this special issue of Zebrafish as well as meetings that unite toxicologists, developmental biologists, and biomedical researchers such as the Zebrafish Disease Model Society annual meeting will be steps toward new insights from toxicology.

Disclosure Statement

No competing financial interests exist.

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