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Beyond Mortality: Why Molecular Toxicogenomics Should Redefine Pesticide Risk Assessment in Aquatic Ecosystems

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Abstract

As the world becomes more dependent on pesticides, their use has significantly boosted agricultural output but also raised concerns about ecological health and environmental contamination. Pesticide contamination is often found in surface waters through agricultural runoff, leaching, spray drift, and wastewater discharge, making aquatic ecosystems among the most vulnerable environmental compartments. Traditional pesticide risk assessment approaches are primarily based on apical endpoints, including mortality, growth inhibition, reproductive impairment, and developmental abnormalities. These endpoints are still critical for hazard characterization but may not adequately capture early molecular events that precede detectable adverse effects. New molecular toxicology and omics studies have shown that environmentally relevant levels of pesticides can trigger extensive changes in gene expression, protein production, metabolic pathways, oxidative stress responses, endocrine signaling, immune regulation, and developmental processes, leading to effects without immediate death. With the advent of toxicogenomics, especially transcriptomics, proteomics, metabolomics, and multi-omics integration, new opportunities are now available to discover sensitive biomarkers, clarify mechanisms of toxicity, and enhance ecological risk prediction. The zebrafish (Danio rerio) is a closely related, fast-growing, and convenient vertebrate model for studying the molecular effects of pesticides in high-throughput screening. This opinion paper suggests that the traditional risk assessment of pesticides based on mortality should be replaced with a mechanism-based approach using toxicogenomic data. This transition would be beneficial for environmental protection, improved regulatory decision-making, and a better understanding of pesticide effects on aquatic biodiversity in an increasingly complex chemical landscape.

Keywords: Zebrafish Toxicogenomics; Aquatic Ecotoxicology; Environmental Risk Assessment; Multi-omics; Developmental Toxicity; Ecological Risk Assessment

Introduction

Global agricultural production has grown to a scale unparalleled in history, leading to unprecedented pesticide use. Pesticides, such as fungicides, insecticides and herbicides, are essential tools in protecting yields and ensuring food security for an ever-increasing global population. But at the same time, the use of pesticides has raised significant concerns for the environment, as high levels of these substances eventually reach non-target ecosystems. Agricultural runoff, spray drift, atmospheric deposition, and wastewater discharge can be serious problems in surface waters, rivers, lakes, estuaries, and wetlands. Aquatic organisms are thus exposed to environmental concentrations of complex combinations of pesticides, which are likely to have adverse biological effects over both short- and long-term time scales Yu, Wang, Yao, & Zhou [1].

Historically, traditional EC risk assessment has focused on apical endpoints, including mortality, growth inhibition, failure to reproduce, behavioral changes, and developmental abnormalities. Acute toxicity measures, such as LC50 and EC50, remain a key aspect of regulatory initiatives globally. While much research has gone into these endpoints, they provide little insight into the biological pathways that lead to toxicity. More importantly, they often fail to consider subtle molecular perturbations that may occur before any visible physiological or phenotypic change. This means that threats to the environment from chronic, low-level exposures can be underestimated, even though substantial cellular and molecular perturbations occur Rocha, Félix, & Farias [2].

There is growing evidence that many pesticides and other chemicals can cause toxicity in ways that do not always align with the traditional endpoints of toxicity testing based on death. Exposure to environmentally relevant concentrations has been linked to reproductive impairment, immunotoxicity, neurotoxicity, endocrine disruption, epigenetic changes, oxidative stress and mitochondrial dysfunction in aquatic organisms. These disruptions in molecules occur at concentrations well below those needed to kill them and could also lead to long-term effects at the population level. Lately, studies have focused on reproductive toxicity, developmental abnormalities, and transgenerational effects that can result from chronic pesticide exposure, which is why there is a need for more sensitive, mechanistically relevant evaluation methods. Emerging contaminants and residues are increasingly a concern, which has driven interest in novel environmental risk assessment approaches. It is worth stressing that traditional ecological evaluations cannot accurately represent the complexity of interactions among chemicals in aquatic ecosystems and may underestimate cumulative ecological effects under chronic exposure scenarios. Moreover, the growing diversity of contemporary agrochemicals introduces new issues for regulatory bodies that must assess environmental safety using traditional testing approaches. It is therefore critical to develop new methods that can identify early biological responses before irreversible ecological damage occurs Zhou, Chen, Liu, & Wang [3].

The zebrafish (Danio rerio) is among the most useful vertebrate models for aquatic toxicology and environmental risk assessment Dai [4]. The zebrafish is a popular organism for study because it has several special properties: its embryos develop quickly, it is optically transparent, it is highly fecund, has a short generation time, and has significant genetic homology with humans. These features support the simultaneous assessment of developmental, physiological, behavioral and molecular endpoints. Zebrafish has recently emerged as an excellent model for evaluating developmental, neurotoxicity, reproductive toxicity, and endocrine-disruption induced by pesticides, and it is an ideal model for environmental monitoring and mechanism-based toxicology studies Khan & Alhewairini [5]; Scholz [6].

Toxicogenomics has revolutionized the field of toxicological research, enabling comprehensive studies of biological responses at the molecular level. Toxicogenomics combines genomics, transcriptomics, proteomics and metabolomics to provide a comprehensive understanding of the complex interactions between environmental contaminants and biological systems. Toxicogenomics differs from traditional toxicity testing, which is more concerned with observable adverse outcomes, in that it enables the detection of molecular signatures of exposure and toxicity early in the testing process Li [7]. This type of information can help to elucidate affected biological pathways, molecular initiating events, and adverse outcome pathways that eventually lead to organismal and population-level effects. Transcriptomic technologies have had a tremendous impact on aquatic ecotoxicology. With high-throughput RNA sequencing, thousands of genes can be sequenced simultaneously, providing a detailed picture of cellular responses to chemical stressors. A large body of studies has shown that exposure to pesticides can alter genes that regulate oxidative stress, apoptosis, xenobiotic metabolism, immune function, endocrine signaling, and embryonic development. These transcriptomic responses often occur before any physiological abnormalities are observed, making them good candidates for early-warning markers in environmental monitoring and risk assessment Schirmer, Fischer, Madureira, & Pillai [8].

Together with advances in toxicology, proteomics and metabolomics are additional techniques that can contribute to understanding toxic mechanisms by providing insights into protein-level changes after exposure to chemicals and their metabolic pathways. Transcriptomics can be used to determine changes in gene expression. In contrast, proteomics and metabolomics can be used to determine changes at the functional level (biological consequences) at later levels of organization. Multi-omics studies have recently shown that combining multiple datasets can greatly enhance the identification of toxic mechanisms, the discovery of new biomarkers and the prediction of adverse effects. It is now widely appreciated that these methods can be useful in advancing environmental toxicology toward a more predictive and mechanistic science Chen [9].

The application of omics technologies in ecological risk assessment aligns with the overall shift towards New Approach Methodologies (NAMs). Scientists in regulatory agencies and the environment are now interested in alternative methods that can reduce uncertainty while simultaneously increasing understanding of mechanisms. Multi-omics platforms can offer sensitive exposure markers, aid in the development of AOP, and augment the predictive toxicology framework Min [10]. These advances can eventually help establish more accurate environmental quality standards and better protect the diversity of aquatic life. Even with significant scientific advances, regulators' use of toxicogenomic information in pesticide assessment remains a work in progress. Difficulties include data standardization, reproducibility, the complexity of bioinformatics, the interpretation of large datasets, and the setting of regulatory acceptance criteria. However, recent developments in computational biology, machine learning, systems toxicology and adverse outcome pathway frameworks are quickly overcoming these limitations. The increased availability and reduced cost of omics technologies will make their incorporation within the

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environmental risk assessment process more likely Nam, Bae, Ki, Ahn, & Rhee [11].

However, in today's environmental issues, the use of mortality as an endpoint seems increasingly inadequate. Survival of organisms is a key component of ecosystem health, but so is their ability to maintain reproductive success, developmental integrity, immune competence, behavioral function, and adaptive capacity. Molecular toxicogenomics is a revolutionary tool that can transform the way pesticides are assessed for risk, from post-exposure detection of damage to pre-exposure detection of biological perturbations that could lead to irreversible damage. This would help strengthen environmental protection, improve regulatory decision-making, and play a role in the sustainable management of agrochemicals in aquatic ecosystems.

Conclusion

Pesticide risk assessment in the future should not be limited to mortality but should focus more on molecular and mechanistic toxicity. Traditional endpoints, such as LC50, are important for hazard identification but are not sufficient to measure subtle biological disturbances that may result in long-term ecological effects. The emerging field of toxicogenomics, along with transcriptomics, proteomics, metabolomics, and multi-omics integration, has shown unprecedented promise for developing new tools to identify early indicators of pesticide exposure and elucidate the mechanisms of pesticide-induced toxicity. Zebrafish is now an essential model for linking molecular responses to developmental, physiological, and ecological outcomes. Incorporation of toxicogenomic tools into the regulatory process would enhance its sensitivity, predictiveness, and ecological relevance, and aid in the development of future generations of environmental risk assessment strategies. In an era of escalating threats from pesticide pollution and new contaminants in aquatic environments, the use of molecular toxicogenomics is more than a scientific tool; it is an essential step in the evolution of a more effective approach to environmental protection and sustainable chemical management.

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