

# Omic-based fingerprinting of neurotoxic and microtubule-disrupting pesticides in zebrafish embryos

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## Introduction

Plant protection products play a pivotal role in agriculture, yet their impact on non-target organisms and the environment raises growing concerns due to some of their non-selective harmful effects. Conventional environmental risk assessments of pesticides primarily focus on lethal concentrations, neglecting the intricate influence of sublethal effects and exposure timing during critical developmental phases, potentially leading to developmental abnormalities. This study employs transcriptomic analysis to uncover the molecular fingerprints of two pesticides – the neurotoxic insecticide endosulfan and the microtubule assembly inhibitor herbicide benfluralin - in zebrafish embryos. In the present study, zebrafish embryos were exposed to different sublethal concentrations of endosulfan and benfluralin in a modified 96 hours zebrafish embryo toxicity test (zFET).

## Methods

A modified version of the OECD 236 fish embryo toxicity test was employed to assess substance-induced gene expression alterations. At 96 hpf, total RNA was extracted and subjected to Illumina TruSeq library preparation for mRNA sequencing. mRNA-Seq reads were aligned to the zebrafish genome (version GRCz11). Differentially expressed genes (DEGs) were identified using three biological replicates per condition, including BH-adjusted  $p$ -value  $\leq 0.05$  and absolute  $\log_2$  fold change  $> 0.1$  using DESeq2.

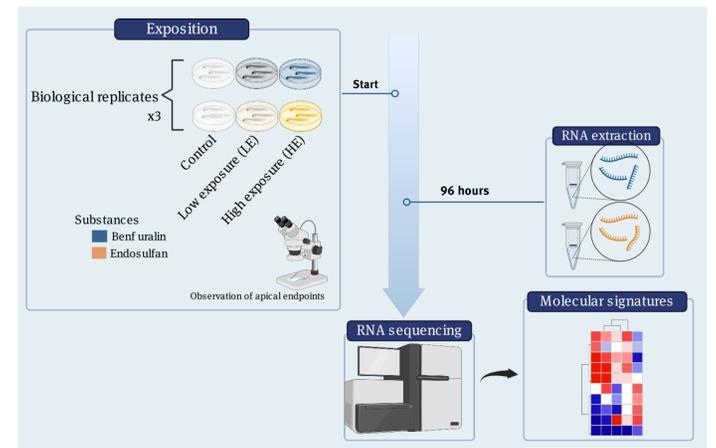


Figure 1: Experimental Setup. Created using Biorender.com

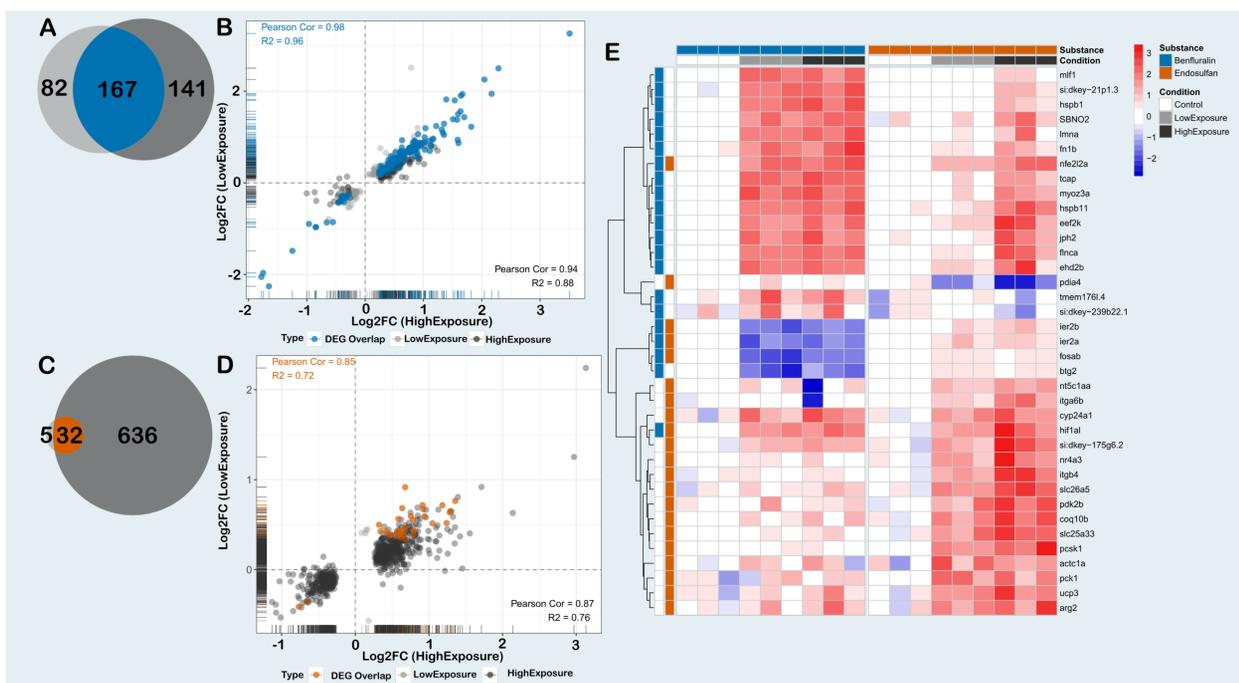


Figure 2: Illustrates transcriptomic responses to sublethal concentrations of the neurotoxic insecticide endosulfan and the microtubule assembly inhibitor herbicide benfluralin in zebrafish embryos at 96 hpf. The Venn diagram depicts the overlap and unique sets of differentially expressed genes (DEGs). Scatterplots compare the  $\log_2$ -fold change (lfc) values of DEGs observed at low and high sublethal test concentrations of benfluralin and endosulfan. (E) Heatmap showing patterns of DEGs.

## Results

Despite the absence of significant effects on survival or hatching rates compared to the control group, transcriptomic analysis revealed distinct molecular responses in zebrafish embryos exposed to each pesticide (Figure 2 A,B,C,D), providing insights into their specific impacts on biological processes. Benfluralin exposure resulted in the differential expression of genes associated with muscle cell differentiation, heart contraction, and skeletal muscle development. In contrast, exposure to endosulfan targeted genes crucial for hormone response, neuron apoptotic processes, and neuron death, which are pivotal for nervous system development (Figure 3). These findings underscore the complexity of sublethal effects induced by these compounds on non-target organisms, highlighting the importance of conducting a more comprehensive assessment that extends beyond survival-focused evaluations. Understanding the intricate molecular mechanisms underlying such sublethal effects is essential for conducting better-informed risk assessments and developing more effective mitigation strategies.

## Conclusion

Integrating these molecular insights into risk assessment frameworks is imperative, particularly in establishing transcriptomic points of departure (tPODs) that are protective against chronic effects. This integration recognizes the complexities that extend beyond traditional endpoint measurements and provides additional weight-of-evidence for a comprehensive assessment of pesticide toxicity. By incorporating -omic data, regulators and policymakers can better understand the underlying molecular mechanisms driving adverse effects, thereby improving the accuracy and reliability of risk assessments. Moreover, this approach facilitates the identification of early molecular markers of toxicity, enabling proactive and targeted mitigation strategies to protect the environment, human health, and enhance pesticide regulatory decision-making processes.

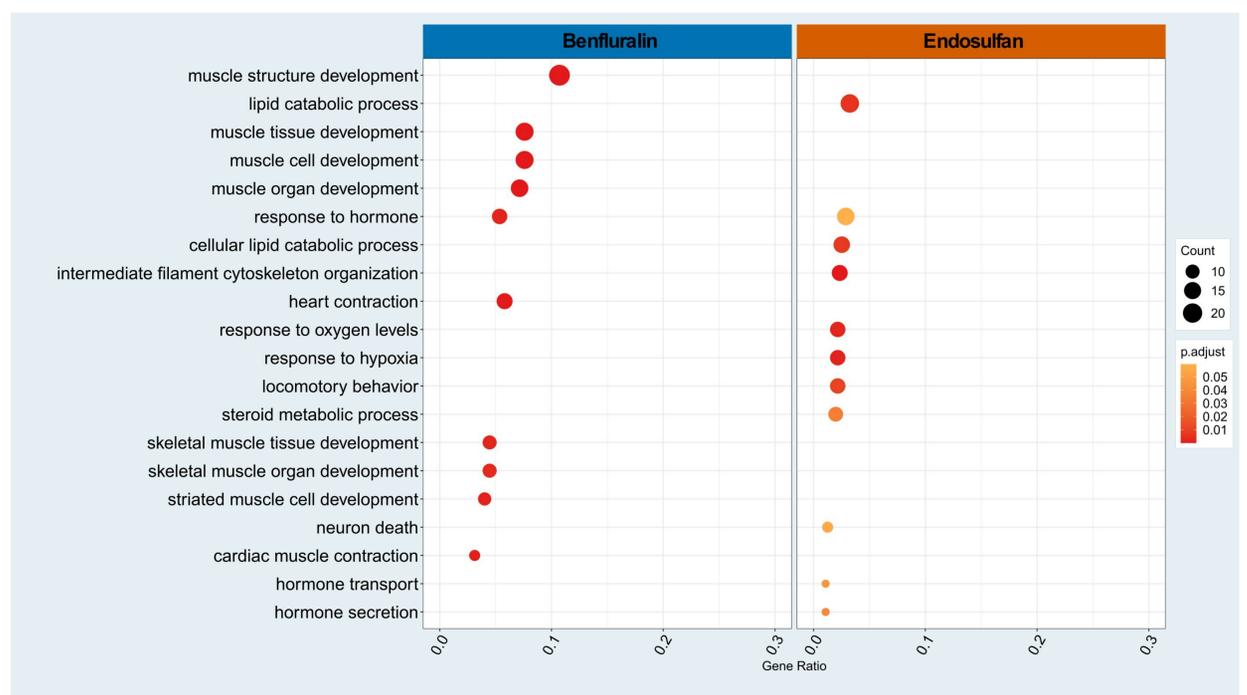


Figure 3: Bubble plot illustrating the results of overrepresentation analysis (ORA) for significantly enriched biological processes in the gene expression profiles observed after exposure of zebrafish embryos to benfluralin and endosulfan at 96 hpf.