National Institute of Environmental Health Sciences
Division of Translational Toxicology

Abstract 3527
Poster N680

Understanding the Range of Phenotypic Responses for the Embryonic Zebrafish Developmental Toxicity Assay

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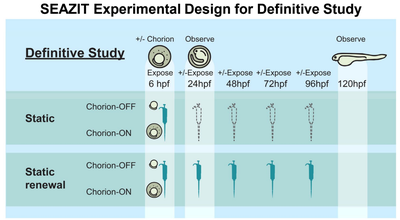
Background

- Embryonic zebrafish assays are used to screen chemicals for potential developmental toxicity.
- Many research groups employ different experimental protocols and often report toxicity as a single combined mortality and malformation metric. However, specific information on malformations is useful in chemical hazard assessments and can potentially inform on mechanism of action.
- The Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) explored how different experimental conditions can impact assessment of toxicological response to optimize zebrafish protocols (1).
  - The study specifically focused on the effects of varying two protocol elements identified by zebrafish experts: experimental media renewal ("Static" vs. "Static Renewal") and chorion status of the embryo ("Chorionated" vs. "Dechorionated").
  - Data from SEAZIT is publicly available via the SEAZIT-DIVER resource (QR code below).
- This poster summarizes our findings on the variability of malformations induced by the positive or vehicle controls within three laboratories (Lab A, Lab B, Lab C) that used varying experimental protocol conditions.

Explore SEAZIT-DIVER:

Protocol information, data, visualizations!
https://manticore.niehs.nih.gov/seazit

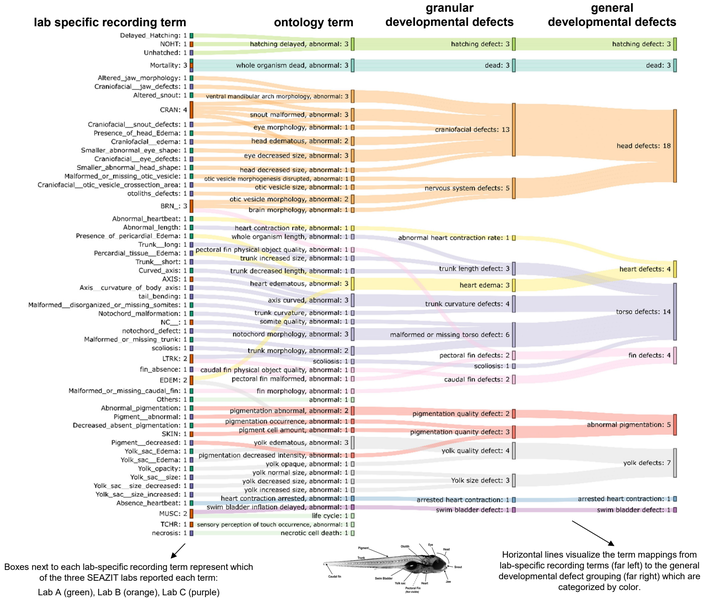
SEAZIT Experimental Design for Definitive Study



Labs participating in the study exposed embryos under four exposure conditions: static exposure (-, dashed pipette), static renewal of exposure media every 24 hours (+, filled pipette), using both chorionated (ON) and dechorionated (OFF) embryos.

Ontology Mapping and Hierarchical Grouping of Laboratory-specific Phenotype Recording Terms

This diagram, taken from Hsieh et al. (2), shows our harmonization approach for phenotype terms to enable comparisons between labs, since each of the labs utilized different terms to describe phenotypes. This process started with the lab-specific recording terms, which were mapped to ontology terms gathered from the Zebrafish Phenotype Ontology (3). Terms were then grouped by granular and general developmental defects based on anatomical region.



Boxes next to each lab-specific recording term represent which of the three SEAZIT labs reported each term:
Lab A (green), Lab B (orange), Lab C (purple)

Horizontal lines visualize the term mappings from lab-specific recording terms (far left) to the general developmental defect grouping (far right) which are categorized by color.

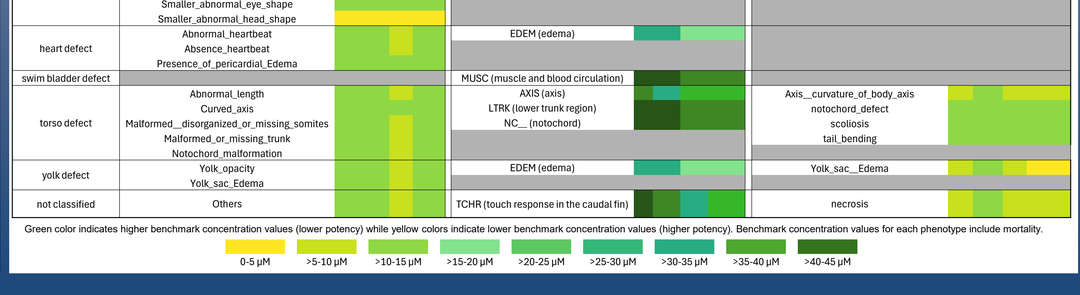
Vehicle and Positive Control Altered Phenotypes Differ Among Experimental Condition and Lab

In general, head defects were among the most sensitive endpoints across both positive and negative controls and all three labs, with some lab-specific terms such as "abnormal head shape" (Lab A), "snout defect", and "jaw defect" (Lab C) driving the head defect finding. There were also some notable differences in percent effect and benchmark concentration values among the labs, which may have been due to experimental handling or genetic variation in the zebrafish strain. Lab C reported a higher number of altered phenotypes in both the negative and positive control groups compared to the other two labs, and potency of the positive control was markedly lower in Lab B compared to the other labs.

The heatmaps below summarize the altered phenotypes observed by the three labs after treatment with vehicle and positive controls. Colors indicate severity of effects, as detailed below, per lab-specific recording term, which are furthered grouped by general developmental defect. Grey shading indicates that there was no additional lab-specific phenotype term or that a lab did not include that phenotype in their assessments of development defects. Vertical lines indicate that the phenotype could not be assessed due to removal of the chorion. Bolded and underlined terms indicate a significant difference between results observed under different experimental conditions within one lab (chi-squared test for vehicle control, with asterisks indicating phenotypes that were not statistically tested due to small sample sizes, and ANOVA test for the positive control endpoints alive with altered phenotype(s) and mortality).

S-C=Static-Chorionated, S-DC=Static-Dechorionated, SR-C=Static Renewal-Chorionated, SR-DC=Static Renewal-Dechorionated

Vehicle Control, 0.5% Dimethyl Sulfoxide, Percent Affected



Dark purple color indicates a lower percentage of affected larvae while light turquoise color indicates a higher percentage affected.

0-0.5% affected | >0.5-1% affected | >1-3% affected | >3-5% affected | >5-8% affected | >8-10% affected | >10-15% affected

Positive Control, 3,4-Dichloroaniline, Benchmark Concentrations

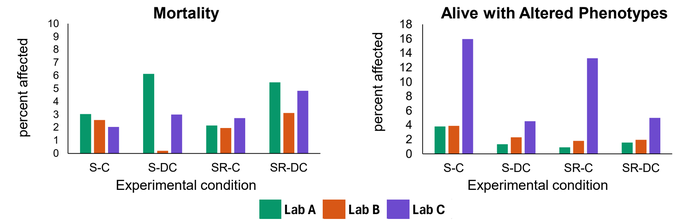
Green color indicates higher benchmark concentration values (lower potency) while yellow colors indicate lower benchmark concentration values (higher potency). Benchmark concentration values for each phenotype include mortality.

0-5 µM | >5-10 µM | >10-15 µM | >15-20 µM | >20-25 µM | >25-30 µM | >30-35 µM | >35-40 µM | >40-45 µM

Altered Phenotypes Varied After Exposure to Vehicle Control

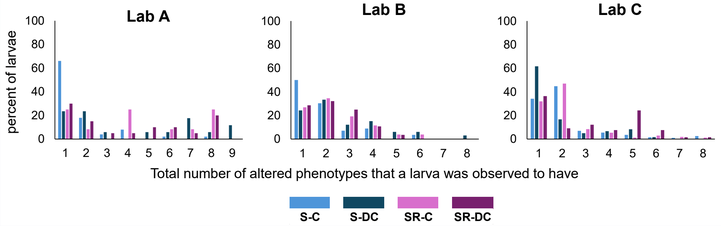
While Labs A and C observed decreased survival of dechorionated embryos, there were few other commonalities among altered phenotypes observed in the three labs. The bar charts below depict, for each lab: the percent mortality among larvae (number dead/total number tested; left chart) and the percent of the total number of altered phenotypes observed (number of larvae alive with altered phenotype(s)/total number alive; right chart).

S-C=Static-Chorionated, S-DC=Static-Dechorionated, SR-C=Static Renewal-Chorionated, SR-DC=Static Renewal-Dechorionated



Co-occurrence of Phenotypes

To demonstrate co-occurrence of phenotypes, we further analyzed data on live embryos according to how many altered phenotypes a larva was reported to have. In the bar graphs below, percent of larvae was calculated as the number of larvae with the number of observed altered phenotypes divided by the total number of live larvae with altered phenotypes. While the results varied among the different experimental conditions, for each lab on average 52-70% of larvae were observed to have 1 or 2 altered phenotypes among the vehicle control larvae, demonstrating that there was not a large co-occurrence of the reported altered phenotypes.



Key Takeaways and Future Directions

- These results help clarify the landscape of variability within and across laboratories that implement unique zebrafish testing protocols.
- The range of phenotypes reported by each lab indicates that inclusion of refined phenotypes, such as those used to describe head defects in assessments, can enable sensitive findings.
- Static-renewal exposure generally led to increased phenotypic effects after exposure to the positive control for all three labs, but some differences were noted between chorion status for the vehicle control.
- The SEAZIT definitive interlaboratory study included testing of a broader set of 42 chemicals. Future work will expand analyses to include between-lab comparisons, evaluations of phenotype alterations within this tested chemical set, and comparisons of variability among other models utilized for developmental toxicity screening approaches.

References and Acknowledgements

(1) Hamm et al. Interlaboratory Study on Zebrafish in Toxicology: Systematic Evaluation of the Application of Zebrafish in Toxicology's (SEAZIT's) Evaluation of Developmental Toxicity. Toxics 2024, 12, 93. https://doi.org/10.3390/toxics12010093
(2) Hsieh et al. Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT): Developing a Data Analysis Pipeline for the Assessment of Developmental Toxicity with an Interlaboratory Study. Toxics 2023, 11, 407. https://doi.org/10.3390/toxics11050407
(3) Zebrafish Phenotype Ontology: https://www.ebi.ac.uk/ols/ontologies/zp, accessed on 10 April 2023

Check out other SEAZIT related presentations:

Poster N673 Ryan et al. Evaluating the Impacts of Experimental Parameters on Chemical Potency in the Zebrafish Developmental Toxicity Assay: Findings from the SEAZIT Interlaboratory Study

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