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

Bridgett Hill[1], Jonathan Hamm[1], Jui-Hua Hsieh[2], Emily N. Reinke[1], Helena T. Hogberg[2], Kristen Ryan[2]

[1]Inotiv, RTP, NC, United States; [2]NIH/NIEHS/DTT RTP, NC, United States

Background and Purpose

Embryonic zebrafish assays have been utilized to screen chemically induced developmental toxicity. Many research groups employ different experimental protocols and often report toxicity as a combined mortality and malformation metric. Such an approach can provide a sensitive indication of toxicity potential or serve specific research needs (e.g., excluding any malformed larvae from behavioral analyses). 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 protocols can impact assessment of toxicological response. Since the completion of two study phases, SEAZIT has produced data harmonization approaches (e.g., phenotypic ontology mapping), data analysis pipelines, and a publicly available web application, SEAZIT-DIVER (https://manticore.niehs.nih.gov/seazit). Here we investigate the variability of malformations induced by the positive or vehicle controls within and between laboratories across varying experimental protocol conditions.

Methods

A definitive study with three participating laboratories tested a suite of 42 blinded chemicals in concentration response using four combinations of experimental conditions: embryos with chorion intact or dechorionated, and static or static-renewal media exposure. Briefly, single zebrafish embryos (approximately 4-6 hours postfertilization) were placed into individual wells of a 96-well plate for exposure to blinded chemicals, the positive control substance (3,4-dichloroaniline, DCA, 0.1–50 µM, n = 1–2 embryos per concentration per plate), and the vehicle control (0.5% dimethyl sulfoxide, n = 11–12 embryos per plate) for 5 days. Malformations were first recorded using laboratory-specific phenotype recording terms. Due to variations in how these were reported, these laboratory-specific terms were annotated to Zebrafish Phenotype Ontology terms and further grouped into granular and general categories based on anatomical region to assist with cross-laboratory comparisons. Benchmark concentrations (BMC) per phenotype term and experimental conditions for each laboratory were calculated, and incidence rates were calculated for the vehicle control. Phenotypic responses that were significant at different concentrations than those inducing mortality were determined and were termed "specific developmental responses".

Results

This presentation discusses the incidence rate and BMC of phenotype data for positive and vehicle controls to characterize the range of responses among laboratories and experimental conditions. The BMC values for the positive control DCA (3–45 µM) and incidence rates for the vehicle control (0–8%) varied across laboratories and experimental conditions. For DCA, the most specific altered general phenotype across all three laboratories was head defects, including

abnormal head shape and snout/jaw defects. Some of these responses were dependent on the exposure scenario, with lower BMC values observed under static-renewal conditions for two laboratories. In addition, one laboratory noted heart and yolk defects induced by lower concentrations of DCA when testing with static-renewal conditions. For the vehicle control, one laboratory reported higher incidence rates for altered phenotypes (0–8% affected, with 36% of assessed phenotypes at least 3%) compared to the other two laboratories (0–3% affected), suggesting that experimental handling may have been a factor. For the laboratory with the higher range of incidence rates, some of the phenotypes observed at rates greater than 3% included craniofacial deformities and scoliosis. Incidence of these phenotypes appeared to be more correlated with chorion status than solution renewal conditions. Other differences included high mortality for dechorionated embryos in two laboratories. Delayed hatching under static conditions was also noted by one laboratory.

Conclusions

Overall, these results can help to understand the landscape of variability within and across laboratories that implement unique zebrafish testing protocols. While there were similarities in general phenotype responses for the selected positive and vehicle controls, the range of different phenotypes reported by each laboratory indicates that inclusion of refined phenotypes such as head defects in assessments can enable sensitive findings. Differences in static-renewal exposure generally led to increased phenotypic effects for all three laboratories, but some differences were noted between chorion status for the vehicle control. Future work will expand analyses to include phenotype alterations within the tested chemical set and compare variability among other models utilized for developmental toxicity screening approaches. All screening data can be accessed and explored through SEAZIT-DIVER. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.