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

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Embryonic zebrafish assays have been utilized to screen for chemically induced developmental toxicity. Many research groups employ different experimental protocols and often report toxicity as a combined mortality and altered phenotype metric. However, specific information on phenotypes is useful in chemical hazard assessments and can potentially inform on mechanisms of action. Here we investigate the incidence variability of phenotypes that can occur within and between laboratories across varying experimental protocol conditions. An interlaboratory study with three participating laboratories tested a suite of 42 blinded chemicals at five or more concentrations each using four combinations of experimental conditions: embryos with chorion intact or removed (dechorionated) and exposure to test chemical via a static or static-renewal protocol. Briefly, single zebrafish embryos (approximately 4-6 hours postfertilization) were placed into individual wells of a 96-well plate for a 5-day 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). Phenotypes 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 for all chemicals. The BMC values for DCA varied between 3–45 µM 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. Ongoing analysis for the remainder of the 42 blinded chemicals will determine if head defects remain a sensitive indicator for developmental toxicity or if phenotype patterns are chemical- or laboratory-specific. An additional evaluation of vehicle-treated embryos also demonstrated variability across laboratories and experimental conditions. One laboratory reported higher incidence rates for altered phenotypes (0–16% affected) compared to the other two laboratories (0–3% affected), suggesting that experimental handling may have been a factor. For the laboratory with the higher incidence rates, some of the phenotypes observed at rates greater than 3% included craniofacial deformities and scoliosis. 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 responses for the selected positive and vehicle controls, the range of different phenotypes reported by each laboratory indicates that inclusion of refined phenotypes in assessments can enable sensitive findings. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C. The views expressed above do not necessarily represent the official positions of any federal agency.