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Using the Larval Zebrafish Locomotor Assay in Functional Neurotoxicity Screening: Light Intensity and the Order of Stimulus Presentation Affect the Outcome. S. Padilla[1], K.A. Jarema[2] and D. L. Hunter[1]. [1]Integrated Systems Toxicology Division, [2]Toxicology Assessment Division, NHEERL, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

The U.S. Environmental Protection Agency is evaluating methods to screen and prioritize large numbers of chemicals using 6 day old zebrafish (*Danio rerio*) as an alternative test model for detecting neurotoxic chemicals. We use a behavioral testing paradigm that simultaneously tests individual larval zebrafish under both light and dark conditions in a 96-well plate using a video tracking system. By controlling the duration and level of intensity of light during the test session, we are able to manipulate the locomotor activity of the fish, assessing changes in locomotion during either dark or light periods. Our ability to manipulate different variables allowed us to explore whether the light level used affected our ability to detect effects of chemical exposure on locomotor activity. Using a testing paradigm that employed light levels covering four orders of magnitude (0.01 lux to 51.3 lux), we assessed the activity of larvae dosed acutely with ethanol (a known neurotoxic chemical and disruptor of locomotor activity). We found that the highest ethanol concentration (2% ethanol; n=46) affected activity regardless of the level of the light stimulus. The other two ethanol concentrations (1% or 0.5%; n=46 and 47, respectively) showed the largest hyperactive effects during the periods with the lowest light levels (i.e., in the dark or at 0.01 lux). At higher light levels (0.5 lux or 51.3 lux), a completely different pattern emerged with no difference in activity between the controls (n=44) and the 0.5% ethanol group, and little to no hypoactivity in the 1% group. Moreover, the order of stimulus presentation also affected the outcome: in the 0.5 lux group that was subsequent to a 0.01 lux exposure period, there was no difference in activity among the 1%, 0.5% and control groups, but if that same light level (0.5 lux) was subsequent to a much brighter light presentation (51.3 lux), the 1% ethanol group exhibited marked hypoactivity. Therefore, in the zebrafish larval locomotor test, the level of light used and the order of presentation of the stimuli will affect the sensitivity of the assay and the direction of the effect (i.e., either hyper- or hypo-activity). *This abstract may not necessarily reflect official Agency policy.*