Rapid In Vivo Assessment of Bioactivity in Zebrafish: High Content Data for Predictive Toxicology

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Early developmental life stages are often uniquely sensitive to environmental insults, due in part to the enormous changes in cellular differentiation, proliferation and migration required to form the required cell types, tissues and organs. Molecular signaling underlies all of these processes. Thus, most toxic responses result from disruption of molecular signaling; making early developmental life stages the ideal time to determine if a chemical can perturb the expression or activity of essential molecular pathways. A central goal of our group is to identify bioactive compounds, and to identify their molecular targets that are acted upon to disrupt vertebrate development. We developed an efficient screening process using embryonic zebrafish to assess chemical effects on behavior, morphology and gene expression. As a proof of concept, we obtained the EPA phase I and II Toxcast chemicals that consist of 1,078 compounds made up of pesticides, drugs, “green” chemicals, chemicals in cosmetics and other consumer products. A static non-renewal exposure paradigm was initiated beginning at 6 hours post fertilization (hpf) using a range of concentrations in 96-well plates. A total of 32 individual animals were assessed at each concentration. We also kept exposed embryos completely in the dark until 24 hpf, and assessed a simple photo-motor response using the Photo-motor Response Assessment Tool (PRAT). PRAT quantifies individual embryonic photo-motor response following two pulses of bright light. The initial pulse normally results in pronounced movement, and the second light pulse usually produces no activity. At 120 hpf we also assessed photo-induced larval locomotor activity using Viewpoint Zebrabox to determine if chemical exposure impacted CNS-dependent motor responses. Finally, each larva was assessed for changes in a suite of 20 morphological endpoints at 120 hpf. We have successfully conducted the phenotypic and behavioral screening on all 1,078 compounds and a summary of the results will be discussed.