Toxicity Screening of the ToxCast Phase II Chemical Library Using a Zebrafish Developmental Assay

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As part of the chemical screening and prioritization research program of the US EPA, the ToxCast Phase II chemicals were assessed using a vertebrate screen for developmental toxicity. Zebrafish embryos (*Danio rerio*) were exposed in 96-well plates from late-blastula stage (6hr postfertilization, pf) through day 5pf (1-2 days post-hatch). All exposures were by immersion and renewed daily. The 700 chemicals included food additives, consumer use product ingredients, pesticides, failed pharmaceuticals, and "green" plasticizers (http://epa.gov/ncct/toxcast/chemicals.html). Intra- and inter-plate replicates were included for quality control. Developmental toxicity was initially assessed using a single nominal concentration of 80 µM: positives and a selection of negatives were confirmed by concentration-response determinations. On day 5pf, larvae were moved from exposure solution to a control solution without chemical, and on day 6pf were assessed for overt toxicity (*i.e.,* death, non-hatching and dysmorphology; n=4 embryos per chemical). Dysmorphology was a combined score using both in-life observation and brightfield, high-content image analysis. Overt toxicity was noted with 46% of the chemicals tested, compared to 62% positive chemicals when the ToxCast Phase I library, consisting of mostly pesticide active ingredients, was previously tested. As with the Phase I library, the octanol-water partition coefficient ($\log_{kow}$) of the Phase II library chemicals was positively correlated with overt toxicity: there were 18% positive chemicals with $\log_{kow}$ <0; 41% positive chemicals with $\log_{kow}$ of 0 to 4; and 67% positive chemicals with a $\log_{kow}$ >4. All chemicals positive at the single concentration were further assessed for potency using a Dose-Response Study (8-point, semi-log concentration curve: n=3 embryos per concentration). These data demonstrate the utility of zebrafish in medium-throughput chemical testing programs for detection of adverse developmental outcomes. *This abstract may not necessarily reflect official Agency policy*.