



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

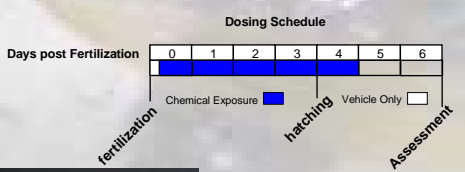
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Introduction

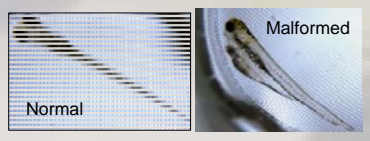
The zebrafish model is becoming popular in developmental biology. These vertebrates share 70-80% genetic homology, as well as structural similarities, with humans. Its small size, ability to produce a large number of progeny, and rapid development make this model ideal for high-throughput screening of toxic compounds. Our overall goal was to determine if the zebrafish embryo assay will be able to identify the developmentally toxic compounds in a group of chemicals; these compounds can then be sent on for further testing in mammalian models. This present group of studies assessed whether the results from the zebrafish embryo development assay correlate with the results from the mammalian toxicity studies. *This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.*

Methods

Outbred, wildtype, zebrafish eggs were placed into individual wells of a microtiter plate at approximately 8 hours after fertilization. The eggs were exposed by immersion to each of the ToxCast320 chemicals in the water (10% Hanks balanced salt solution; 0.4% DMSO final concentration) at a final concentration of 80µM for the first 4 days of development. On the fifth day, the larvae were removed from the chemical and placed in 10% Hanks solution. On Day 6, each embryo was assessed for malformations and lethality by someone blinded to the treatment group of each animal. Positives were defined as a dead animal or an animal with frank malformations.



6-8 Hr post fertilization

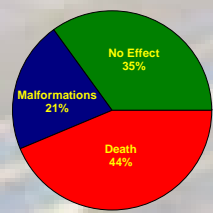


6 days post fertilization

Results and Conclusions

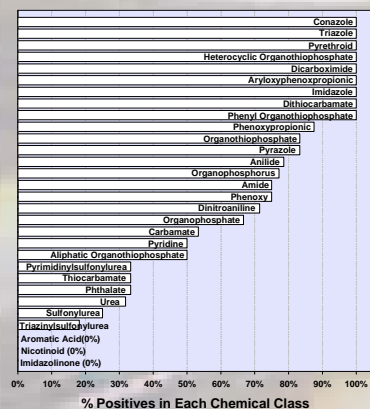
A. Biological Activity:

Approximately 65% of the ToxCast_320 chemicals (administered at 80 µM) produced malformations or lethality in the Zebrafish Embryos.



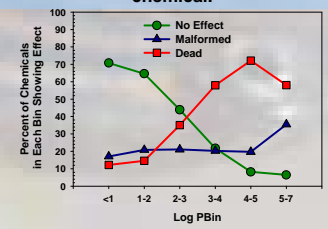
C. Biological Activity is Related to Chemical Class

Below are all chemical classifications that had >5 members. Some classes caused little to no toxicity (e.g., aromatic acid, nicotinoid, imidazolinone) while all members of other classes caused malformations or lethality (e.g. conazole, pyrethroid, dithiocarbamate, subclasses of the organophosphates, etc.).



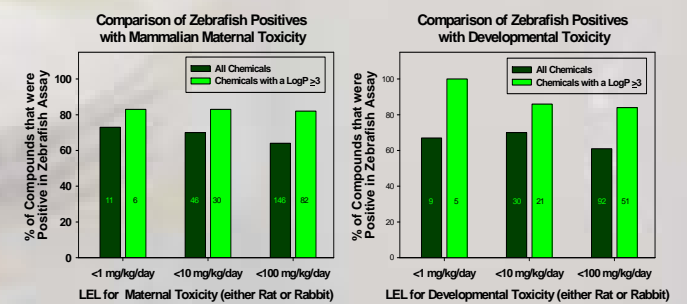
B. Biological Activity is Related to Hydrophobicity

As the hydrophobicity (LogP) of the chemicals increased, so did the likelihood of causing death. Conversely, it is much more likely to have no effect with a more hydrophilic (LogP≤3) than with a hydrophobic chemical.



D. Biological Activity in the Zebrafish Assay Correlates with Mammalian Toxicity

Data from rat and rabbit prenatal studies (<http://www.epa.gov/ncct/toxrefdb>) were compared to the Zebrafish assay data. The Zebrafish assay was positive for ≥ 70% of the more potent chemicals [LOEL(LEL) ≤100 mg/kg/day]. The number in each bar is the number of chemicals in each bin. The degree of concordance increases when comparing only with the chemicals that have a LogP≥3 (lighter green bars).



E. Future Studies

A dose response assessment is ongoing and should be completed within three months.

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