

## High-throughput screening examines multiple effects of 1060 compounds on zebrafish

By Nancy Lamontagne

Researchers led by Oregon State University Superfund Research Program grantee Robert Tanguay, Ph.D., used high-throughput screening to analyze 1,060 unique compounds for 22 possible effects on zebrafish embryos.

Researchers said this is one of the largest systematic *in vivo* toxicological studies to date. Using zebrafish to test a large number of chemicals with known structures, and look at a large number of effects, will allow the identification of groups of chemicals that may share the same mechanism of toxicity. Chemicals that show a response in zebrafish can also be further studied using other systems, such as cell-based testing.

"Our study demonstrates that it is now possible to rapidly evaluate the bioactivity of a large number of chemicals in the whole animal," Tanguay (<http://tanguaylab.com/?q=user/33>) said. "The ability to screen more of the chemical space will help the field move closer to relevant whole animal chemical structure-response relationships for predictive toxicology."

The highly automated and streamlined screening approach developed by the researchers is detailed in a [paper](http://www.ncbi.nlm.nih.gov/pubmed/24136191) (<http://www.ncbi.nlm.nih.gov/pubmed/24136191>) published in the January issue of *Toxicological Sciences*.

### A comprehensive screening approach

Using their new approach, the researchers conducted developmental and neurotoxicity screening of 1,060 unique ToxCast phase 1 and phase 2 chemicals. The U.S. Environmental Protection Agency (EPA) National Center for Computational Toxicology [ToxCast program](http://www.epa.gov/ncct/toxcast/) (<http://www.epa.gov/ncct/toxcast/>) is assessing a large number of chemicals, using a diverse set of *in vitro* tests, with the goal of developing cost-effective ways to prioritize the thousands of chemicals for which there is no toxicity information. ToxCast phase 1 chemicals are well-studied chemicals, such as pesticides, and phase 2 chemicals come from a broad range of sources, including industrial and consumer products, food additives, green products, cosmetics, and pharmaceutical drugs.

Using automation to streamline the screening process, investigators employed an automated embryo placement system to load 6-hour-old zebrafish embryos into 96-well plates. Then the researchers added one chemical per well, at six concentrations, with 32 embryos used for each concentration. They monitored various developmental, behavioral, and morphological endpoints, at time points up to 120 hours after fertilization.

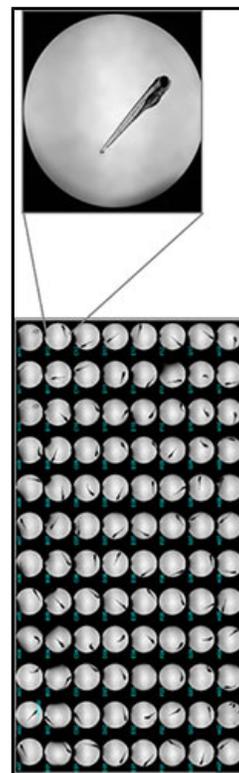
The diversity of the measured endpoints, and the large number of animals per test, increased the screening's sensitivity to detect hazardous chemicals. Also, by measuring 18 of the 22 endpoints simultaneously over time, the researchers could determine relationships between the endpoints and embryonic development. David Reif, Ph.D., (<http://genetics.sciences.ncsu.edu/index.php/people/david-m.-reif>) a study collaborator from North Carolina State University, was instrumental in developing novel analysis and visualization tools, such as a custom photomotor response assessment tool to quantify an embryo's response to pulses of light.

### Biological responses

Of the 1,060 unique chemicals evaluated, 487 showed significant biological responses. The data demonstrated that mortality alone was not a good determinant of toxicity, because of the high number



Tanguay said that the goal in screening chemicals with zebrafish is not simply to identify hazardous compounds, but to also generate data that will allow prediction of toxicity. (Photo courtesy of Robert Tanguay)



Each well, above, contains a zebrafish embryo. "This was an enormous study that required a multidisciplinary team

of false negatives. Global patterns of variation across the tested chemicals revealed high correlation among endpoints. However, some chemicals, such as the pesticides thiram, ziram, and sodium dimethyldithiocarbamate, affected only a single developmental endpoint in the zebrafish. The researchers concluded that their approach detected adverse responses that other methods would miss.

The scientists continue to refine their experimental approach and to expand the number of chemicals tested. They are working with the EPA, the National Toxicology Program, and others to compare their zebrafish findings with data collected from mammalian cells and whole animal models. The results will allow them to determine the chemical classes for which the zebrafish model is predictive, and to identify the limitations of the model.

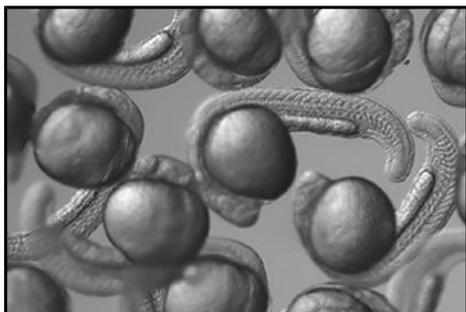
*composed of mechanical engineers, software engineers, toxicologists, statisticians, and informatics specialists to complete," Tanguay said. (Photo courtesy of Robert Tanguay)*

*Citation: Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL.*

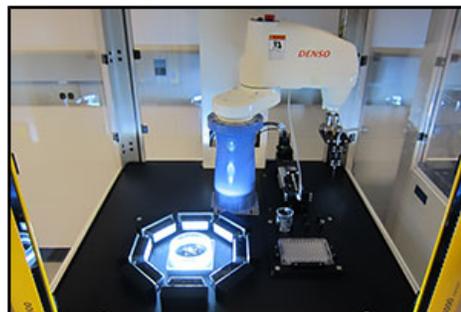
*(<http://www.ncbi.nlm.nih.gov/pubmed/24136191>)*

2014. Multidimensional in vivo hazard assessment using zebrafish. *Toxicol Sci* 137(1):212-233.

(Nancy Lamontagne is a science writer with MDB Inc., a contractor for the NIEHS Division of Extramural Research and Training.)



*Zebrafish have several advantages as a model system for toxicology screening. They develop rapidly, have a short life cycle, and are clear for the first few days of life. Also, approximately 84 percent of human genes involved in disease are present in zebrafish. The zebrafish embryos above are 24 hours old. (Photo courtesy of Robert Tanguay)*



*Automation helped improve accuracy and streamline the high-throughput zebrafish screening. The researchers used this robot to load the embryos into 96-well plates. (Photo courtesy of Robert Tanguay)*

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