NTP talk explores zebrafish as a vertebrate model in toxicity screening

By Kristen Ryan

Arantza Muriana, Ph.D., gave a presentation Aug. 19 on the utility of zebrafish for toxicity testing, speaking by telephone to a capacity audience of NTP and NIEHS scientists with interests in the advancement of predictive toxicology. Her talk, "Zebrafish as a Tool for Screening and Prioritization of Chemicals for Toxicity Testing," was hosted jointly by the NTP Toxicology Branch (TB) and Biomolecular Screening Branch (BSB).

Muriana is director of Research and Development Management for Biobide S.L., an international contract research organization offering preclinical testing for early drug development. Biobide's unique specialty is providing the opportunity to rapidly assess numerous and complex aspects of drug-induced or chemical-induced toxicity in zebrafish.

Linked Video

Watch 'Drawn to Science: Zebrafish - a new model for drug discovery,' a charming video about this tiny fish that is having an enormous impact on toxicology and drug development (03:32).

"The NTP has been interested in identifying new strategies for screening and prioritizing compounds for *in vivo* testing, especially with the increasing nominations of classes of compounds such as flame retardants, PAHs, and phenolic benzotriazoles (PBZT)," said NTP contract toxicologist Mamta Behl, Ph.D., presentation host. "The zebrafish is a powerful tool to screen compounds, especially for early development, since it is a vertebrate, has a high genetic homology with humans, and the assays can be automated to provide relatively high-throughput along with high-content analysis."

An emerging model for toxicology and drug development

As Muriana explained, the adult and developing zebrafish are well established as model organisms for studies of vertebrate *(http://en.wikipedia.org/wiki/Vertebrate)* development, gene expression, and behavior, with over 12,000 research papers published within the last 10 years. In particular, zebrafish embryos have many applications, including the assessment of chemicals for their potential acute toxicity and teratogenicity, as well as organ-specific toxicities, including cardiotoxicity, hepatotoxicity, and neurotoxicity. At Biobide, she said, these assays have been developed to perform with a

Partnerships in zebrafish screening

NTP has several collaborations underway to examine the utility of zebrafish for screening and prioritizing chemicals, to ultimately serve as a model for in vivo hazard characterization:

-Robert Tanguay, Ph.D., at Oregon State University has tested over 3,000 compounds of interest to NTP, to complement the ongoing high-throughput screening efforts in the U.S. government's multiagency Tox21 research program. His research focuses on examining the effects of selected chemicals and chemical classes on zebrafish development and associated gene expression pathways (see related story).

-Christopher Weis, Ph.D., toxicology liaison in the NIEHS Office of the Director, and Stephanie Padilla, Ph.D., of the U.S. Environmental Protection Agency, are investigating the effects of flame retardants on zebrafish development, as well as behavior.

high degree of sensitivity and specificity, and can also be conducted under GLP (Good Laboratory Practice) testing regulations.

The zebrafish model is also adaptable to automated, high-throughput technologies, which are currently of interest to NTP (see text box).

During the seminar, Muriana highlighted the wide range of applications for toxicity testing in zebrafish, as well as Biobide's approach to refine and reduce the use of animals, while saving time and expense. At Biobide, a MultiTox Assay was developed to narrow down a large chemical set, by screening zebrafish through a particular sequence of assays, rather than performing one assay at a time.

Can the model work for NTP?

While zebrafish seem to be ideal for drug development, individuals such as NTP contract pathologist Deepa Rao, Ph.D., questioned how well this strategy would work for environmental chemicals, since very little is known about their toxicity profiles at the onset of testing. Muriana pointed out that Biobide can customize assays. In response to a question on throughput from NTP molecular toxicologist Scott Auerbach, Ph.D., she estimated that nearly 100 compounds could be tested across three assays within three months' time.

A lively discussion led to several interesting questions from the audience, including one by TB head Paul Foster, Ph.D., about whether or not these assays, using embryos at day five, can accurately capture necessary windows of development for the urogenital system. Foster wondered whether longer-term assays can be conducted, to evaluate the effects of chronic exposures.

Overall, the seminar strengthened the growing appreciation for the value of zebrafish in toxicity testing, and brought insight to the program for future alternative and complimentary testing strategies within NTP.

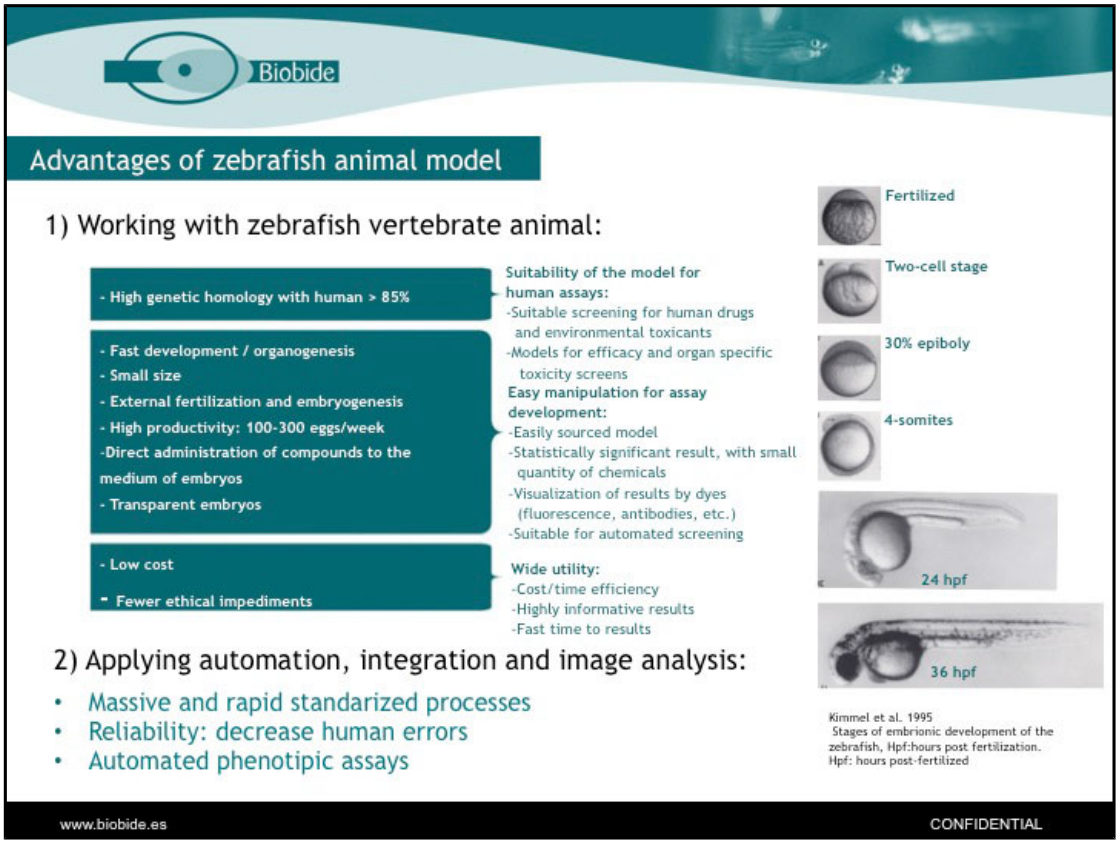
(Kristen Ryan, Ph.D., is an Intramural Research Training Award Fellow in the NTP Toxicology Branch.)

Behl introduced Muriana, whose talk was broadcast from Spain. Behl also moderated the question-and-answer session. (Photo courtesy of Michael Garske)

BSB head Raymond Tice, Ph.D. , described goals of the NTP predictive toxicology program. Seated beside him is Warren Casey, Ph.D., acting director of the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, which has an explicit interest in refining and reducing the use of animals in testing. (Photo courtesy of Michael Garske)

Rachel Goldsmith, Ph.D. , of the NTP WormTox Group, asked about characterization of COX2 genes in zebrafish, something Muriana said her company has yet to do. (Photo courtesy of Michael Garske)

Like some of her colleagues, Rao wanted assurance that the drug development screening model would work as well in toxicity screening. (Photo courtesy of Michael Garske)



Muriana used this slide to summarize the many advantages of the zebrafish model. (Image courtesy of Arantza Muriana)
