charles river

Summary

The zebrafish is a valuable model for developmental toxicity testing due to its efficiency in detecting mammalian toxicants. It maintains a high concordance with traditional mammalian assay results while supporting the principles of the 3Rs.

SAFETY ASSESSMENT

Zebrafish as an Alternative Model for Developmental Toxicity Testing

Approaches

Compared to standard mammalian embryo-fetal development (EFD) studies, zebrafish embryo-larval assays provide a screening and investigative tool capable of testing a much larger number of compounds.[1] Use of this model has become increasingly common in drug discovery to screen compounds for lead selection and optimization, and in drug development for investigation of developmental toxicity mechanisms and issue resolution.

The five-day zebrafish developmental toxicity assay is based on well-tested methods[2] with demonstrated value in pharmaceutical and chemical compound screening.[3,4,5] In addition, the assay has been designed to predict the outcome of standard developmental toxicity testing methods defined in guidance from the US FDA and ICH for pharmaceuticals and the US EPA and OECD for chemicals.

A typical screen consists of:

- Treatment with a range of concentrations of the test compound (e.g., 0.1 to 100 μM)
- Stock solution of test compound in either DMSO or an aqueous vehicle added to zebrafish embryo medium
- Continuous exposure of zebrafish embryos/larvae in the treated embryo medium between approximately 4 and 120 hours post-fertilization (developmental periods generally analogous to the period of treatment in mammalian EFD studies)
- Assessment of viability, growth, morphology and functional endpoints

The results of these tests are then used for identification of potential developmental toxicity hazards. The data are used to predict whether the test compound is likely to produce developmental toxicity in standard mammalian EFD studies or human exposures.

Performance

The concordance between the outcome of zebrafish screening and mammalian developmental toxicity has been evaluated in several studies and may be higher than 80%.[3,4] The types of effects observed in zebrafish were generally correlated with those found in mammals. The model is especially efficient at detecting strong mammalian toxicants, demonstrating the utility of the assay in prioritizing and refining testing in traditional models.

Zebrafish and the 3Rs

The biomedical research community consistently pursues the 3Rs principles of replacement, reduction and refinement. Zebrafish research supports each of these principles as follows:

- **Refinement:** Using the results of zebrafish screening to design definitive mammalian studies that will provide the most useful information (e.g., collection of additional endpoints, shifting timing of studies in the drug development process)
- **Replacement:** Testing chemicals under REACH regulations in a nonmammalian model rather than rodents or rabbits
- **Reduction:** Reducing the number of compounds that need to be tested in mammalian models by facilitating the choice of candidates with a greater likelihood of success

References

1. Brannen, K.C., Panzica-Kelly, J.M., Charlap, J.H., Augustine-Rauch, K.A., "Zebrafish: A Nonmammalian Model of Developmental Toxicology" in *Developmental Toxicology, Third Edition* (ed. Hansen, D.K., Abbot, B.D) 215-242 (Informa HealthCare, 2009).
2. Brannen, K.C., Charlap, J.H., Lewis, E.M. "Zebrafish Teratogenicity Testing" in *Teratogenicity Testing: Methods and Protocols (Methods in Molecular Biology Series)* (ed. Barrow, P.C.) 383-401 (Humana Press, 2013).
3. Brannen, K.C., Panzica-Kelly, J.M., Danberry, T.L., Augustine-Rauch, K.A. "Development of a Zebrafish Embryo Teratogenicity Assay and Quantitative Prediction Model." *Birth Defects Research Part B, Developmental Reproductive Toxicology*. 89, 66-77. (2010).
4. Nagel, R. "DarT: The Embryo Test with the Zebrafish *Danio rerio* - A General Model in Ecotoxicology and Toxicology." *ALTEX*. 19, Suppl 1:38-48 (2002).
5. Ball, J.S. et al. (2014). "Fishing for Teratogens: A Consortium Effort for a Harmonized Zebrafish Developmental Toxicology Assay." *Toxicological Sciences*. (Epub, February 20, 2014).

charles river

askcharlesriver@criver.com • www.criver.com