

Opening Session

Aquatic Animal Models – Not Just for Ecotox Anymore

Daniel L. Villeneuve, PhD

Mid-Continent Ecology Division, U.S. Environmental Protection Agency

A wide range of internationally harmonized toxicity test guidelines employing aquatic animal models has been established for regulatory use. For fish alone, there are over a dozen internationally harmonized toxicity test guidelines that have been or are being validated. To date, nearly all of these guidelines focus on direct observation of apical toxicity outcomes (i.e., impacts on survival, growth, or reproduction) and are intended solely to support ecological risk assessment. However, with increased attention to understanding mechanisms of toxicity, describing adverse outcome pathways, and collecting pathway-based data, there is increased opportunity to blur the lines between ecological and human health toxicity testing and risk assessment and take a more integrated approach that effectively employs available data, including data from alternative models.

This presentation will introduce some of the most widely used fish-based toxicity tests. It will highlight some of the barriers that have prevented more widespread use of pathway-based data in a regulatory context. It will then describe the adverse outcome pathway framework and how formalizing that framework can support scientifically defensible extrapolation of pathway-based data across levels of biological organization and among taxa. Examples illustrating use of the adverse outcome pathway framework to support a more integrated approach to toxicity testing and chemical hazard assessment will be provided.

The contents of this abstract neither constitute nor necessarily reflect official Environmental Protection Agency views or policy. All studies using animals or animal tissues referred to in this presentation were carried out in accordance with applicable animal care laws, regulations, and guidelines and approved by an Institutional Animal Care and Use Committee.

Zebrafish Embryos in Drug Safety Assessment

Jyotshna Kanungo, PhD

*Division of Neurotoxicology, National Center for Toxicological Research,
U.S. Food and Drug Administration*

Zebrafish embryos are routinely used in chemical toxicity assessments and are considered excellent preclinical models. As an alternative animal model, zebrafish embryos are mostly considered suitable for noninvasive developmental toxicity testing and for rapid screening of small molecules. Additionally, for mechanistic studies, zebrafish embryos are better suited than higher order vertebrates and may be more predictive than *in vitro* cell culture systems. Several FDA-regulated drugs show effects (alone or in combination with other drugs) in these embryos that are similar to those observed in humans. However, in contrast to mammalian models, the mode(s) of action (MOA) using phenotypic, biochemical, and genomic approaches can be conveniently elucidated in zebrafish embryos. Drug absorption (accumulation) in these embryos is an important aspect for determining whether the observed effects are specific, dose-dependent and comparable with those of mammalian models as well as humans. Emphasis on potential therapeutic intervention points and translatability is further supported as efforts are made to identify the effector molecule(s) of drug actions. Dose–response and MOA studies, as well as reversal of adverse outcomes based on the MOA studies, are critically compared with the information available from other mammalian models with the specific goal in mind that such studies may provide clues as to how the adverse outcomes of these drugs might occur in humans thus leading to better risk characterization and assessment.

The Use of Zebrafish for Drug Safety Assessment Within the Pharmaceutical Industry: *An (Ex) Insider's Perspective*

Matthew J. Winter, PhD

*School of Biosciences, College of Life and Environmental Sciences
University of Exeter*

(Former affiliation: AstraZeneca Research and Development)

Over recent years the zebrafish has emerged as a credible nonmammalian vertebrate model for human drug safety assessment. In particular, the embryo–larval form is 3Rs-friendly and possesses a range of attributes (e.g. small size, rapid development, transparent organogenesis) that facilitate its use with low amounts of test compound in microwell plates. These factors are particularly desirable in the development of higher throughput assays for use in early-stage drug safety assessment ('frontloading'). Unsurprisingly, the zebrafish has attracted the interest of the pharmaceutical industry as a potentially useful additional model.^{1,2}

The range of safety assessment disciplines for which zebrafish-based assays have been proposed is wide: from developmental toxicity to cardiovascular function, from ototoxicity to behavioral-based assays addressing adverse effects such as seizure and addiction potential. In our experience, however, there are more applications for which the zebrafish could be utilized routinely within safety assessment screening programs than is actually the case. Here, using our own experiences and examples from the literature, we explore areas where the zebrafish appears to have been successfully deployed to meet early-stage drug safety assessment demands, and touch upon possible reasons for underutilization in others, within the context of pharmaceutical industry expectations for new models. These expectations include: adequate prediction of (pre)clinical outcome; desired levels of throughput and compound requirements; required levels of automation; and the recurrent issues of relative exposure concentrations, therapeutic versus assay effect levels, metabolic capability and clinical relevance.

The overall aim of this presentation is to provide the audience with a better understanding of which properties are attractive in the context of human drug safety assessment, where the zebrafish model could and has proven useful, and what is expected of a zebrafish-based assay before it is suitable for application within a pharmaceutical-industry safety assessment setting.

References

- ¹ Redfern W.S., Waldron G., Winter M.J., Butler P., Holbrook M., Wallis R., and Valentin J-P. (2008) Zebrafish assays as early safety pharmacology screens: Paradigm shift or red herring? *Journal of Pharmacological and Toxicological Methods*, **58**, 110-117.
- ² Fleming A. and Alderton W.K. (2013) Zebrafish in pharmaceutical industry research: finding the best fit. *Drug Discovery Today: Disease Models*, **10**, e43–e50.