www.toxgurukul.org

TOXGURUKUL FOUNDATION

Pictorial Abstract

TOXICOLOGY NEWSLETTERS

"Progress in science comes when experiments contradict theory."

- Richard Feynman

October, 2020

Important Announcement

ToxGurukul Foundation Presents

1. ToxGurukul's Webinar Series

Resource person: **Dr. Helena Kandarova**
(Senior Scientist, Centre of Experimental Medicine, Slovak Academy of Sciences, Slovakia)

Topic: 'Alternative methods and 3D tissue models'

Date and Time: **Nov. 7th, 2020, 8:00 p.m.** Indian Standard Time (IST).
Duration: 90 minutes (1hr+30 min Q&A)

Registration Link: Click Here

2. ToxGuruQuiz-2020

For more details and Registration Link: Click Here

Request for donations: Any sponsorship(s) to cover the cost of webinar conferencing/ student sponsorship/ prize for upcoming **Tox Quizzes, please contact the undersigned (**drawn towards **Toxgurukul Foundation).**

~ Dr. K.S. Rao
toxrao@gmail.com
+91-733-783-0074

Editor:
Sapna Gupta
editor.toxgurukul@gmail.com

Zebrafish counterparts for non-clinical safety studies: a snapshot review

Dr. Pushkar Kulkarni

Center for Innovation in Molecular and Pharmaceutical Sciences, Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500046, Telangana, India. Email: kk.pushkar@gmail.com, pushkark@drils.org

ABSTRACT

"What, Why, How and When" are the quintessential questions for any field of research. Keeping this spirit, the present article is an attempt to provide a short appraisal on the state of zebrafish safety methods and suggest directions for the future. Although, zebrafish models have attracted curiosity and research interest over last two decades, a snapshot on their counterparts for the non-clinical safety studies is long overdue. This article provides short descriptions of various methods and their stage of utility in drug safety evaluation. It aims to help toxicologists from academia and industry to the choose appropriate zebrafish methods to address issue of safety assessment when there is a need for a quick and cost-effective alternative.

INTRODUCTION

In last couple of decades Zebrafish have gained popularity in pharmaceutical research. These developments have followed from their use in research for environmental toxicity, developmental biology, genetic research, evolution biology and other disciplines of fundamental research [1-3]. In pharmaceutical research, they are being used widely for development of disease models to understand pathobiology, discover and/or validate new druggable targets, and, creation of screening assays for pharmacology and toxicology [1-3].

Over the years, zebrafish toxicology screens have been developed for various toxicity endpoint encompassing almost all the aspects of non-clinical safety assessment studies.

Zebrafish (*Danio rerio*)

- Tropical freshwater fish
- Native to water bodies of the Indian subcontinent
- Larvae optically transparent
- Amenable to large sample studies
- Several genomic, cellular, and physiological features are conserved



In this review, we will try to understand the various zebrafish counterparts for the safety assessment studies. There will be differences in protocols published by various research groups across the world, however, in this article, the general perspective on these assays has been presented. **Table 1** provides a snapshot glance at zebrafish counterparts for routine safety assessment studies.

Table 1: Routine safety studies and their zebrafish counterparts

Category	Routine Non-Clinical Safety Studies	Zebrafish Counterparts
Genotoxicity	▪ Ames ▪ Mouse Micronucleus ▪ Chromosomal Aberration	▪ Micronucleus ▪ Chromosomal aberration ▪ Comet assay ▪ $EGFP^{mut}$ reverse mutation assay
Carcinogenicity	▪ 2-year rat study ▪ 2-year mouse study	▪ Tumorigenesis in long terms studies ▪ Short term studies in p53 heterozygotes
CNS Safety Pharmacology	▪ Irwin (modified) test ▪ Functional observation battery in repeated dose toxicity	▪ Behavioural analysis (group of assays) ▪ Adult fish EEG ▪ Developmental neurotoxicity assay
Cardiovascular Safety Pharmacology	▪ hERG ▪ Dog/Primate telemetry ▪ Purkinje fibre	▪ Larval heart rate, AV block ▪ Adult fish ECG
Respiratory Safety Pharmacology	▪ Plethysmograph	▪ Swim bladder assessment
General Toxicity	▪ Acute toxicity ▪ Repeated dose toxicity (14 days to 6 months) ▪ Vital organs assessment (in vitro, exploratory)	▪ Acute fish toxicity ▪ Vital organ studies by simple phenotype assessment for: – Neurotoxicity – Hepatotoxicity – Nephrotoxicity – Immunogenicity
Reproductive Toxicity	▪ Fertility ▪ Teratogenicity ▪ Pre- and post-natal development	▪ Fertility ▪ Male Vitellogenin ▪ Teratogenicity & Developmental toxicity

Overall strengths and weaknesses of zebrafish from safety assessment perspective

Strengths:

Zebrafish are the organisms with over 70% conserved genome as compared to humans and organ systems and functions similar to humans. Over 80% disease causing genes in humans have zebrafish counterparts [4]. A single zebrafish female produces large number of viable and healthy eggs allowing large sample studies for statistical power. Embryo-larval stages of zebrafish are translucent allowing observation of several safety evaluation endpoints in an intact organism, including in live organism in real time. The translucency also allows to observe endpoints using simple staining, immunohistochemistry, in-situ techniques, etc. Zebrafish are convenient and cost effective to maintain, e.g., 50 fish can be maintained in a space equivalent to one mouse cage. For embryo-larval based studies, these can be arrayed in multi-well plates and observed under simple stereo-zoom microscope. Small size also allows to conduct toxicity assessments with low quantities of the candidate drugs.

Weaknesses:

The major limitation of zebrafish for human safety assessment is that it's a fish, therefore have major physiological differences when compared to other mammalian animal models. These differences make it difficult to correlate data and establish predictivity. The predictive aspect for zebrafish, similar to any research model, is a tricky question, as none of the models predict clinical outcomes accurately. In fact, pivotal clinical trials also fall short of predicting outcomes of definitive clinical trials.

The second important weakness is the duplication of some of the genes in zebrafish that increases complexity of functional impact of these genes, thus making interpretation difficult. However, the fact that the entire zebrafish genome is sequenced, such aspects can be now factored in safety evaluation.

The third major drawback is lack of widely standardized protocols and their validation. Although, many major pharmaceutical companies and reputed academic laboratories use zebrafish models and there are over 40,000 publications using them, regulatory agencies will still depend on standardization of study protocols and widespread validation of these

models. Therefore, good quality data generation and its analysis are key to establish the regulatory acceptance of any model for predictions on clinical outcomes.

DESCRIPTION OF SOME IMPORTANT METHODOLOGIES

Genotoxicity

Comet assay, micronucleus test and chromosomal aberration test are standard clastogenicity and DNA damage tests that have been published in zebrafish [5]. The principles of these methods are similar to the conventional methods used in-vitro or in rodent models. While these tests offer the general advantages of zebrafish, the classical models, along with in-silico approaches, have the benefit of extensive data and validation performed over several decades and are known to have substantial predictive value. Zebrafish standard battery assays may be useful in very early stages of a discovery program where potentially pro-genotoxic agents will require in-vivo assessment with very low compound available in a quick turnaround time.

One promising approach for genotoxicity assessment using zebrafish is the reverse mutation assays proposed in a European Patent titled “Method for determining genotoxicity using non-fluorescent proteins” [6]. This assay is based on the principle of reverse mutagenesis wherein transgenic zebrafish lines are created that express a non-fluorescent variation of a protein, e.g. EGFP (enhanced green fluorescent protein) variant ($EGFP^{mut}$), and exposure of zebrafish larvae (between 24 – 96 hours post fertilization) to genotoxic test substances results in a reverse mutation to green fluoresce that could be observed and analysed using simple microscopic and image analysis techniques [6]. This method needs specific attention as it provides the in-vivo environment and possibilities of DNA repair (either from the organism or external introduction of human enzymes). Organ specific target proteins that might be susceptible to certain mutagenic candidate drugs could be developed using similar approach and relevant proteins. This assay promises to be an important addition/alternative to pharmaceutical risk assessment for genotoxicity.

Carcinogenicity

ICH S1 Expert Working Group (S1 EWG) started discussion on changes to the ICH S1 guidelines for assessment of carcinogenicity using the 2

rodent species, 2-year assay [7]. The major considerations of the S1 EWG is to study and suggest ways to predict the outcome of a 2-year rat carcinogenicity assay based on knowledge of toxicological pathways and other classical toxicology study data. Other considerations include the possibility of using only one species i.e. rat and waive of requirement for second species, use of transgenic mouse models such as rasH2 or p53 heterozygote mice with a 6-9 months study and using other weight of evidence (WOE) approaches [7]. This discussion and effort on part of ICH suggests that regulators are very keen on reducing the time of the carcinogenic risk assessment. The EWG is evaluating 50 representative compounds as part of their assessment to propose changes to the S1 guidelines.

Zebrafish carcinogenicity studies in wild type and p53 heterozygotes have been reported in substantial numbers now. These suggests that 3-6 months evaluation in zebrafish models could be used to predict the carcinogenic potential of candidate drugs [8-10]. Furthermore, several biomarker studies can be conducted for mechanistic evaluation with large sample size. Although the protocols for zebrafish carcinogenicity assays need standardization and validation, the potential benefits of developing these assays are substantial. Especially a zebrafish carcinogenicity assay in p53 heterozygotes will not just have cost and time implications but an exploratory study, with very low quantities of test compound, in early stages of drug development can avoid late stage attrition of candidate drugs, which has far more value.

CNS Safety Pharmacology and Neurotoxicity

Neurobehavior is an important aspect of safety assessment that must be studied in whole organisms; as in-vitro or 3D organoids are less likely to depict any of these phenotypes. The major CNS safety pharmacology effects that have been studied well in zebrafish are locomotor activity, sedation/excitation, anxiogenic/anxiolytic behaviour, startle response, convulsions and EEG measurements [11, 12]. The major advantages of these endpoints are that almost all of them can be performed in both larval (high throughput) and adult zebrafish, are non-invasive and there are automated systems available with defined quantitative parameters. Several publications have reported good correlation between zebrafish

models and human behavioural end points. A set of 5-6 endpoints (locomotion, sedation/excitation, anxiety, startle response, convulsions and shoaling) in zebrafish could act as surrogate for the modified Irwin test in rodents.

In terms of general neurotoxicity, the National Centre of Toxicology Research (NCTR) of the US FDA have been routinely publishing papers on zebrafish developmental neurotoxicity including standard protocol for the same [13, 14].

Neurobehavioral effects and neurotoxicity can have go/no-go impact in a drug discovery program and thus early assessment of these risks is valuable. Several major pharmaceutical companies are known to use zebrafish for such screens either in-house or at CROs/academia suggesting that zebrafish models for neurotoxicity have higher acceptance in the industry for early screening [15].

Cardiac Safety Pharmacology

Cardiac safety pharmacology, especially for prediction of potential to cause QT-prolongation is a major aspect of safety assessment for pharmaceuticals. A “thorough QT study” in non-rodent (commonly dog telemetry study) is conducted for this aspect of risk assessment. The major focus in this area has shifted to developing in-silico predictions for hERG inhibition and by implication QT liability. Availability of a good in-vitro method like the hERG channel assay have subdued the interest in simpler zebrafish assays on heart rate and AV block. However, the in-vitro hERG channel assay along with adult zebrafish ECG could provide a substantial advantage for early prediction of pre-clinical and clinical QT risk [16-18].

Furthermore, zebrafish ECG study can be used as an in-vivo system for drug effects on specific sub-populations of congenital long QT syndrome (LQTS). There is increasing understanding that three major LQTS genes KCNQ1, KCNH2, and SCN5A have been associated with this condition and such individuals are at higher risk of drug induced QT prolongation [19,20]. Furthermore, several single nucleotide polymorphisms (SNPs) are responsible for making certain set of individuals more susceptible to such drug induced effects [20]. Zebrafish models using genetic manipulations

of LQTS genes have been now reported and they suggest high conservation for major pathways [21-24].

QT-prolongation liability is a fatal risk at pharmacodynamic doses and hence requires to be assessed in order to ensure that none of the known suspectable populations is at a drug induced risk. Adult zebrafish ECG model, with and without genetically modified strains, offer to be valuable model system to address this aspect of cardiac safety assessment.

Respiratory Safety Pharmacology and Toxicity

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are assessed in classical toxicity studies in rodents and also in respiratory safety pharmacology. The zebrafish swim bladder is becoming very appealing organ system with structural and functional similarities with mammalian lungs [25]. Fluorescence fish lines or other florescence and imaging techniques can be used to assess ALI or ARDS in transparent zebrafish larvae in real time. There are recent reports of modelling chronic obstructive pulmonary disease (COPD) and other inflammatory conditions in zebrafish swim bladder [25-27]. More reports of standardization and validation of studies in swim bladder would help develop protocols for respiratory safety evaluation.

Hepatotoxicity

Drug induced liver injury (DILI) in zebrafish can be assessed using histopathology and biomarkers of liver injury in both adults and larvae. The most promising quick, simple and early detection assay for is use of simple morphological endpoints in 3 – 7 days old larvae wherein liver size, liver optical density and yolk absorption are considered good markers of hepatotoxic effect of a candidate drug [28]. Yolk absorption especially is a good indicator of liver function as it is a sole source of nutrition for larvae till day 7 of birth. A set of these endpoints were studied by Janssen and Pfizer in collaboration with Evotec for 50 selected compounds and the sensitivity, specificity and predictivity was claimed to be over 80% when correlated to classical 28-day toxicity in rats [28]. Further analysis and publications in last few years have suggested that zebrafish being a whole animal model can be used to understand mechanisms using follow-up experiments and thus aid in better risk assessment.

Nephrotoxicity

There are two good methods to evaluate renal safety of candidate drugs in zebrafish. One is a simple renal function assessment using rhodamine-dextran dye [29]. The principle is simple, a normally functioning zebrafish renal system will clear the dye at a certain rate; however, in case of renal injury this rate of clearance will be reduced significantly (in exceptional cases it might be increased). This assay can be performed in 5 – 7 days old larvae and end point can be observed under fluorescence filters of simple microscope.

Another recently published assay is a high throughput imaging system wherein detailed morphological measurements of glomerular and tubular structures in larval zebrafish kidneys has been shown. The authors of this publication have screened 1,280 known drugs at single concentration. While the authors have not performed in calculations on sensitivity, specificity and predictivity, they have demonstrated a large correlation between mammalian nephrotoxic classes and zebrafish [30].

Immunogenicity

Zebrafish immunobiology has been an area of increasing curiosity in recent years. The innate and adaptive immune systems in zebrafish are highly conserved with organ systems having species differences [31, 32]. Several publications show the utility of zebrafish in developing models for infection, immune oncology, auto-immune conditions and vaccine research [33]. Although specific drug-based studies on immunogenicity are infrequent, there is a possibility of assessing immunological end points in a detailed developmental toxicity study.

General Toxicity

Repeated dose general toxicity study in zebrafish is very rarely reported. While several vital organ toxicity methods have been popular (discussed in subsequent sections), this is a major missing piece in utilizing zebrafish for drug toxicity screening. However, a detailed and well conducted developmental toxicity study in embryo-larval stages with sufficient morphological details could be quite predictive of general toxicity endpoints. The anxiety amongst toxicologists would be the high sensitivity of developing embryos as compared to developed adults. Suitable factors

of extrapolation, after generating large amount of data, can be developed to address this issue.

Fertility Assays

Routine fertility assays by pre-treatment of chemical agents in evaluation for endpoints of fertility, fecundity, sex organs histopathology and morphology of gametes have been well reported [34]. However, the most significant assay that has become very popular is the male zebrafish vitellogenin assay to determine endocrine disruption and estrogenic effect on male fertility. This particular method has been adopted as OECD guideline 234 [35].

Teratogenicity and Developmental Toxicity

Teratogenicity and developmental toxicity assessment are one of the most well researched method in zebrafish research. Zebrafish developmental toxicity assay (ZEDTA) has been a collaborative effort by major biopharmaceutical companies like AstraZeneca, Bristol-Mayers Squibb, Pfizer, Amgen, and others in different projects. Various publications from these efforts suggest a predictive potential of over 80% for zebrafish teratogenicity and developmental toxicity potential [36, 37]. A novel automated morphological assessment methodology has also been recently published [38]. The OECD guideline TG 236 is a good simple protocol of potential effects of a candidate drug on embryo-foetal development [39]. However, a detailed morphological scoring system proposed by Panzica-Kelly et al. is the most detailed and descriptive methodology that could be useful for predicting toxicities in several vital organs using phenotypic end points [40].

CONCLUSIONS AND FUTURE DIRECTIONS

Zebrafish models for pharmaceutical safety assessment can complement the present safety assessment landscape. The field has been evolving and several innovative methods are developing routinely. A well conducted detailed evaluation of phenotypes in a developmental toxicity study can actually predict several vital organ toxicities. Over several years it has been observed that zebrafish larval studies for several endpoints have good predictivity even for adults. Similarly, adult models have major advantages, especially from perspective of drug administration and important safety pharmacology end points (ECG, EEG, behaviour, etc.),

and as more of these are developed and understood, the utility of zebrafish will further improve.

A few areas of importance that need specific focus are as follows:

Drug exposure data:

A major lacuna in present research has been paucity on data on pharmacokinetics and internal drug concentrations in zebrafish toxicity studies. Protocols and methods for such assessments are being published now and as more data is available there will be better correlation between zebrafish data and classical toxicology data [41-44].

Consortium approach for standardization and validation:

While individual laboratories and companies develop their own protocols and validate them for specific use, there is a need for coordinated efforts to identify and carry out large scale validation of these methods. Furthermore, the research has reached a stage where industry, regulators and academicians need to engage more to understand and work on sensitivity, specificity and predictivity of different models for human safety assessment. The consortium for zebrafish developmental toxicity assay is a perfect example on the way forward for standardization and validation of zebrafish methods for drug safety evaluation.

Utilization in personalised medicine:

Zebrafish 'avatars' or 'human equivalents' have attracted major attention in personalised medicine in recent years. Zebrafish telomer biology closely resembles humans, even better than rodents, this along with the data on conservation of cancer associated genes have made 'zebrafish cancer avatars' very popular in precision medicine for cancer [45, 46]. A prospective co-clinical trial (ClinicalTrials.gov Identifier: NCT03668418) is being conducted, wherein chemotherapy administered to cancer patients will be concurrently administered to zebrafish embryos xeno-transplanted with cancer cells of the specific patients (patient derived xenografts (PDX)) in order to demonstrate the ability to predict the therapeutic regimen and efficacy [47]. Many such studies and their correlation with human data are planned or underway.

Similarly, personalized medicine methods are also being utilized to treat rare diseases. Fenfluramine became the first drug based on zebrafish

screens approved by US FDA for the treatment of Dravet Syndrome, a rare debilitating epileptic disease [48, 49]. There are presently 10 rare diseases where drugs developed based on zebrafish screens are being investigated clinically [50]. Several of these rare diseases occur in specific sub population of a certain condition that cannot be treated by routine therapies and this is due to genetic reasons attributable to these sub populations. The ease of modelling rare genetic diseases in zebrafish makes it useful to create screening models for repurposing drugs or optimizing therapeutic regimens of symptomatic drugs for precision medicine to such sub-populations.

As personalised precision medicine approaches increase for therapeutic purposes, there will be requirement for personalised safety assessment as well. Furthermore, this assessment will have to be quicker, robust and an addition to the routine safety evaluation. Zebrafish models have the potential to fulfil this requirement.

REFERENCES:

1. Yoganantharjah P, Gibert Y. The Use of the Zebrafish Model to Aid in Drug Discovery and Target Validation. Curr Top Med Chem. 2017; 17: 2041-2055.
2. Wiley DS, Redfield SE, Zon LI. Chemical screening in zebrafish for novel biological and therapeutic discovery. Methods Cell Biol. 2017; 138: 651-679. doi: 10.1016/bs.mcb.2016.10.004.
3. MacRae CA, Peterson RT. Zebrafish as tools for drug discovery. Nat Rev Drug Discov. 2015; 14: 721-731.
4. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, et al. The zebrafish reference genome sequence and its relationship to the human genome. Nature. 2013; 496: 498-503.
5. Chakravarthy S, Sadagopan S, Nair A, Sukumaran SK. Zebrafish as an in vivo high-throughput model for genotoxicity. Zebrafish. 2014; 11: 154-166.
6. Choy HE, Choi SY. Method for determining genotoxicity using non-fluorescent proteins. European Patent Application EP 2 574 925 A2. WO 2011/122864.
7. The ICHS1 Regulatory Testing Paradigm of Carcinogenicity in rats. Status Report 2019.

8. Storer NY, Zon LI. Zebrafish models of p53 functions. Cold Spring Harb Perspect Biol. 2010; 2: a001123.
9. Parant JM, George SA, Holden JA, Yost HJ. Genetic modeling of Li-Fraumeni syndrome in zebrafish. Dis Model Mech. 2010; 3: 45-56.
10. Zhao S, Huang J, Ye J. A fresh look at zebrafish from the perspective of cancer research. J Exp Clin Cancer Res. 2015; 34: 80.
11. Khan KM, Collier AD, Meshalkina DA, Kysil EV, Khatsko SL, Kolesnikova T, et al. Zebrafish models in neuropsychopharmacology and CNS drug discovery. Br J Pharmacol. 2017; 174: 1925-1944.
12. Kulkarni P. Zebrafish Models for Neuroscience Drug Discovery. PhD Thesis submitted to the Birla Institute of Technology and Science (BITS) – Pilani. 2018.
13. Lantz-McPeak S, Guo X, Cuevas E, Dumas M, Newport GD, Ali SF, et al. Developmental toxicity assay using high content screening of zebrafish embryos. J Appl Toxicol. 2015; 35: 261-72.
14. Planchart A, Mattingly CJ, Allen D, Ceger P, Casey W, Hinton D, et al. Advancing toxicology research using in vivo high throughput toxicology with small fish models. ALTEX. 2016; 33: 435-452.
15. Fleming A, Alderton WK, Zebrafish in pharmaceutical industry research: finding the best fit. Drug Discovery Today: Disease Models. 2013; 10: e43-e50.
16. Milan DJ, Jones IL, Ellinor PT, MacRae CA. In vivo recording of adult zebrafish electrocardiogram and assessment of drug-induced QT prolongation. Am J Physiol Heart Circ Physiol. 2006; 291: H269-73.
17. Chaudhari GH, Chennubhotla KS, Chatti K, Kulkarni P. Optimization of the adult zebrafish ECG method for assessment of drug-induced QTc prolongation. J Pharmacol Toxicol Methods. 2013; 67: 115-20.
18. Zhao Y, Yun M, Nguyen SA, Tran M, Nguyen TP. In Vivo Surface Electrocardiography for Adult Zebrafish. J Vis Exp. 2019; 10.3791/60011.
19. Al-Khatib SM, LaPointe NMA, Kramer JM, Califf RM. What Clinicians Should Know About the QT Interval. JAMA. 2003; 289: 2120–2127.
20. Shinozawa T, Nakamura K, Shoji M, Morita M, Kimura M, Furukawa H, et al. Recapitulation of Clinical Individual Susceptibility to Drug-Induced QT Prolongation in Healthy Subjects Using iPSC- Derived Cardiomyocytes. Stem Cell Reports. 2017; 8: 226-234.

21. Arnaout R, Ferrer T, Huisken J, Spitzer K, Stainier DY, Tristani-Firouzi M, Chi NC. Zebrafish model for human long QT syndrome. Proc Natl Acad Sci U S A. 2007; 104: 11316-21.
22. Leong IU, Skinner JR, Shelling AN, Love DR. Zebrafish as a model for long QT syndrome: the evidence and the means of manipulating zebrafish gene expression. Acta Physiol (Oxf). 2010; 199: 257-76.
23. Tanaka Y, Hayashi K, Fujino N, Konno T, Tada H, Nakanishi C, et al. Functional analysis of KCNH2 gene mutations of type 2 long QT syndrome in larval zebrafish using microscopy and electrocardiography. Heart Vessels. 2019; 34: 159-166.
24. Pott A, Bock S, Berger IM, Frese K, Dahme T, Keßler M, et al. Mutation of the Na+/K+-ATPase Atp1a1a.1 causes QT interval prolongation and bradycardia in zebrafish. J Mol Cell Cardiol. 2018; 120: 42-52.
25. Zhang Y, Liu H, Yao J, Huang Y, Qin S, Sun Z, et al. Manipulating the air-filled zebrafish swim bladder as a neutrophilic inflammation model for acute lung injury. Cell Death Dis. 2016; 7: e2470.
26. Ljujic M, Varga M, Bartel S, Krauss-Etschmann S, Rankov AD. Zebrafish as a model for study of developmental origins of chronic lung diseases. Eur Respir J. 2018; 52: PA1389;
27. Lee G, Cheng N, Yu H, Tsai JN, Liu T, Wen ZH, Chen BH. A novel zebrafish model to emulate lung injury by folate deficiency-induced swim bladder defectiveness and protease/antiprotease expression imbalance. Sci Rep. 2019; 9: 12633.
28. Hill A, Mesens N, Steemans M, Xu JJ, Aleo MD. Comparisons between in vitro whole cell imaging and in vivo zebrafish-based approaches for identifying potential human hepatotoxicants earlier in pharmaceutical development. Drug Metab Rev. 2012; 44:127-140.
29. Mo D, Ihrke G, Costa SA, Brilli L, Labilloy A, Halfter W, et al. Apical targeting and endocytosis of the sialomucin endolyn are essential for establishment of zebrafish pronephric kidney function. J Cell Sci. 2012; 125: 5546-5554.
30. Kroeger PT Jr, Wingert RA. Using zebrafish to study podocyte genesis during kidney development and regeneration. Genesis. 2014; 52: 771-792.

31. Martins RR, Ellis PS, MacDonald RB, Richardson RJ, Henriques CM. Resident Immunity in Tissue Repair and Maintenance: The Zebrafish Model Coming of Age. Front Cell Dev Biol. 2019; 7: 12.
32. Renshaw SA, Trede NS. A model 450 million years in the making: zebrafish and vertebrate immunity. Dis Model Mech. 2012; 5: 38-47.
33. Bailone RL, Fukushima HCS, Ventura Fernandes BH, De Aguiar LK, Corrêa T, Janke H, et al. Zebrafish as an alternative animal model in human and animal vaccination research. Lab Anim Res. 2020; 36: 13.
34. Hoo JY, Kumari Y, Shaikh MF, Hue SM, Goh BH. Zebrafish: A Versatile Animal Model for Fertility Research. Biomed Res Int. 2016; 2016: 9732780.
35. OECD Guideline for the Testing of Chemicals. Test No. 234. Fish Sexual Development Test. 2011.
36. Ball JS, Stedman DB, Hillegass JM, Zhang CX, Penzica-Kelly JM, Caburn A, et al. Fishing for teratogens: a consortium effort for a harmonized zebrafish developmental toxicology assay. Toxicological Sciences: An Official Journal of the Society of Toxicology. 2014; 139: 210-219.
37. Panzica-Kelly JM, Zhang CX, Augustine-Rauch KA. Optimization and Performance Assessment of the Chorion-Off [Dechorinated] Zebrafish Developmental Toxicity Assay. Toxicological Sciences: An Official Journal of the Society of Toxicology. 2015; 146: 127-134.
38. Teixidó E, Kießling TR, Krupp E, Quevedo C, Muriana A, Scholz S. Automated Morphological Feature Assessment for Zebrafish Embryo Developmental Toxicity Screens. Toxicological Sciences: An Official Journal of the Society of Toxicology. 2019; 167: 438-449.
39. OECD Guideline for the Testing of Chemicals. Test No. 236. Fish Embryo Acute Toxicity (FET) Test. 2013.
40. Panzica-Kelly JM, Zhang CX, Danberry TL, Flood A, DeLan JW, Brannen KC, Augustine-Rauch KA. Morphological score assignment guidelines for the dechorionated zebrafish teratogenicity assay. Birth Defects Res B Dev Reprod Toxicol. 2010; 89: 382-395.
41. Kulkarni P, Chaudhari GH, Sripuram V, Banote RK, Kirla KT, Sultana R, et al. Oral dosing in adult zebrafish: proof-of-concept using pharmacokinetics and pharmacological evaluation of carbamazepine. Pharmacol Rep. 2014; 66: 179-83.

42. Kulkarni P, Medishetti R, Nune N, Yellanki S, Sripuram V, Rao P, et al. Correlation of pharmacokinetics and brain penetration data of adult zebrafish with higher mammals including humans. J Pharmacol Toxicol Methods. 2017; 88: 147-152.
43. Alsop D, Wilson JY. Waterborne pharmaceutical uptake and toxicity is modified by pH and dissolved organic carbon in zebrafish. Aquat Toxicol. 2019; 210: 11-18.
44. Morikane D, Zang L, Nishimura N. Evaluation of the Percutaneous Absorption of Drug Molecules in Zebrafish. Molecules. 2020; 25: E3974.
45. Costa B, Estrada MF, Mendes RV, Fior R. Zebrafish Avatars towards Personalized Medicine-A Comparative Review between Avatar Models. Cells. 2020; 9: 293.
46. Fazio M, Ablain J, Chuan Y, Langenau DM, Zon LI. Zebrafish patient avatars in cancer biology and precision cancer therapy. Nat Rev Cancer. 2020; 20: 263-273.
47. NCT03668418. Xenotransplantation of Primary Cancer Samples in Zebrafish Embryos (xenoZ). ClinicalTrials.gov.
48. Sourbron J, Partoens M, Scheldeman C, Zhang Y, Lagae L, de Witte P. Drug repurposing for Dravet syndrome in scn1Lab-/- mutant zebrafish. Epilepsia. 2019; 60: e8-e13.
49. US FDA. Press Announcements. FDA Approves New Therapy for Dravet Syndrome. Released on June 25, 2020.
50. Cully M. Zebrafish earn their drug discovery stripes. Nat Rev Drug Discov. 2019; 181: 811-813.

QUESTIONS

1. What are the benefits of using Zebrafish over traditional Toxicity methods?
2. What are the major developments in Preclinical/Non-Clinical Safety Studies?
3. What are the strengths/ weakness of using various Zebra fish studies for genotoxicity?
4. Write a brief note on various well established and accepted Zebrafish Preclinical/Non-Clinical Safety Studies.

---------End of the Document---------

Author Profile

Dr. Pushkar Kulkarni
BVSc & AH, MVM, PhD, DABT

Dr. Pushkar Kulkarni is a veterinarian by training, PhD in pharmacology & toxicology and a Diplomat of the American Board of Toxicology (DABT). He has done his bachelors from Bombay Veterinary College, Mumbai, India; masters from Swedish University of Agricultural Sciences, Uppsala, Sweden; and; PhD from Birla Institute of Sciences and Technology (BITS) - Pilani, Hyderabad, India.

Presently, he is working as a Senior Research Scientist at Dr. Reddy's Institute of Life Sciences (DRILS), Hyderabad where he has set up a zebrafish laboratory from scratch. His past record includes working at Ranbaxy Laboratories, Gurgaon and GVK Bio, Hyderabad. He has got entrepreneurial experience as Co-founder & Director of two start-ups: Zephase Therapeutics (zebrafish services company) and Vegrandis Therapeutics (nanotechnology company).

He has filed two international patents and published 45 research papers (including 1 book chapter) in reputed peer reviewed journals that have been cited around 650 times till date, with several of them been cited by Key Opinion Leaders in the field.

Disclaimer from Newsletter Editor

Sapna Gupta,
M.Sc., CLAS
editor.toxgurukul@gmail.com

Ms. Sapna is a trained Toxicologist and working with Shriram Institute for Industrial Research, Delhi since 2008. This newsletter editing is done by her voluntarily for no monetary gains.

These articles are for educational purposes only and experiences shared by our authors in an easily understandable reference for readers. We are not working with any publishing house. And, this contribution may not add academic benefits to your profile. But this would indeed be supporting the knowledge and understanding in various topics to shape the careers in Toxicology.

Please note: Dr. K.S. Rao started writing newsletter through email from his experience to a small group of Toxicologists in India back in 2014-2015. This initiative was to promote them reading more and prepare for DABT exam. In 2016, Dr. Ramesh Subramani started editing these articles and turned these emailed letters experiences to a properly shaped newsletter article. Till then only Rao Sir used to write an email loaded with his vast experience. Afterwards Mr. Alex Thomas worked for newsletter editing and some more authors joined in with Rao sir to contribute for these articles.
Ms. Sapna has started working as Newsletter editor since Nov. 2019. However, Due to Pandemic Situations and Our involvement in Webinar series not much have been done in past few months.
(All the previous articles are available on our Website: https://www.toxgurukul.org/library/).

Kind regards,
Sapna

ToxGurukul Foundation

ToxGurukul Foundation is a registered non-profit organization for professionals in the field of toxicology who are in search of a platform to learn and share the vast knowledge in this area. This syndicate belongs to independent professionals from different backgrounds of toxicology who share their knowledge to un-puzzle the Rubik's cube that each face in their daily work routine.

Website: ***www.toxgurukul.org***
Email: ***toxgurukul.india@gmail.com; editor.toxgurukul@gmail.com***

Important Links – ToxGurukul Foundation

Follow us

Join us

Subscribe

Library

Feedback

ToxGurukul Foundation (A Non - Profit Organization)
Regd. Office: Fl. No.10, New Ajanta Avenue, Building-4, Wing- A1,
S. No. 135/136 Part, Kothrud, Pune-411038
Corporate Identity Number: U80904PN2019NPL182886
Email Id: toxgurukul.india@gmail.com