Zebrafish

Methods for Assessing Drug Safety and Toxicity

Edited by Patricia McGrath

WILEY
Zebrafish
Zebras

Methods for Assessing Drug Safety and Toxicity

Edited by

Patricia McGrath

Phylonix, Cambridge, MA, USA
9. Whole Zebrafish Cytochrome P450 Assay for Assessing Drug Metabolism and Safety

9.1 Introduction 103
9.2 Background and Significance 104
9.3 Materials and Methods 105
9.4 Results 107
9.5 Conclusions 113
Acknowledgment 113
References 113

10. Methods for Assessing Neurotoxicity in Zebrafish

10.1 Introduction 117
10.2 Limitations of Current Neurotoxicity Testing 118
10.3 Assessing Neurotoxicity in Zebrafish 118
10.4 Summary 130
Acknowledgments 131
References 131

11. Zebrafish: A Predictive Model for Assessing Cancer Drug-Induced Organ Toxicity

11.1 Introduction 135
11.2 Materials and Methods 136
11.3 Results 139
11.4 Conclusions 149
Reference 149

12. Locomotion and Behavioral Toxicity in Larval Zebrafish: Background, Methods, and Data

12.1 Introduction 151
12.2 Background 152
12.3 Locomotion 153
12.4 Zebrafish Models 154
12.5 Analyzing Larval Locomotion 155
12.6 Chemical Effects on Larval Locomotion 158
12.7 Conclusions 161
Acknowledgments 162
References 162

13. Zebrafish: A Predictive Model for Assessing Seizure Liability

13.1 Introduction 165
13.2 Materials and Methods 167
The zebrafish model organism is increasingly used for assessing compound toxicity, safety, and efficacy and numerous studies confirm that mammalian and zebrafish toxicity profiles are strikingly similar. This convenient, predictive animal model can be used at an intermediate stage between performing cell-based assays and conventional animal testing. Although in vitro assays using cultured cells are commonly used to evaluate potential drug effects, they are frequently not predictive of the complex metabolism that affects drug efficacy and causes toxicity in animals. Therefore, many compounds that appear effective in vitro fail during costly animal trials.

Currently, there is no single reference source for toxicity testing using this emerging model organism. Investigators seeking general information on toxicity methods and results currently refer to toxicology textbooks that focus on mammalian models. The target readership of this timely book includes students (undergraduates and graduate level) and professionals in all biomedical sciences, including drug research and development, environmental testing, and product safety assessment.

This initial volume describes methods for assessing compound-induced toxicity in all major organs, including heart (Chapters 4, 5, 6, and 11), liver (Chapters 8, 9, and 11), kidney (Chapter 11), central nervous system (Chapters 10, 11, 12, 13, and 14), eye (Chapters 15 and 16), ear (Chapter 19), hematopoietic system (Chapter 7), and overall development (Chapters 2 and 3).

This vertebrate model offers several compelling experimental advantages including drug delivery directly in the fish water, small amount of drug required per experiment, statistically significant number of animals per test, and low cost. Animal transparency makes it possible to visually assess compound-induced effects on morphology and fluorescently labeled probes and antibodies can be used to localize and quantitate compound effects in physiologically intact animals. Compounds can be assessed using wild-type, mutant, transgenic, knockdown, and knock-in animals. In addition, several chemical-induced disease models, phenocopies, designed to identify potential drug candidates, are described (Chapters 14, 16, 17, 18, 19, and 21). Assays used to develop disease models can also be used to assess compound-induced toxicity on specific end points. Several widely used cell-based assay techniques have been adapted for use with this small model organism and quantitative morphometric image analysis (Chapters 10, 14, and 18) and microplate formats (9, 16, and 17) offer unprecedented throughput for assessing compound effects in whole animals. Additional analytical tools adapted for use with zebrafish, including ECG (Chapter 6) and motion detectors (Chapters 10, 12, 13, 15, and 18), are described.

Improvements in breeding and spawning, which address requirements of industrial scale screening, are discussed (Chapter 1). As a reference source to be used as a companion document for assessing data presented in individual chapters, we have
reprinted a description of zebrafish stages during organogenesis. An interesting recent development that successfully pairs this emerging model with an emerging market need is the use of zebrafish for assessing safety of nanoparticles (Chapter 20), which are now incorporated in virtually all product categories. In addition, the unique ability of this animal to regenerate tissue and organs offers potential for compound screening for cell-based therapies (Chapter 22).

An important recent development impacting wider use of zebrafish for toxicity testing is that the Organization for Economic Cooperation and Development (OECD), an international organization helping governments tackle the economic social and governance challenges of the globalized economy, is developing standards for using zebrafish to assess chemical toxicity.

Further supporting wider use of this emerging model organism, the European Union recently enacted Registration, Evaluation, Authorisation and Registration of Chemicals (REACH) legislation that requires toxicity assessment for any chemical imported or manufactured in the region and is expected to have far-reaching impact on new product introductions and animal testing, including zebrafish.

Confounding interpretation of drug-induced toxicity and limiting wider acceptance of this model organism, reported results show that inter- and intralaboratory standards vary widely, although cooperation among academic and industry laboratories to develop standard operating procedures for performing compound assessment in zebrafish is increasing. Understanding all aspects of current toxicology testing will facilitate more uniform approaches across industries and enhance acceptance from regulatory authorities around the world. Full validation of this model organism will require assessment of large numbers of compounds from diverse classes in a wide variety of assays and disease models. I hope that methods and data reported here will facilitate standardization and support increased use of zebrafish for compound screening.

PATRICIA McGRATH

Cambridge, MA
Contributors

Wendy Alderton, CB1 Bio Ltd, Cambridge, UK
Jessica Awerman, Phylonix, Cambridge, MA, USA
Florian Beuerle, The Institute für Organische Chemie, Universität Erlangen-Nürnberg, Erlangen, Germany
Louis D’Amico, Phylonix, Cambridge, MA, USA
Myrtle Davis, NCI, NIH, Bethesda, MD, USA
Anthony DeLise, Sanofi-Aventis, Bridgewater, NJ, USA
Adam P. Dicker, Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, PA, USA
Elizabeth Glaze, NCI, NIH, Bethesda, MD, USA
Maryann Haldi, Phylonix, Cambridge, MA, USA
Maegan Harden, Phylonix, Cambridge, MA, USA
Uwe Hartnagel, The Institute für Organische Chemie, Universität Erlangen-Nürnberg, Erlangen, Germany
Adrian Hill, Evotec (UK) Ltd, Abingdon, Oxfordshire, UK
Andreas Hirsch, The Institute für Organische Chemie, Universität Erlangen-Nürnberg, Erlangen, Germany; and C-Sixty Inc., Houston, TX, USA
Deborah L. Hunter, Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA
Terra D. Irons, Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC, USA
Gabor Kari, Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, PA, USA
Christian Lawrence, Aquatic Resources Program, Children’s Hospital Boston, Boston, MA, USA
Russell Lebovitz, C-Sixty Inc., Houston, TX, USA
Chunqi Li, Phylonix, Cambridge, MA, USA
Yingxin Lin, Phylonix, Cambridge, MA, USA