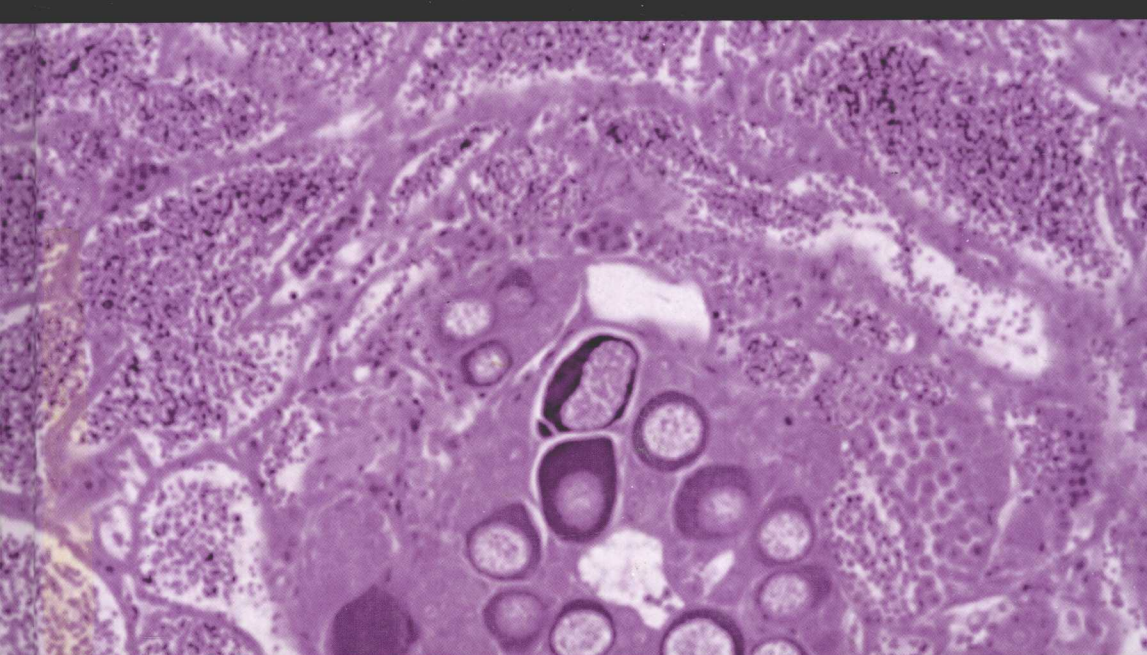
Histological Analysis of Endocrine Disruptive Effects in Small Laboratory Fish

Daniel R. Dietrich and Heiko O. Krieger



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PREFACE

In view of the ever-expanding literature on xenobiotic [endocrine-disrupting compounds (EDCs)]-induced endocrine disruption in fish populations one is:

1. Easily overwhelmed and thus prone to losing focus.
2. Often unsure whether the parameters and changes reported in the literature are really important or even relevant for the task(s) defined at the outset.
3. More and more left with the impression that many of the deleterious population effects reported are not as ubiquitous as reported but rather, represent a very restricted local issue.

In view of these concerns latter and the fact that most studies employ fish gonadal histopathology in the assessment of compound-induced endocrine-disruptive effects, the European Chemical Industry Council–Long Range Initiative (CEFIC-LRI) sponsored a laboratory fish gonad histopathology workshop. This workshop was organized by the Department of Human and Environmental Toxicology, University of Konstanz, Germany, and the company IPMC-TMC GmbH in Switzerland, and was carried out in Hannover, Germany in February 2004 with the participation of the leading fish histopathologists in Europe and the United States: Helmut Segner (University of Bern, Switzerland), Leo van der Ven (RIVM, The Netherlands), Jeff Wolf (EPL Inc., Sterling, Virginia), John Fournie (U.S. Environmental Protection Agency), and Daniel Dietrich (University of Konstanz, Germany), as well as with statistical support by Nelly van der Hoeven, EcoΣtat, Leiden, Netherlands.

The workshop clearly identified several issues that appeared to hamper understanding of experimental results with EDCs in fish:

- Limited understanding of normal physiology and reproduction of the fish species used
- Variant terminology in describing effects
- Insecurities as to the relevance of the parameters determined for evaluating EDC-mediated effects
- Limited understanding of the natural variation of the parameters determined
- Flaws in the actual technical determination of parameters (variant limits of detection or quantification)

- Flaws in the experimental design (i.e., number of parameters determined, statistics used, replication and/or number of animals used per replicate, etc.)

In view of these issues and in the spirit of a successful workshop, the experts who were assembled at the Hanover workshop with Daniel Dietrich in the lead wanted to develop a book on fish gonadal histopathology that addresses just these points. Meanwhile, over 1200 publications and reports were reviewed, multiple book chapters and tables generated, and a book assembled that hopefully intuitively leads the reader through basic fish biology to fish sexual reproduction, female and male gonad development and histopathology, EDC-mediated effects, methods for histopathological processing and quantification of effects, experimental design issues, and finally, a short conclusion chapter reiterating the most important findings. The book aims to provide a thorough overview and, where possible, more in-depth information to students, researchers, and experts who seek information on the state-of-the-art of research in the field of endocrine disruption in fish.

Power point files of the illustrations that appear in this book are provided in the attached CD-ROM.

Konstanz, Germany
March 2009

DANIEL R. DIETRICH

ACKNOWLEDGMENTS

This book and all the research, reviewing, and editing work could not have been carried out without the support, understanding, patience, and love of our families. In view of this, it is especially difficult when one of our loved ones passes away prematurely. This book is therefore dedicated to Maike Wester, whose death has made us realize how precious time is when it is dedicated to research in lieu of spending it with loved ones.

We would also like to acknowledge Nelly van der Hoeven, EcoΣtat, Leiden, the Netherlands, for her support in the preparation of Chapter 9, as well as the numerous researchers who made tissue slides and artwork available. Last but not least, Karin Rieder, secretary at the Human and Environmental Toxicology Department at the University of Konstanz, and the team at John Wiley & Sons, Inc., are thanked for their patience and support in making this book happen.

We have organized the book to enlighten scientists interested in endocrine disruption and gonadal histopathology in small fish. The information presented represents the combined knowledge of the contributing authors and data from published information, and as such is part of a rapidly developing field. In this sense:

> The minute one utters a certainty, the opposite comes to mind.
> —May Sarton, *Mrs. Stevens Hears the Mermaids Singing*, 1965

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CONTENTS

CHAPTER 1

Introduction

Histopathology is a tool employed routinely in disease diagnosis for the detection and assessment of xenobiotically mediated adverse effects in laboratory animals [1–19]. Recently, histopathological analysis has become a method applied more frequently for the assessment of potential effects of endocrine-active compounds (capable of endocrine modulation, often referred to as EDCs (*endocrine-disrupting chemicals* [20]) in fish. EDCs have been defined by the WHO-IPCS [21] as follows: "An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" and "A potential endocrine disruptor is an exogenous substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations."

EDCs can have an effect at both the cellular and whole-organ levels and have been demonstrated to induce histopathological changes within the gonads of exposed fish within the confines of routine aquaculture procedures or in an environmental risk assessment (ecotoxicology) setting (see Figure 1.1) [20,22–26]. Chemicals with endocrine-active properties and proven potential for adverse effects are considered chemicals of concern and are subject to authorization under recent European legislation.

Concurrent with the definition of EDCs, effects of EDCs on the level of the cell or the organ could then be derived. Exposure of small laboratory fish to estrogens [e.g., 17β-estradiol (E_2) and 17α-ethinylestradiol (EE_2)] has been reported to induce gonadal changes, such as atresia of vitellogenic oocytes [27–33] and inhibition of spermatogenesis (via various modes of action) [28,33–45]. Additional effects have been reported, such as pronounced interstitial fibrosis [34,46] and induced interstitial accumulation of protein, in some cases identified as vitellogenin [47,48], or Sertoli cell hypertrophy [49]. However, systematic evaluation of the effects of hormonally active compounds, or any other xenobiotic for that matter, on the gonads of small laboratory fish based on peer-reviewed publications has been hampered to some extent by procedural restrictions, interpretational problems (i.e., no clear nomenclature of observed structures and effects), and graphical issues (figures inadequate for proper assessment of the effect), thus limiting the use and extrapolation potential of the data

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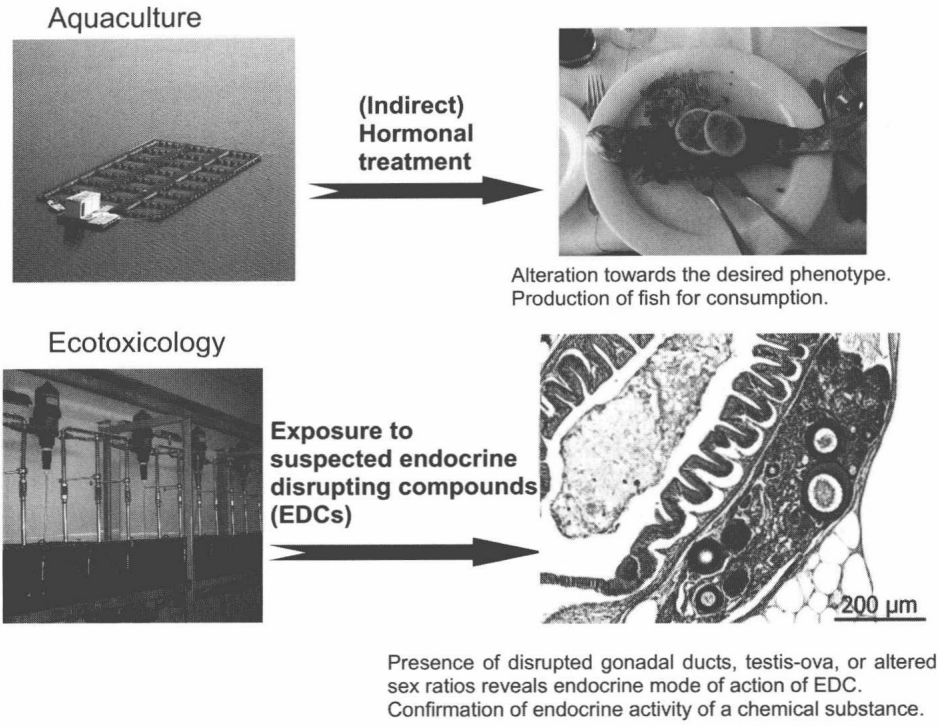

FIGURE 1.1 Control of sexual differentiation in fish: various applications. (Picture upper left courtesy of Kevin Frost, upper right courtesy of Susanne Beeck, pixelio).

provided. High stringency and standardization in the evaluation process of effects are prerequisites if the findings from different studies with endocrine-active xenobiotics in small fish are to be compared reliably [22].

Comprehensive, reproducible, and objective histopathological analysis will be greatly facilitated if the following basic standards of pathological reporting are included, while by the same token, lack of the following factors will restrict the quality and thus the value of the histopathological analysis:

1. A standardized and optimized histological gonad preparation method (fixation, embedding, sectioning, and staining techniques), which would allow clear distinction of individual gonadal cell types and interpretation of changes.
2. A standardized gonadal staging and evaluation system and a clear nomenclature system for the various cell types, which would allow a qualitative and/or quantitative assessment of the effects of endocrine-modulating compounds.
3. A standardized methodology to allow quantification of the occurrence and frequency of individual gonadal cell types for effect assessment.
4. Agreement on reporting pathological findings using representative photographs with adequate resolution and magnification and, wherever possible, the inclusion of a size bar.

In the Appendix we provide standardized protocols and procedures as well as guidance for possible quantification and reporting approaches for points 1 to 4 above, and in the main body of the book we focus on an understanding of the processes visualized via histopathological changes and the interpretation of these findings. Indeed, a thorough assessment of histopathological tissue changes can provide key diagnostic information and will form the basis for an understanding of the underlying mechanism(s) of endocrine active substances [50,51]. A more detailed morphological and mechanistic understanding of effects observed will allow enhanced reproducibility and comparability of the studies conducted. However, the description of chemically induced changes in gonadal histology can be of profound value only if these changes can be put into context with and interpreted in light of the normal reproductive biology of the particular fish. Consequently, a prerequisite for the histopathological analysis of gonadal changes in fish is an understanding of the reproductive biology and normal gonadal histology of the fish such that an induced (e.g., chemical-mediated) effect can be distinguished from naturally occurring gonadal changes in fish during development, spawning, gonadal regression or recrudescence, or during aging. The distinction of such effects will then also allow better prediction of the ramifications (type and magnitude of consequences) of the chemically mediated gonadal effects for fish reproductive capacity and thus probably also for the fish population. It must be noted, however, that beyond the obvious—the complete lack of gonadal development or the destruction of the reproductive tissue and thus reproductive capacity *in toto*—no agreement has been found to date as to what small or moderate gonadal changes, whether the occurrence of intersex (testis–ova/ovo–testis) or outright pathological changes, in an individual's gonad will translate into at the level of the population.

Our goal in this book is to provide a detailed understanding of the normal gonad physiology, development, anatomy, and histopathology as well as of changes induced by xenobiotics (e.g., endocrine-active substances) primarily in three small fish species: fathead minnow (*Pimephales promelas*), medaka (*Oryzias latipes*), and zebrafish (*Danio rerio*). These three species are employed predominantly in routine experiments used for environmental risk assessment, including hazard and consequently, risk assessment of chemicals (e.g., endocrine-active compounds) [52]. However, wherever possible and considered of additional value for understanding differences or exceptions to the general concept developed with the three species chosen, references to other species, notably carp, trout, bream, stickleback, guppy, platyfish, eelpout, and sheepshead minnow are included.

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