ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

GUIDANCE DOCUMENT ON HISTOPATHOLOGY TECHNIQUES AND EVALUATION (PART 3)
FOR THE LARVAL AMPHIBIAN GROWTH AND DEVELOPMENT ASSAY (LAGDA)

Series on Testing & Assessment
No. 228

Cancels & replaces the same document of 25 September 2015

JT03386648

Complete document available on OLIS in its original format
This document and any map included herein are without prejudice to the status of or sovereignty over any territory, to the delimitation of international frontiers and boundaries and to the name of any territory, city or area.
OECD Environment, Health and Safety Publications

Series on Testing and Assessment

No. 228

Guidance Document on Histopathology Techniques and Evaluation (Part 3)

Environment Directorate

ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT

Paris 2015
About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 34 industrialised countries in North and South America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD’s work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD’s workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in ten different series: Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides and Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and Safety of Manufactured Nanomaterials. More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD’s World Wide Web site (www.oecd.org/ehs/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.
This publication is available electronically, at no charge.

Also published in the Series on Testing and Assessment (link):

For this and many other Environment, Health and Safety publications, consult the OECD’s World Wide Web site (www.oecd.org/chemicalsafety/)

or contact:

OECD Environment Directorate,
Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France

Fax: (33-1) 44 30 61 80

E-mail: ehscont@oecd.org

© OECD 2015
Applications for permission to reproduce or translate all or part of this material should be made to: Head of Publications Service, RIGHTS@oecd.org. OECD, 2 rue André-Pascal, 75775 Paris Cedex 16, France
FOREWORD

The project to develop a Larval draft Amphibian Growth and Development Assay (LAGDA) was initiated by Japan and the United States and included in the work plan of Test Guidelines Programme in 2009.

The Integrated Summary Report and first draft TG were submitted to the Working Group of the National Coordinators of the Test Guidelines Programme (WNT) in 2014, followed by subsequent commenting rounds in 2014. The draft guidance document on amphibian histopathology was prepared to accompany the draft Test Guideline and help users of the test become more proficient in applying tissue sampling and preparation techniques, evaluation techniques and in the interpretation of the slides.

The guidance document on amphibian histopathology techniques and evaluation was approved by the WNT at its 27th meeting in April 2015. The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology agreed to the declassification of the guidance document on 10th July, 2015.

This document presents Part 3 of the guidance document which in total consists of three parts.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.
GUIDANCE DOCUMENT ON HISTOPATHOLOGY TECHNIQUES AND EVALUATION (PART 3)

FOR THE LARVAL AMPHIBIAN GROWTH AND DEVELOPMENT ASSAY (LAGDA)
Ovary, Germ Cell Degeneration. The term “germ cell degeneration” is used rather than “atresia” to characterize the in vivo deterioration of stem cells or oogonia, or the deterioration of phenotypically undifferentiated cells in early stage gonads. Ovarian germ cell degeneration in the form of apoptotic-like cells (arrows) was observed as a treatment-related response in the Grade 2 image. Bar = 25 microns.
Oviduct Staging. As the oviduct (Müllerian duct) matures in the female, it becomes progressively larger, with an expanded lumen and a more convoluted mucosal lining. Conversely, oviducts regress over time in male frogs. Control male frogs typically have Stage 1 or 2 oviducts 10 weeks post NF stage 62 (LAGDA termination), and the average stage in males is usually less than the average stage in same-aged females of the same study. In borderline cases, the size of the oviduct relative to the Wolffian duct is a useful criterion for differentiating between Stage 2 and Stage 3 oviducts. Bar = 50 microns (Stages 1, 2, and 3), 100 microns (Stage 4).
Oviduct Staging, Additional Examples of Stage 1 Oviducts. Stage 1 oviducts (arrows) range from small fibrous protuberances, to suspensory ligaments in which there is no apparent residual oviduct (e.g., Fig. D). These oviducts do not have lumens or mucosal linings. Bar = 50 microns.
Oviduct Staging, Additional Examples of Stage 2 Oviducts. Stage 2 oviducts (arrows), which are comparable in size to the Wolffian ducts, all have a visible lumen and are lined by one to a few layers of mucosal epithelial cells, with or without slight mucosal folding. Bar = 50 microns (Figs. A-C), 25 microns (Fig. D).
Oviduct Staging, Additional Examples of Stage 3 Oviducts. Stage 3 oviducts (arrows), which greater than 1.5 times the size of Wolffian ducts, have intricate mucosal folding. Bar = 500 microns (Fig. A), 100 microns (Figs. B and C), 25 microns (Fig. D).
Oviduct Staging, Additional Examples of Stage 4 Oviducts. Stage 4 oviducts (arrows) are markedly larger than Wolffian ducts, and the mucosal epithelium is forming, or has formed, large glandular structures. Fig. D is enlarged relative to the other figures. The oviduct in Fig. D represents the borderline between Stages 3 and 4. Bar = 500 microns (Figs. A and B), 100 microns (Fig. C), 50 microns (Fig. D).
Wolffian Duct Staging. The Wolffian ducts, which are located within the lateral extremities of the right and left kidneys, also function as ureters in *Xenopus* spp. With maturity, the Wolffian ducts become progressively larger, although not to the extent of oviducts in female frogs. The mucosal lining of the ducts also becomes thicker and contains more mucous cells with age. Generally, mean Wolffian duct scores are slightly higher in control males than in control females of the same study. Bar = 25 microns (Stages 1-3), 50 microns (Stage 4).
**Normal Kidney from a Control Frog.** Glomeruli (G) are located in the ventral third of the kidneys, whereas proximal tubules (T) occupy the dorsal portions. Bar = 100 microns (Fig. A), 50 microns (Fig. B).
Proteinaceous Fluid. Proteinaceous fluid (arrows) appears as homogenous dark pink material within the renal interstitium, blood vessels, tubules, and/or Bowman’s spaces. Bar = 25 microns (Grade 1), 100 microns (Grade 4).
Proteinaceous Fluid, Grade 4, Additional Examples. Tubules, glomeruli, and the renal interstitium are flooded by proteinaceous fluid in these examples. BS = dilated Bowman’s space. Bar = 500 microns (Fig. A), 100 microns (Fig. B), 50 microns (Figs. C and D).
**Fibrosis.** This finding, which is characterized by excessive amounts of fibrous connective tissue (F) within the renal interstitium, usually occurs as a sequel to chronic inflammation or other long standing parenchymal damage. Bar = 100 microns (Grade 3).
Glomerulomegaly and Glomerular Hypercellularity. This finding can be a consequence of low level chronic glomerular damage. The arrow indicates a small deposit of proteinaceous material within a glomerular capillary. Bar = 20 microns (Grade 3).
Regenerative Blast Cell Hyperplasia. This finding, which is another consequence of chronic renal damage and tubular loss, is characterized by streaming proliferations of cells with large, hyperchromatic (dark) nuclei (arrows). Figure B is a higher magnification of Figure A. This particular case was assigned a severity score of Grade 2 (mild). Bar = 50 microns (Fig. A), 25 microns (Fig. B).
Mineralization with Tubular Dilation. Mineralization appears to be a common background finding in laboratory reared X. laevis, and the severity of this finding appears to vary from facility to facility. Husbandry factors (e.g., issues involving feed and/or water composition) are suspected causes. The occurrence of mineralization is associated with focal to diffuse renal tubular dilation, presumably due to obstruction of urine flow. Tubular dilation and mineralization are usually graded separately. In a given frog, the severity grade for tubular dilation is typically one grade less than that of mineralization (see example below). Bar = 25 microns (Grade 1), 100 microns (Grades 2 and 3).
Mineralization, Additional Examples. Figure A represents another case of Grade 3 mineralization and Grade 2 tubular dilation. Figure B illustrates a small amount of mineral (arrow) within a multinucleated giant cell macrophage. Bar = 100 microns (Fig. A), 25 microns (Fig. B).
References.


