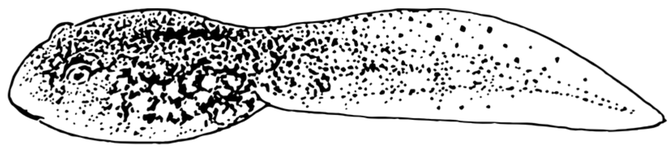
Histological Lesions in African Clawed Frogs (*Xenopus laevis*) used in the Amphibian Metamorphosis Assay



Olivia Richard, Klaus Weber, Laura Polledo, Tanja Razinger, Bastian Zingg

AnaPath Services GmbH, Hammerstrasse 49, 4410 Liestal, Switzerland
AnaPath Services GmbH, Buchsweg 4, 4625 Oberbuchsiten, Switzerland

Introduction

Xenopus laevis tadpoles are widely used in the Amphibian Metamorphosis Assay (AMA) to identify substances which may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis. Although Xenopus laevis have been used extensively in research, there is a paucity of information in the current literature describing background lesions and their relevance. We retrospectively assessed data from 42 AMA studies from five institutions located in North America and Europe. To our knowledge, this is the first report documenting spontaneous background lesions in Xenopus laevis. This document serves as a resource to pathologists and will aid in interpretation of findings and differentiation of background from test article–related changes.

Material and Methods

Forty-two studies have been used for this compilation. The in-life part was performed in four different European and one North American CRO's. All studies comprised a dilution (water) control group, whereas solvent controls where use for each acetone and TEG (triethylene glycol) in one study, and DMF (dimethylformamide) in 14 studies. In each study, there were 4 replicates per study and at least 20 tadpoles per group for histology evaluation. This resulted in the following number of control animals: water control – 843; DMF – 280; TEG – 25; acetone -20. From 23 studies, thyroid glands have been evaluated only. In 19 studies, also liver and/or kidney sections (transversal sections through body) were present, whereby all present organs have been screened in addition. The studies have been evaluated by three different pathologists from AnaPath Services GmbH (Table 1).

Results

In thyroid glands, the findings were made according to Grim et al. (2009). The findings are summarized for the water control in Table 1. Differences were mainly for thyroid hypertrophy and atrophy which is likely driven by cut-of-plane artefacts. Differences in the number of diagnoses made for follicular cell hypertrophy or atrophy are due to single affected animals within control groups. The findings of increased or decreased follicular area are deemed to be arbitrary in nature and related to stage and physiological status of thyroid glands as well as to follicular cell hypertrophy/atrophy. Follicular cell hyperplasia was found in one animal from a DMF group only. No differences were found for thyroid gland alterations between control groups from different CRO's (Table 2, Figures 1-4).
Background lesions of degenerative and/or inflammatory nature, as well as infections and parasites have been encountered in different organs: liver, kidneys, abdominal cavity, gills, gastrointestinal tract pancreas, skeletal muscle, skin, eyes, and oral cavity (Table 2, Figures 5-12)

Summary

All inflammatory and degenerative background lesions may be encountered as induced findings, however, at higher severity/incidences. Up to now, renal tubular necrosis, renal granulomatosis inflammation, and metaplasia of gill epithelia (Figures 7, 8) have not been diagnosed in control animals. Different solvents did not cause different lesions. There were no major differences between different CRO's.

Table 1. Findings in Thyroid Glands from Control (Water)Tadpoles made by Three Pathologists

Number of Studies evaluated by	Finding	% Incidence (total 843 tadpoles)	% Affected Studies (by Pathologist)		
			A	B	C
Pathologist A: 25	Thyroid hypertrophy	2.37	0	41.7	0
	Thyroid atrophy	1.19	16	25	0
Pathologist B: 12	Follicular hypertrophy	2.25	12	33.3	100
	Follicular atrophy	0.71	4	16.7	0
Pathologist C: 5	Increased area	1.42	8	16.7	20
	Decreased area	0.24	4	0	0

Table 2. Selected findings in Non-Target Organs from Control (Water) and Solvent Control Group Tadpoles made by Three Pathologists

Group	Control		DMF		TEG		Acetone	
	n	%	n	%	n	%	n	%
Thyroid								
Numbers of animals examined	843		280		25		20	
Follicular cell atrophy	6	0.71	1	0.36	0	0.00	0	0.00
Follicular cell hypertrophy	19	2.25	12	4.29	0	0.00	0	0.00
Follicular lumen area increased	12	1.42	12	4.29	0	0.00	0	0.00
Thyroid gland atrophy	10	1.19	0	0.00	2	8.00	0	0.00
Thyroid gland hypertrophy	20	2.37	6	2.14	0	0.00	0	0.00
Liver								
Numbers of animals examined	425		140		25		0	
Mononuclear cell foci	87	20.47	31	22.14	8	32.00	0	0.00
Vacuolation (glycogen type)	18	4.24	11	7.86	0	0.00	0	0.00
Vacuolation (lipid type)	3	0.71	0	0.00	0	0.00	0	0.00
Vacuolation decreased	72	16.94	38	27.14	4	16.00	0	0.00
Kidney								
Numbers of animals examined	345		140		25		0	
Mononuclear cell foci	34	9.86	8	5.71	0	0.00	0	0.00
Tubular basophilia, focal	15	4.35	0	0.00	0	0.00	0	0.00
Gill								
Numbers of animals examined	843		280		25		20	
Epithelial hypertrophy / hyperplasia	4	0.47	0	0.00	0	0.00	0	0.00
Inflammatory cell infiltrate	2	0.24	0	0.00	0	0.00	0	0.00
GIT								
Numbers of animals examined	345		120		25		0	
Ciliates, NOS	10	2.90	0	0.00	0	0.00	0	0.00
Flagellates (*Protoopalina spec.*)	20	5.80	0	0.00	0	0.00	0	0.00
Larvae, nematode, NOS	20	5.80	0	0.00	0	0.00	0	0.00
Protozoa, NOS	22	6.38	0	0.00	0	0.00	0	0.00
Infiltration, stomach/intestine, mononuclear/mixed	8	2.32	3	2.14	0	0.00	0	0.00
Inflammation, stomach/intestine, histiocytic/mixed	13	3.77	10	7.14	0	0.00	0	0.00
Single cell apoptosis	27	7.83	16	11.43	0	0.00	0	0.00
Pancreas								
Numbers of animals examined	345		120		25		0	
Inflammation, focal, mixed	3	0.87	1	0.71	0	0.00	0	0.00
Necrosis, multifocal	4	1.16	0	0.00	0	0.00	0	0.00
Single cell apoptosis	6	1.74	0	0.00	0	0.00	0	0.00
Skeletal muscle								
Numbers of animals examined	843		280		25		20	
Degeneration, focal	7	0.83	5	3.57	0	0.00	0	0.00
Oral cavity								
Numbers of animals examined	843		280		25		20	
Basophilic intranuclear inclusion bodies, oral mucosa	66	7.83	4	1.43	0	0.00	0	0.00
Inflammation, mucosa	6	0.71	0	0.00	0	0.00	0	0.00
Single cell apoptosis	80	9.49	11	3.93	0	0.00	3.00	15.00

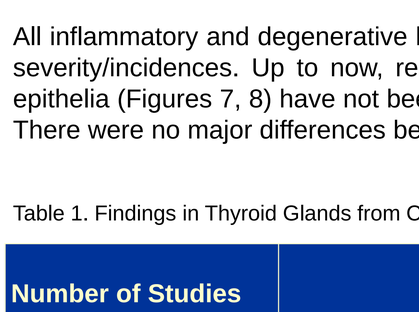


Figure 1. Thyroid. Normal

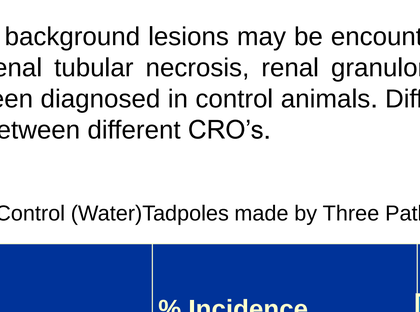


Figure 2. Thyroid. Follicular cell atrophy.

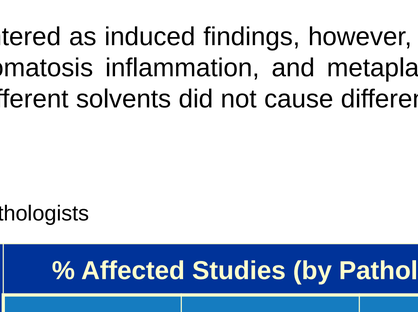


Figure 3. Thyroid. Follicular cell hypertrophy.

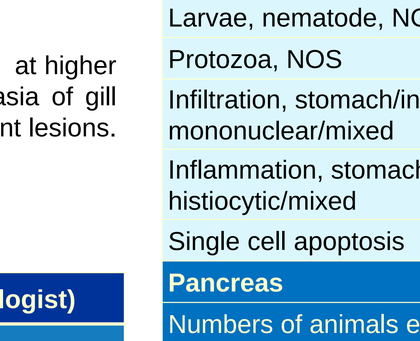


Figure 4. Thyroid. Follicular cell hyperplasia.

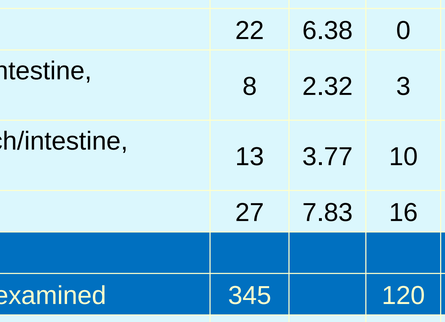


Figure 5. Gill. Apoptosis, single cell necrosis.

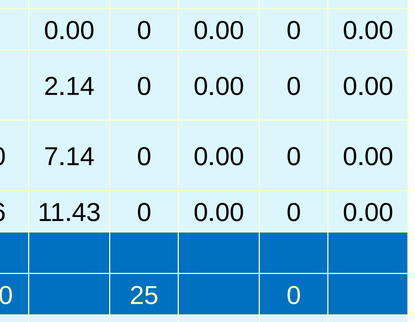


Figure 6. Gill. Hyperplasia.

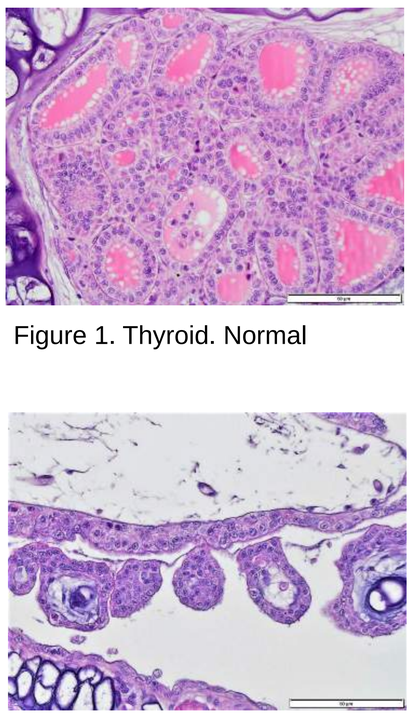


Figure 7. Gill. Hyperplasia/ metaplasia.

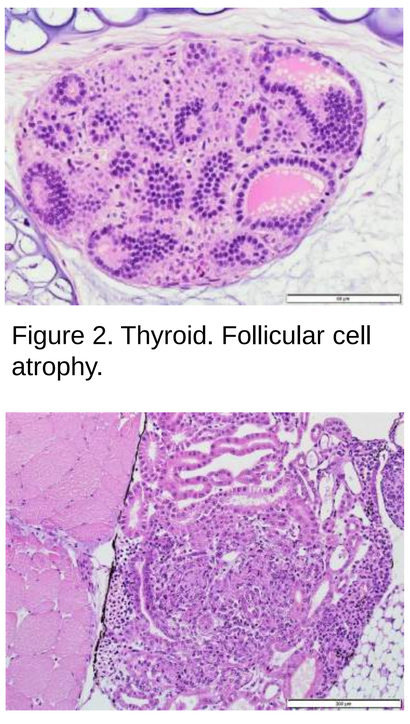


Figure 8. Kidney. Granulomatous inflammation.

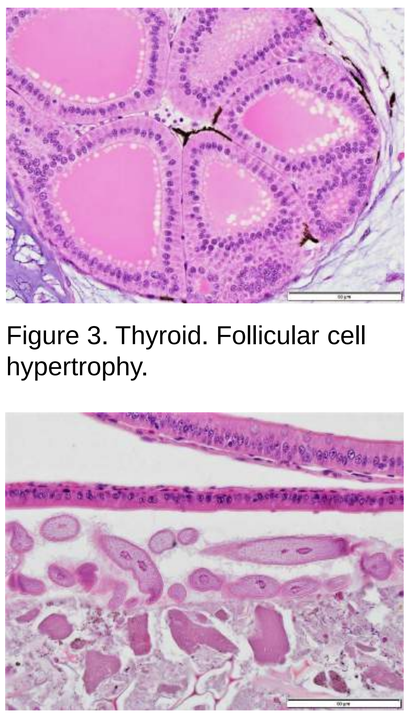


Figure 9. Intestine. *Paraopalina spec.*

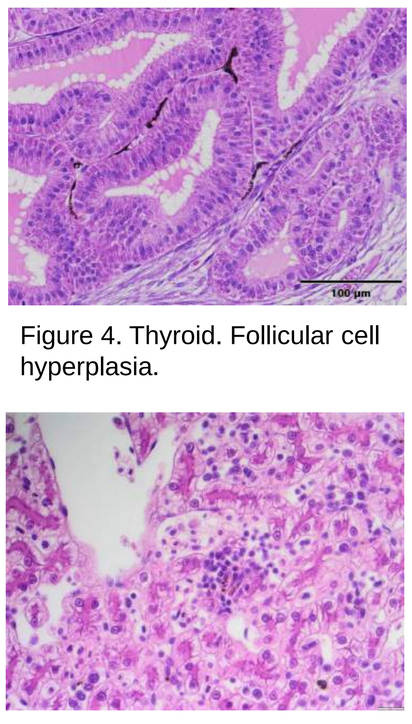


Figure 10. Liver. Mononuclear cell focus

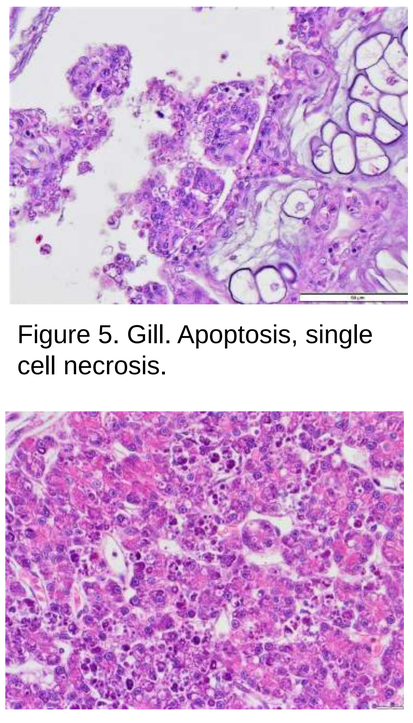


Figure 11. Pancreas. Single cell necrosis.

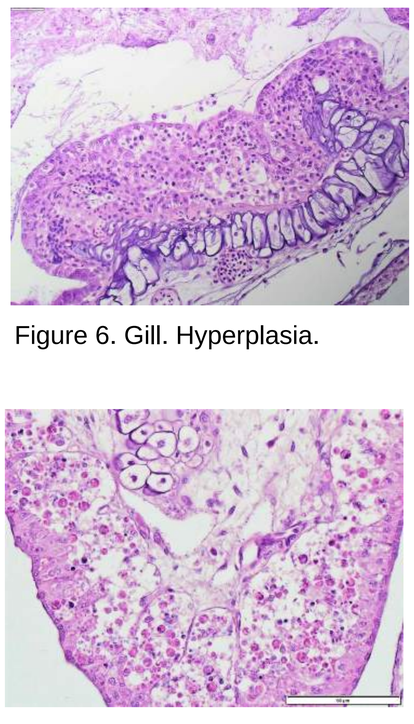


Figure 12. Oral cavity mucosa. Necrosis.

Grim KC, Wolfe M, Braunbeck T, Iguchi T, Ohta Y, Tooi O, Touart L, Wolf DC, Tietge J. Thyroid histopathology assessments for the amphibian metamorphosis assay to detect thyroid-active substances. Toxicol Pathol. 2009. 37: 415–424.

Publication in Progress