Inhibition of frog development from the larva of African clawed frogs (*Xenopus laevis*)

Bright Chima Megbo
Department of Plant Physiology, Faculty of Science, Charles University Prague, Czech Republic.

ABSTRACT
In this study, the positive control (1µg/L thyroxine) triggered notable acceleration of metamorphic development compared to all treatments with thyroid hormone inhibitors or antagonists. Thyroxine treatment led to progressive development in the tadpoles which lasted throughout the 30 days of this study. Thirty-day (30-day) exposure of frog larva (*Xenopus laevis*) to Propylthiouracil (PTU) led to considerable reduction in tadpole growth rates and the developmental stage as well as hind limb length. Propylthiouracil caused a complete inhibition of metamorphosis as tadpoles did not develop after stage 54 within 30 days of exposure to chemical. Propylthiouracil induced the inhibition of tail elongation as compared to the control. Methimazole and Propylthiouraci separately impeded larval development and morphological changes in the thyroid gland in the form of reduced colloid, cellular hyperplasia, hypertrophy and glandular hypertrophy. Ammonium perchlorate disrupted iodide uptake by the follicular cells of the thyroid gland and inhibited forelimb emergence, hind limb development as well as tail resorption. During tadpole transformation into frog secreted thyroid hormone induced morphological modification of larval tissues. Ethylenthiourea (ETU) caused a striking retardation of tadpole development which resulted in lower developmental stages compared to the control tadpoles. All chemical treatments (Propylthiouracil, Ethylenthiourea; Methimazole; Fluoxetine; and Ammonium perchlorate) used in this study caused significant depletion in thyroxine levels.

KEYWORDS: Metamorphorsis; Propylthiouracil, Ethylenthiourea; Methimazole; Fluoxetine; and Ammonium perchlorate; *Xenopus laevis*; Thyroxine

1.0 INTRODUCTION

This study investigates the effects of some chemicals on the perturbation of the frog development cycle from the tadpole (larval stage) of the African clawed frogs (*Xenopus laevis*) in fresh water habitat. The frog thyroid glands, located between the eyes show various morphological changes during metamorphosis and when exposed to certain chemicals. Amphibian metamorphosis is divided roughly into 3 stages, pre-metamorphosis, pro-metamorphosis and climax. The pre-metamorphic stage is the period until the appearance of the hind limbs, and the pro-metamorphic period is from their appearance to that of the forelimbs. During the period of metamorphic climax, resorption of tail and gills and development of lungs occur. The thyroid gland secretes thyroxine (T4), which is converted to a more biologically active form, 3,3′,5-triiodothyronine (T3), mainly in the peripheral target tissues. The inhibition of 5-deiodinase in the peripheral tissue is related to reduction of conversion from T4 to T3. The endocrine-disrupting chemical effects have been employed to study the complex processes of metamorphosis of the aquatic larvae into an adult tetrapod regulated by the endocrine system. Frog tadpoles have high sensitivity to their environment, especially in terms of the substances that occur in their habitat. Amphibian tadpoles possess a thin, permeable skin and live in aquatic environments. Considering this factor, they are susceptible to toxicants through both skin and dietary routes. In

addition, a variety of contaminants in wastewater emanating from agricultural fields and from industrial sites constitute a disruption to their habitats. Thyroid hormones can trigger striking changes during the metamorphosis of tadpoles into frogs, which may lead to structural and functional changes in larval tissues. There are a variety of mechanisms determining thyroid hormone balance or disruption directly or indirectly. In this study, the careful observations of the structural and functional changes of larval tissues have be used to provide valuable information for the elucidation the manner and extent of endocrine disruption caused by following substances: Sertraline ("Zoloft") a selective serotonin re-uptake inhibitor (SSRI); Propylthiouracil (PTU) inhibits deiodinase; Ethylenthiourea (ETU); Methimazole; Fluoxetine; and Ammonium perchlorate. Some endocrine-disrupting chemicals may act directly to cause changes in thyroxine synthesis or secretion in thyroid through effects on peroxidases, thyroidal iodide uptake, deiodinase, and proteolysis. On the other hand, indirect action may arise from biochemical processes such as sulfation, deiodination and glucuronidation. Both inhibition of thyroidal iodide uptake and suppression of thyroidal peroxidase activity can disrupt thyroid hormone synthesis and secretion.

2.0 MATERIALS and METHODS

Tadpole (larval stage) of the African clawed frogs (*Xenopus laevis*) which were 18 days post-fertilization, were treated with 100 mg/l of MS-222 buffered with 200 mg/l of sodium bicarbonate, and grouped by stage (Nieuwkoopand Faber, 1994). After recovery from the anesthesia, 600 stage 51 tadpoles were randomly placed into 24 glass aquaria exposure tanks for the six (6) treatments, i.e. 25 tadpoles per tank replicated four times). The larvae were observed daily and any dead larva was removed. The concentration of Propylthiouracil (PTU), Ethylenthiourea (ETU); Methimazole; Fluoxetine; and Ammonium perchlorate applied separately in this study was 50mg/L. The positive control with 1 µg/L of thyroxine (T4) was employed. The larva of the African clawed frogs (*Xenopus laevis*) was exposed to these thyroid hormone inhibitors or antagonists for thirty (30) days. Experiments were conducted at room temperature (25ºC-30ºC) for 30 days. Tadpole larvae of each group were morphologically examined under stereoscopic binocular microscope to ascertain the changes in the size of thyroid gland. Changes in thyroid glands were observed at different developmental stages pre-metamorphic, pro-metamorphic and metamorphic climax stages.

3.0 RESULTS AND DISCUSSION

Embryonic and larval development in *X. laevis* has been divided into 66 stages (Nieuwkoop and Faber, 1994), and the relationship between the functional state of the thyroid system and the progress of metamorphic development is well-documented in this species. The metamorphosis processes are systemically controlled by thyroid hormones (Tata, 1998). The premetamorphic period (stage 37 to stages 53/54) is characterized by rapid growth of tadpoles with only minor morphological changes occurring during this period. Thyroid follicular formation starts around stages 46/47 in *X. laevis* tadpoles (Jayatilaka, 1978) and the thyroid gland develops into a

functional gland until stage 53 (Nieuwkoop and Faber, 1994). At NF stage 60, metamorphic climax begins which is characterized by a rapid increase in thyroxine synthesis and the dramatic morphogenetic events, including remodeling of structures such as the craniofacial region and gut, differentiation of the liver, and resorption of the gill and tail (Dodd and Dodd, 1976). Substances which inhibit thyroid function or antagonize thyroid hormone action cause reductions in developmental rates of tadpoles (Lim, et al. 2002) whereas substances that mimic or enhance thyroid hormone activity accelerate metamorphosis (Shi, Y, B., 1999). Thyroid stimulating hormone travels via systemic circulation to the thyroid gland and stimulates thyroid cells to synthesize and release thyroid hormones into systemic circulation. In this study, the positive control (1µg/L thyroxine) triggered notable acceleration of metamorphic development compared to all treatments with thyroid hormone inhibitors or antagonists. Thyroxine treatment led to progressive development in the tadpoles which lasted throughout the 30 days of this study. Thirty-day (30-day) exposure of frog larva (*Xenopus laevis*) to Propylthiouracil (PTU) led to considerable reduction in tadpole growth rates and the developmental stage as well as hind limb length. Propylthiouracil caused a complete inhibition of metamorphosis as tadpoles did not develop after stage 54 within 30 days of exposure to chemical. Propylthiouracil induced the inhibition of tail elongation as compared to the control. Methimazole and Propylthiouraci separately impeded larval development and morphological changes in the thyroid gland in the form of reduced colloid, cellular hyperplasia, hypertrophy and glandular hypertrophy. The above result of this study is supported by the work of (Engler et al., 1982a,b;) which reported that both methimazole and propylthiouracil have the ability to inhibit thyroxine synthesis by blocking thyroid peroxidase coupling of iodine to the tyrosine precursor contained within thyroglobulin. Ammonium perchlorate disrupted iodide uptake by the follicular cells of the thyroid gland and inhibited forelimb emergence, hind limb development as well as tail resorption, linked to significant hypertrophy of the thyroid follicular epithelium. According to O'Connor et al. (1999) histopathological change of the thyroid gland is the most valuable information regarding thyroid toxicants. In this study, during tadpole transformation into frog, secreted thyroid hormone induced morphological modification of larval tissues. Ethylenthiourea (ETU) caused a striking retardation of tadpole development which resulted in lower developmental stages compared to the control tadpoles. This study further shows that ethylenthiourea inhibited the development of thyroid gland and suppressed thyroidal activity. The T3 and T4 hormonal levels in plasma serum affected the metamorphosis in all stages of tadpole larvae. All chemical treatments (Propylthiouracil, Ethylenthiourea; Methimazole; Fluoxetine; and Ammonium perchlorate) used in this study caused significant depletion in thyroxine levels. This result is consistent with that obtained by Callery and Elinson, (2000) as they reported that methmizaole and propylthiouracil were well characterized inhibitors of thyroxine synthesis in a number of species and have been shown to retard the metamorphic process in amphibians. The work by Lim et al. (2002) reported that treatment of tadpoles with synthetic thyroid hormone receptor antagonists (e.g., NH^{-3}) leads to retardation or even complete blockade of *Xenopus laevis* (*X. laevis*) metamorphic development. Many other scientists have reported the effects of exposure to propylthiouracil and

another peroxidase inhibitor ethylenethiourea in *Xenopus laevis*. After exposure to propylthiouracil, also known as inhibitor of deiodinase, *Xenopus tropicalis* tadpoles showed considerable reduction in the developmental stage (Huang, 2001).

4.0 CONCLUSION

In this study, the positive control (1µg/L thyroxine) triggered notable acceleration of metamorphic development compared to all treatments with thyroid hormone inhibitors or antagonists. Thyroxine treatment led to progressive development in the tadpoles. All chemical treatments (Propylthiouracil, Ethylenthiourea; Methimazole; Fluoxetine; and Ammonium perchlorate) used in this study caused significant depletion in thyroxine levels.

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