RESEARCH PAPER | Zoology | Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

Effect of Methyl Parathion on TSH, T_4 And T_3 in Fish, Labeo Rohita

KEYWORDS: Pesticidal stress, methyl parathion, Labeo rohita

S. N. C. Ray	Chandra Mohan	R. C. Sinha
Centre for Environment and Nature Conservation Department of Zoology, Patna University, Patna	Centre for Environment and Nature Conservation Department of Zoology, Patna University, Patna	Centre for Environment and Nature Conservation Department of Zoology, Patna University, Patna

ABSTRACT *Pesticides are often used indiscriminately, in large amount causing environmental pollution. Pesticides cause adverse effects on different body systems, including endocrine system. Many authors have reported the ability of organophosphate pesticides to disturb thyroid function. The aim of this work was to investigate the effect of sub-acute toxicity on TSH, T_4 and T_3 in the fish Labeo rohita. The acute toxicity L_{50} 96 h was 16.8 ppm for methyl parathion and the fish was exposed to 1/3rd of LC_{50} h. (5.6 ppm). TSH decreased by 42.6% in 24 h but increased by 303.9% and 76.5% during 48 & 72 h and then decreased during 96 h by 11.8%. T_3 decreased by 82.2, 56.8, 19.2 and 61.7% in 24, 48,72 hand 96 h respectively than the control. T_4 decreased by 7.2, 13.4 during 24 and 48 h respectively, but increased by 41.6% to that of 48 h in 72 h. The sub-lethal toxicity which cause decrease in serum T_3 & T_4 and TSH may be due to the reduction in fish metabolic rate, indirectly reducing the toxic impact of the pesticides. During pesticidal exposure, for regaining safe homeostasis fish perform many physiological processes and the two important physiological processes which are modulated when fishes are exposed to stress are the hormonal status and immune functions. Suppressed level of thyroid hormones found in Labeo rohita depicts physiological stress*

Introduction:

Pesticides have brought tremendous benefits to mankind by increasing production and controlling the vector of man and animal diseases. At the same time use of these pollutants has posed potential health hazards to life of fishes. Pesticides are major cause of concern for aquatic environment because of their toxicity, persistency, and tendency to accumulate in the organisms (Joseph and Rai, 2010). The impact of these pesticides on aquatic organisms is due to the movement of pesticide from various diffuse or point sources. These pesticides are posing a great threat to aquatic fauna especially to fishes, which constitute one of the major sources of protein rich food for mankind.

Blood is highly susceptible to internal and external environment fluctuations because it is the vehicle for the transport of such pollutants (Blaxtall, 1972). The fish serves as a bioindicator of water quality and the impact of the pesticide can be well understood by analyzing either blood or serum of the fish, because blood is a pathophysiological reflector of the whole body (Sharma and Singh, 2004, 2006; Ray & Sinha, 2015). The toxic effects of pesticides to the blood of the fishes have been studied by many researchers (Kumari *et al*, 2010; Kumari *et al* 2011; Kumari *et al*, 2011; Ray and Sinha, 2015). The response of stress in fish is charaterised by the stimulation of hypothalamus which results in the activation of the neuro- endocrine system and a subsequent cascade of metabolic and physiological changes. These changes enhance the tolerance of an organism to face an environmental variation or an adverse situation while maintaining a homeostasis (Ray and Sinha, 2015). Under the condition of stress, the body of the fish immediate response recognised as primary and secondary responses. The primary response is the perception of an altered state by the central nervous system and the release of stress hormone, cortisol and catecholamines into the blood stream (Randall and Perry, 1992) causing change in the blood and tissue chemistry e. g. an increase in plasma glucose. The entire metabolic pathway produces a burst of energy to prepare the fish for an emergency situation (Rottman *et al*, 1992; Ray and Sinha, 2015). Many researchers considered as a "rule of thumb" that fishes undergoing stressful situation, exhibit plasmatic increases of cortisol and glucose (Hatting, 1977, Barcellos *et al*, 1999; Ray and Sinha, 2015). Cortisol activates glycolysis and gluconeogenesis processes in fish. The whole process increases the substrate level (glucose) to produce enough energy according to the demand of the animal.

In fish, hormones are critical towards maintaining proper physiological function and amongst the many hormones found in fish, the thyroid hormones (T_4) and (T_3) are known to play an important role in fish growth (Hoggs *et al*, 1982; Miwa and Inui, 1985) and early development (Brown, 1997). When the fishes are exposed to stressors, the level of thyroid hormones have been demonstrated to be decreasing (Pickering, 1993; Deane *et al*, 2001) and the chemical pollutants have been reported to detrimentally affecting thyroid hormonal status in a number of fish species (Xu *et al*, 2002; Brown *et al*, 2004; Scott and Sloman, 2004; Vandar Ven *et al*, 2006). Thyroid hormones affect various aspects of metabolism and also play a role in a stress related mobilisation of glucose in fish. Thyroxin treatment caused hyperglycemic effect in some cases (Chan and Woo, 1978). However, other studies observed hypoglycemic responses in thyroxin treatment (Murat and Serfaty, 1970). Thyroidal treatment was found to promote the anabolic effects of growth hormone (lower liver glycogen and high serum cortisol) in rainbow trout (Farlund *et al*. 1980); on the other hand growth hormone treatment significantly elevated plasma T_3 concentration of rainbow trout (Farbridge and Leatherland, 1988).

It is apparent from the literature cited above that there are apparent inconsistencies in these experiments which may potentially reflect the interaction of thyroid hormones with other hormones. The aim of the present paper is to see the effect of acute toxicity of the methyl parathion on the

Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

thyroid hormones (TSH, T_4 and T_3) due to pesticidal stress in the fish, *Labeo rohita*.

Materials and methods:
Labeo rohita, a common carp were obtained from the local hatchery. Fishes were acclimated to laboratory conditions for about 5 days. They were kept in aquarium tank (250 L) and the water was constantly aerated by static system. During the acclimation period, they were given artificial (commercial) feed and grounded shrimp available in the local market to avoid the possible effects of starvation on any other parameters under study. The feeding and maintenance of the fishes and physico-chemical characteristics of the aquarium were measured. Short term test of the acute toxicity over a period of 96 h were performed on these fishes following the renewal assay. Fishes were exposed intracoelomatically with 1/3rd of 16.8 ppm of methyl parathion (LC_{50}) for 96 h to evaluate in vitro concentration of serum TSH, T_4 and T_3 by Chemiluminescence Immuno Assay Reader Neo-Lumax, Model-4901 because it has measuring ranges over many other common assay methods and excellent for detection and quantification of analytes. Serum concentration of T_3 and T_4 and TSH were expressed by ng/ml, ng/ml and µg/dl. The data collected from control and treated groups were represented as mean ± S.D. for (standard deviation) each group.

Blood collection:
The fishes were taken out of aquarium individually through fish net with a minimum possible disturbance. After preliminary investigations, the blood samples were collected from the caudal fin as described by many authors. In the present study, the blood collection from the caudal fin had to be abandoned because there was an unusual increase in the various enzymatic activities, which might have leaked from the surrounding muscle tissues into the blood. Thus, cardiac sampling was the only suitable method available as an alternative to obtain blood under study.

Results and Discussion:
The acute toxicity of methyl parathion (LC_{50} 96 h) was 16.8 ppm and the fishes were exposed to 1/3rd of LC_{50} 96 h (5.6 ppm) and the serum T_3, T_4 and TSH activities of *Labeo rohita* were measured with respect to control fish during 96 h. Thyroid hormones are known to play a crucial role in many metabolic and physiologic processes and are essential for normal growth. Thyroid hormones are implicated in reproduction and appear to be important in the regulation of development. Disruption of the thyroid axis may seriously compromise normal development, differentiation, growth and reproduction in many vertebrate like fishes (Brown *et al*, 2004).

Chemical pollutants have been reported to affect thyroid hormone status in many fishes (Scot and Sloman, 2004). In the present study, it was found that T_3 decreased by 82.2%, 56.8%, 19.2% and 61.7% in 24, 48, 72 and 96 h respectively, as compared to the control whereas T_4 decreased by 7.2%, 13.4% during 24 and 48 h respectively, but increased by 41.2% during 72 h. Decreased serum T_3 was also observed by Brucker (1998) in the fish, *Clarias batrachus* when exposed to cadmium. The reduction in T_3 level when exposed to methyl parathion may be due to reduced metabolic activity, thus indirectly reducing the toxic impact of the pesticide. Thyroid hormones play a role in stress related mobilisation of glucose in fish, *Labeo rohita* (Ray and Sinha, 2015). Mobilisation of readily available energy in the form of glucose enhances the survival of fish (Barton and Iwama, 1991; Pickering, 1993). Wu *et al* (2003) reported the levels of plasma T_3 reduced in *Cyprinus carpio* in hypoxic condition where carbohydrate reserves (glycogen) is depleted to meet the energy demand by pesticidal induced hypoxia. Liver glycogen reserves first get depleted during hypoxia (Kumari *et al*, 2011). Nowadays, most of the research work focused on effects of Environmental Disrupting Chemicals (EDC) on reproduction, sex steroids (Tyler *et al*, 1998) and thyroid hormones (Eales and Brown, 2005). Suppression of the level of thyroid hormones found in Salmonid fishes depicts physiological stress responses (Pickering, 1993; Mc Donald and Millegan, 1999; Pankhurst and Van der Krak, 1999; Shreek *et al*, 1997). Fishes are known to respond to environmental pollutants by altering serum hormones levels especially T_3. Significant reduction of Serum T_3 level was found in *S. mossambicus* after Dimecron exposure (Thangavel *et al*, 2005). Various studies have established that the environmental contaminants affect thyroid hormone levels and cause thyroid dysfunction in fish (Warning *et al*, 1997). Both T_3 & T_4 regulate growth, development and metabolism of fish and also involved in many biological functions (Griffin, 2000). T_4 is the predominantly circulating hormone, through T_3 is metabolically more active and changes according to metabolism. Presence of T_3 in lesser amount may indicate the rapid turnover from T_4 to T_3 usage (Starling *et al*, 1973). A study on Mexican pesticide applicators exposed to ethylenbis (dithiocarbamate) revealed an increase in TSH (Steenland *et al*, 1997).

The other reason for the reduction of T_3 may be due to the indirect action of the pesticide through the interference of cortisol. On the contrary, the decrease in the level of T_4 was much less as compared to T_3 in the present study (Tab.1). The reduction of T_3 due to methyl parathion exposure may be indicative of re-directing of metabolic energy from anabolic processes and towards more vital catabolic processes which are necessary in order to sustain life. Methyl parathion also suppresses the aerobic oxidation of carbohydrate leading to energy crisis in the fish (Ray and Sinha, 2015). The increase of TSH during 48 h in the present study (Tab.1) may be due to the negative feedback and low amount of circulating T_3 &T_4 at the peripheral level of hypothalamus- pituitary- thyroid (HPT) axis. Nutritional status has a strong influence on the thyroid function in fish. It has been observed during the present study that the fishes during pesticidal stress do not consume any food. The reduction in food intake decreases the sensitivity of the thyroid tissue to TSH and decreased hepatic activity. These two phenomena induce a decrease in circulating T_3 and T_4. The thyroid hormones influence intermediary metabolism. Leatherland (1994) suggested that T_3 could act as a permissive factor which would facilitate the direct action of the other anabolic hormones involved in intake control.

Behavioural changes:
In the present study control fishes behaved normally, fish aggregated in the bottom of the aquarium. Irregular, erratic and darting swimming movements, loss of balance and thereafter drowning were quite apparent in the fishes with the increase in exposure period. Fishes secreted copious amount of mucus all over the body as a preventive measure for toxicity effect. Similar reports have been made by Pandey *et al*, 2009; Singh *et al*, 2009; Saxena *et al*, 1997)

Conclusion:
Methyl parathion had a profound impact on the behaviour and thyroid hormone level of *Labeo rohita*, when they were exposed to sub-lethal concentration and also had ad-

Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

verse effects on their physiology. An altered behaviour and thyroid hormone level of exposed fishes was probably due to impaired metabolism and pesticide induced stress.

Tab. 1: Showing the variation in TSH, T_3 and T_4 hormones

Parameter	Control	24 h	48 h	72 h	96 h
TSH	1.275±0.69	0.732±0.44 42.59% wrt C	5.15±1.147 303.92% wrt C	2.25±0.451 76.47% wrt C	1.125±0.36 11.76% wrt C
T3	2.433±1.16	0.433±0.065 82.20% wrt C	1.050± 0.10 56.84% wrt C	1.967±0.306 19.15% wrt C	0.933±0.153 61.65% wrt C
T4	1.121±0.438	1.040±0.288 7.23% wrt C	0.971±0.160 13.38% wrt C	1.587±0.175 41.57% wrt C	

Here; wrt = with respect to, C = Control

Fig.1: Changes in TSH, T_3 and T_4 hormone levels as a function of 24 h interval from 24 h till 96 h.

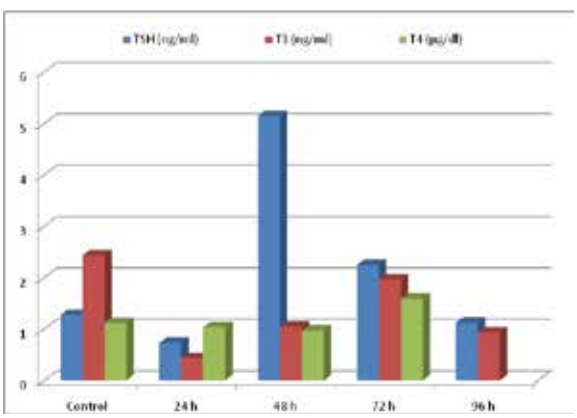


Fig.2: Changes in TSH, hormone level as a function of 24 h interval from 24 h till 96 h.

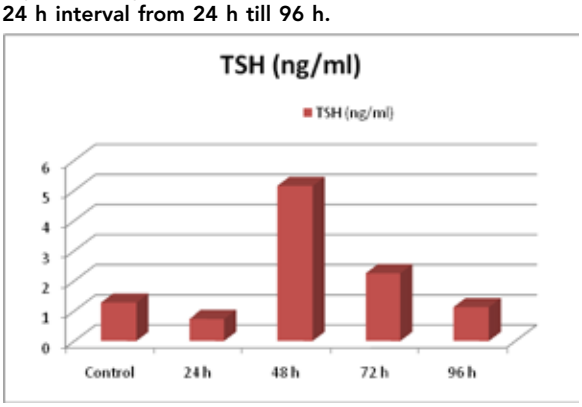


Fig.3: Changes in T_3 hormone level as a function of 24 h interval from 24 h till 96 h.

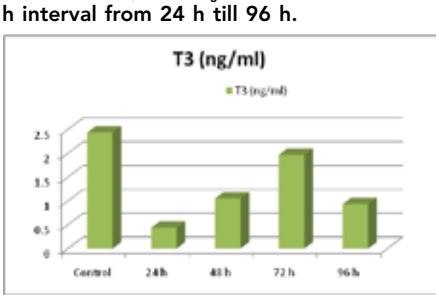


Fig.4: Changes in T_4 hormone level as a function of 24 h interval from 24 h till 72 h.

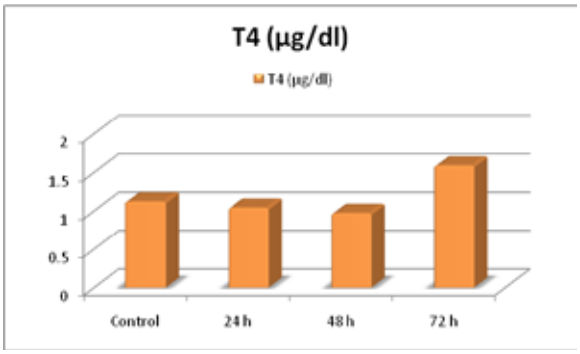


Fig.5: Changes in T_3 and T_4 hormone levels as a function of 24 h interval from 24 h till 96 h.

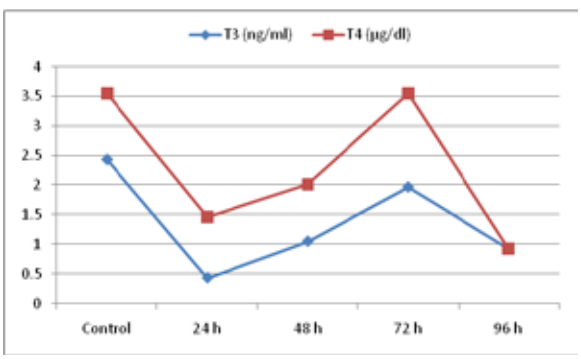
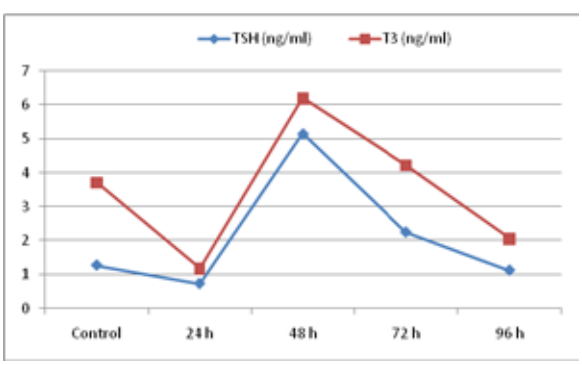


Fig.6: Changes in T_3 and TSH hormone levels as a function of 24 h interval from 24 h till 96 h.



Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

REFERENCE

Blaxhall, P.C. (1972). The haematological assessment of the health of fresh water fish: A review of selected literature. J. Fish. Biol. 4: 593-604. | Brown S.B., Adams, B.A., Cyr, D.G. & Eales, J.G. (2004). Contaminant effects on the teleost fish thyroid. Environ. Toxicol. Chem., 23: 1680-1701. | Brown, S.B., Evans, R.E., Vandenbyllardt, L., Finson, K. W. & Palace, V.P. (2004). Altered thyroid status in lake trout (Salvelinus namaycush) exposed to co-plener 3, 3', 4' 5- pentachlobiphenyl. Aquat. Toxicol. 67: 75-85. | Brown, R.M.S. (1997). U. S. Assessment of the georges bank haddock stock. Woods Hole, M.A. pp. 69. | Chan, D.K.O. & Woo, N.Y.S. (1978). Effect of cortisol on the metabolism of the eel, Anguilla japonica. Gen. Comp. Endocrinol., 35: 2005-2015. | Deane, E.E., Li, J. & Woo, N.Y.S. (2001). Hormonal status and phagocytic activity in sea bream infected with vibriosis. Comp. Biochem. Physiol. B 129: 687-693. | Eales, J.G. and Brown, S. B. (2005). Thyroid hormones In: Mommsen, T.P., Moon, T.W. (Eds). Environ. Toxicol. Biochem. and Mol. Biol. of Fishes Vol. 6. Elsevier, New York. Pp. 397-412. | Fagerlund, U.M., Huggs, D.A., Mc bride., J.R., Plotnikoff, M.D. & Dosanjh, B.S. (1980). The potential for using the anaerobic- hormone 17α-methyltestosterone and or 3,5,3'-tri-iodothyroxine in fresh water rearing of Coho salmon, Oncorhynchus kisutch and affects of subsequent sea water performance. Can. J. Zool., 58: 1424-1432. | Farbridge, K. J. & Leatherland, J. E. (1988). Interaction between ovaine growth hormone and tri-iodothyroxine on metabolic reserves of rainbow trout, Salmon gairdneri. Gen. Camp. Endocrinol, 78: 164-172. | Griffin, J. E. (2006). Thyroid. In: Griffin, J. E., Ojida, S. (eds). Textbook of Endocrine Physiology. Oxford University Press, U. K. pp. 303-327. | Higgs, D.A., Fagerlund, U.H.M & McBride, J.H. (1979). Influence of orally administered L- thyroxine of 3, 5, 3' tri-iodo-L-thyroxine on growth and salanity tolerance of the Atlantic salmon, Salmo solar. Aquaculture, 45: 143-146. | Higgs, D. A., U. H. M. Fagerlund, J. G. Eales & J. R. McBride (1982). Application of the thyroid and steroid hormones as anabolic agents in fish culture Comp. Biochem. Physiol. (B). 73: 143-176. | Joseph, B. & Rai S.J. (2010). Effect of curacron toxicity on the total serum protein content of Cyprinus carpio. Toxicol. Environ. Chem. 92: 1889-1893. | Kumari, K., Ranjan, N. and Sinha R.C. (2010). Effect of endosufan on the absorption ratio of α and β chains of haemoglobin and acetylcholinesterase activity of the fish, Labeo rohiha. J. of V. N. Karazin Kharkiv National University, Russia. 920: 83-89. | Kumari, K., Ranjan, N. & Sinha R.C.(2011). LDH and SDH activity as potential biomarkers in the fish, Labeo rohita. Proc. Int. Conf. on Biodiversity & Aquatic Toxicology, Deptt. of Zoology, & Aquaculture, Acharya Nagarjuna University. Pp 130-138. | Kumari, K., N. Ranjan, and R. C. Sinha (2011). Multiple biomarker response in the fish, Labeo rohita due to hexavalent chromium. Proc. 2nd Int. Conf. on Biotechnology & Food Science: 155-158. | Mc Donald, G. and L. Milligen (1997). Ionic, Osmotic and acid-base regulation in stress. In: Fish Stress and Health in Aquaculture. (Iwama, G.K., Pickering, A.D., Sumptor, J.P. and Schrack, C.B. Eds.), Cambridge University Press, pp. 119-144. | Miwa, S. & Inui, Y. (1985). Effects of L- thyroxine and ovine growth hormone on smoltification of amago salmon (Oncorhynchus roduras). Gen. Comp. Endocrinol., 58: 436-442. | Murat, J. C. & Serfaty, A. (1970). Au subject d'un effect hypoglycemiant de la thyroxione chez la carpe, Cyprinus carpio. Seance Soc. Biol. 164: 1842-1845. | Pandey, R.K., Singh, R.N., Singh, S., Singh, N.N. & Das, V.K. (2009). Acute toxicity bioassay of dimethate on fresh water air breathing catfish, Heteropneustes Fossilis. J. Environ. Biol. 30: 437-440. | Pankhurst, N.W. and Van der Kraak, G. (1997). Effects of stress in reproduction and growth of fish. In: Fish Stress and Health in Aquaculture (Iwama, G.K., Pickring, A.D., Sumptor, J.P. & Schreck, C.B. Eds.), Cambridge University Press. pp. 73-93. | Pickering, A.D. (1993). Growth and stress in fish production. Aquaculture III: 51-63. | Randall & Perry (1992). Catecholamines. In: Fish Physiology. (Randall, D. J. and Hoar, W.S. Eds.), Vol. XII B. The Cardiovascular System, Academic Press, NewYork, pp. 255-300. | Ray, S.N.C. and Sinha, R.C. (2014). Serum Cortisol and Glucose: Reliable Bioindicators of stress in the fish, Labeo rohita. Int. J. Innovative Sci., Engg. and Tech. 1: 6-17. | Ray, S. N. C. and R. C. Sinha (2015). Effect of methyl parathion on the carbohydrate metabolism of the fish, Cirrhinus mrigala. J. of Mat. Sci. & Reser. B, communicated. | Rottman, R.W., Fransis-Floyd, R. and Durborow, R. (1992). The role stress in fish disease. SRAC Publications No. 474, pp. 4. | Saxena, V., Trivedi, S. and Saxena, P.N. (1997). Acute toxicity of two organophosphorus pesticides nuvan and dimecron to fresh water murrel, Channa orientalis. J. Environ. Poll. 4: 79-83. | Schreck, C.B., Olla, B.L. and Davis, M.W. (1997). Behavioural responses to stress. In: Fish Stress and Health in Aquaculture (Iwama, G. K., Pickering, A.D., Sampter, J.P. & Schreck, C. B., Eds.), Cambridge University Press, pp. 145-170. | Scott, G.R. and Sloman, K.R. (2004). The effects of environmental pollutants on complex fish behaviour: Integrating behavioural and physiological indicators of toxicity. Aquat. Toxicol., 68: 369-392. | Sharma, G. & Singh, S. (2004). Studies on the effect of intoxicant indofil on the blood morphology of Channa punctatus. Bionotes, 6: 20. | Sharma, G. and Singh, S. (2006). Assay on some blood parameter of the fish, Channa punctatus after intoxication of Indofil. Bionotes 8: 21. | Sterling, K., Brenner, M.A. & Saldanha, B.F. (1973). Conversion of thyroxine to tri-iodothyroxine by cultured human cells. Science, 179, 1000-1001. | Thangavel, P., Sumathiral, K., Karthikayan, S. & Ramaswamy, M. (2005). Endocrine responses of fresh water teleost, Sarotherodon mossambicus to dimecron exposure. Chemosphere, 61: 1083-1092. | Tyler, C.R., Jobling, S. & Sumptor, J.P. (1998). Endocrine disruption in wild life: a critical review of the evidence. Crit. Rev. Toxicol. 28: 319-361. | Vander Ven, L.T.M., Van den, E.J., Brandhof, J. H.Vos, Power, V. & Wester P.W. (2006). Effects of the anti-thyroid agent propythiouracil is a partial life cycle assay with Zebrafish. Environ. Sci. Technol. 40: 74-81. | Warning, C. P. and Brown, J. A. (1997). Plasma and tissue thyroxin and tri-iodothyronine contents in sub lethal stressed aluminium- exposed brown-trout (Salmon trulta). Gen. Comp. Endocrinol. 106: 120-126. | Wu, R.S.S., Zhou, B.S., Randall, D.J. & Lam, P.K. (2003). Aquatic hypoxia in an endocrine disruptor and impairs fish reproduction. Environ. Sci. technol., 37: 1137-1141. |