International Journal of Biology and Biological Sciences Vol. 4(3), pp. 037-047, December 2015
Available online at http://academeresearchjournals.org/journal/ijbbs
ISSN 2327-3062 ©2015 Academe Research Journals

Full Length Research Paper

Comparative study of the thyroid function in *Heteropneustes fossilis* (Bloch.) under exposure to sub-lethal concentration of chlorpyrifos and 2, 4 Dichlorophenoxyacetic acid

Nazia Khatun* and Rita Mahanta

Department of Zoology, Cotton College, Gauhati University, Assam, India.

Accepted 14 December, 2015

Changes in the concentrations of thyroid hormones in freshwater catfish, *Heteropneustes fossilis* (Bloch), under exposure to sub-lethal concentrations of 2, 4-Dichlorophenoxyacetic acid (usually referred to as 2, 4 D, herbicide) and chlorpyrifos (organophosphate insecticide) were analyzed in this study. A comparison of the effects of 2, 4 D and chlorpyrifos at different concentrations on hormonal activities afforded a promising approach. The experiment was performed for 30 days and the fish were subjected to 1/10th and 1/50th Lethal Concentration (LC_{50}) value of 2, 4 D (3.00 ppm and 0.60 ppm) and chlorpyrifos (1.42 ppm and 0.28 ppm). Both the xenobiotics were found to be effective in increasing the serum concentration of triiodothyronine (T3) and thyroxine (T4) up to the 20th day after which it showed a declining trend at the terminal phase of the experiment. This trend was observed to be dose dependent and time of exposure. The test chemicals exhibited drastic effect on thyroid stimulating hormone (TSH) level as indicated by significantly lower ($p<0.01$) concentration of TSH than that of the normal control group. In comparison to chlorpyrifos, 2, 4 D was observed to be more effective in decreasing the TSH level in the experimental fishes and in altering the hormone profile for the assessment of thyroid function.

Key words: Chlorpyrifos, 2, 4-Dichlorophenoxyacetic acid, thyroid hormones, *Heteropneustes fossilis*.

INTRODUCTION

Environmental pollution caused by pesticides, especially in aquatic ecosystems, has become a serious problem. The surface run-off from cultivated lands brings heavy loads of pollutants into natural and manmade water bodies where inorganic fertilizers, pesticides, insecticides and rich manure are applied. The indiscriminate use of these pesticides may have impacts on non-target organisms either directly or indirectly, and can lead to reduced fish productivity by influencing abnormalities in hormone secretion and action. The rise in concentration of chemicals in edible fish tissue can affect the health of humans consuming these fishes. As pesticides degrade, they may leach into soil and water, or they may windswept or volatilized reaching neighboring or far away areas (Haider and Inbaraj, 2008). But the most harmful are those that either degrade very slowly or do not degrade in nature.

2, 4-Dichlorophenoxyacetic acid (2, 4-D) is an herbicide and secondarily a synthetic auxin (plant hormone). It is a chlorinated phenoxy compound, functions as a systemic herbicide and is used to control many types of broadleaf weeds. Previous studies found that 2, 4-D has negative impact on the male reproductive system including dead and malformed sperm (Lerda et al., 1991) (Figure 1).

Toxicity to fish and aquatic invertebrates varies widely depending on chemical form, with esters being the most toxic (WHO, 1989; Tomlin, 2006). In fish, the greater toxicity of the esters form of 2, 4-D is likely due to the greater absorption rates of the esters through the gills,

*Corresponding author. E-mail: naziakhatun.02@gmail.com. Tel: +919706080330, +919707849187.

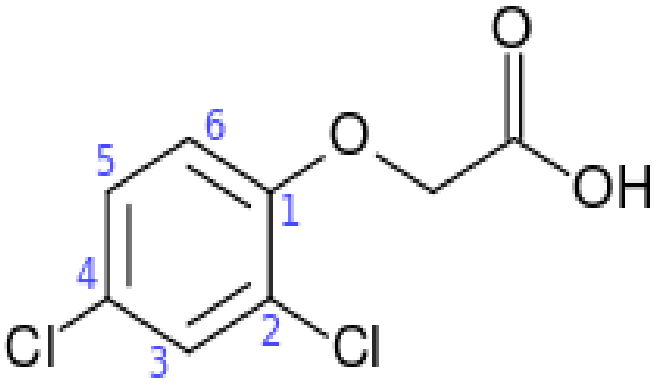

Figure 1. Chemical structure of 2, 4-D.

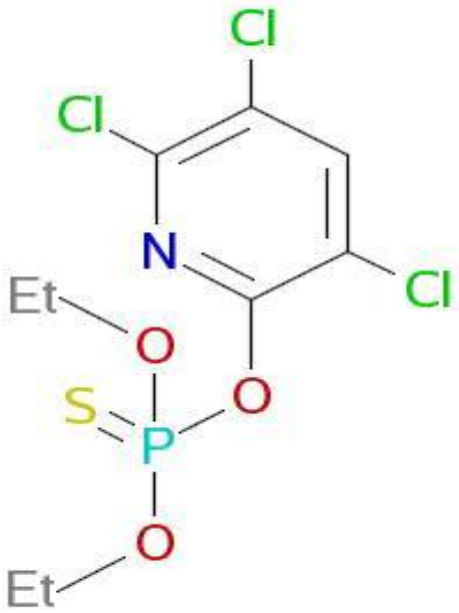

Figure 2. Chemical structure of chlorpyrifos.

where they are hydrolyzed to the acid form. Over 1500 herbicide products containing 2, 4 D as an active ingredient has demonstrated toxic effects on the thyroid and gonads following exposure (United States. Environment Protection Agency (EPA), 2005). 2, 4 D is included in the U.S EPA June 2007 draft list of Chemical for Tier I Screening. Effects of 2, 4 D on human health and the environment depend on the dose of 2, 4 D and the length and frequency of exposure.

Chlorpyrifos (O, O – diethyl O-3, 5, 6 trichloro-2-pyridyl-phosphorothioate) is used as broad-spectrum chlorinated organophosphate insecticide. Since its introduction in 1965 by Dow Chemical Company, the use of chlorpyrifos has greatly been increased (Figure 2).

The EPA classifies chlorpyrifos as Class II: moderately toxic to humans and chronic exposure has been directly linked to neurological complications, developmental disorders and autoimmune disorders. But this substance is highly toxic to aquaculture fish and bees according to National Pesticide Information Center (2009). Using the Globally Harmonised System of Classification and Labelling, the European Union (EU) has categorised chlorpyrifos as Aquatic Acute Tox 1, with the hazard phrase “H400 – very toxic to aquatic life”; and Aquatic Chronic Tox 1, with the hazard phrase “H410 – very toxic to aquatic life with long lasting effects”. A scientific study carried out by US EPA in 2011 showed that as the chemical breaks down naturally in the environment, it releases chlorpyrifos oxon, which has been found to be even more toxic than the original form of the chemical (U.S. Environmental Protection Agency, 2005).

Organophosphate pesticides cause adverse effects by interfering with the body’s hormones or chemical messengers. Fish species are sensitive to enzymatic and hormone disruptors. There is increasing evidence that a wide range of chemicals can interfere with thyroid and adrenal functions (Maranghi et al., 2003; De Angelis et al., 2007) and that the developmental life stages are critically vulnerable to these endocrine disrupting chemicals (EDC) (Mantovani, 2006). Still, reports of investigation on the effect of these xenobiotics on thyroid hormone impairment in *Heteropneustes fossilis* are still fragmentary. Thus, the aim of this study is to compare the impact of chlorpyrifos and 2, 4 D on thyroid hormone.

MATERIALS AND METHODS

Healthy freshwater fish, *H. fossilis*, weighing 10 ± 0.5 g and average length of 11 cm of both sexes were collected from local ponds of Dhubri District, Assam, India and carefully brought to the laboratory avoiding any injury to the fishes during transport. The experimental fish, *H. fossilis*, was selected because of its economic importance as well as its suitability in terms of laboratory maintenance. The experimental fishes were acclimatized in aquariums under necessary laboratory conditions for six to seven days. During the whole process of treatment, no food was supplied, though the water was changed daily. Test pesticide and herbicide were selected based on the wide use in local agricultural field.

Median lethal concentration (LC_{50}) was determined by Probit analysis (Finney, 1952) in the acclimatized fish for chlorpyrifos and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) separately. Sub-lethal dose of chlorpyrifos (1.42 ppm and 0.28 ppm, that is, $1/10^{th}$ and $1/50^{th}$ of LC_{50}) and 2,4 D (3.00 ppm and 0.60 ppm, that is, $1/10^{th}$ and $1/50^{th}$ of LC_{50}) were administered in the experimental species *H. fossilis* for a period of days intervals as 5 days, 10 days, 15 days, 20 days, 25 days and 30 days. The fishes were divided into three groups having ten individual fish of both sexes in each group:

Group I: Non-treated control group.

Table 1. Presenting the significance of differences between Group I and Group II (a) in *Heteropneustes fossilis* (Bloch.).

Groups	Days of treatment	Significance of difference	T3 (ng/ml)	T4 (nmol/l)	TSH (µ IU/ml)
Between Group I and Group II (a)	5 Days	t	19.3578	74.0794	15.2186
		p	<0.01	<0.01	<0.01
		df	18	18	18
	10 Days	t	-1.9928	7.07816	19.448
		p	>0.01	<0.01	<0.01
		df	18	18	18
	15 Days	t	5.53294	59.88	19.5672
		p	<0.01	<0.01	<0.01
		df	18	18	18
	20 Days	t	2.94104	12.0923	16.8659
		p	<0.01	<0.01	<0.01
		df	18	18	18
	25 Days	t	12.567	43.871	14.2397
		p	<0.01	<0.01	<0.01
		df	18	18	18
	30 Days	t	21.0562	-1.4431	8.64215
		p	<0.01	>0.01	<0.01
		df	18	18	18

t = student's t-test value, p = probability value, df = degree of freedom.
If t < 2.55 then p > 0.01 which is not significant.
If t > 2.55 then p < 0.01 which is highly significant.

Group II: Experimental group receiving treatment of chlorpyrifos (Group II (a) - 1.42 ppm and Group II (b) - 0.28 ppm).
Group III: Experimental group receiving treatment of 2, 4 D (Group III (a) - 3.00 ppm and Group III (b) - 0.60 ppm).

Hormone sample collection and storage

After the treatment of the above mentioned day intervals, blood samples were collected from the caudal fins in sterilized vials of the non-treated control group and the treated groups (chlorpyrifos and 2, 4-D treated). The collected blood is handled carefully to prevent haemolysis and then the samples are allowed to clot for ten minutes before centrifugation for 12 - 15 min at 3000 rpm. Serum was removed and assayed immediately for the measurement or estimation of triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) concentrations.

Measurement of thyroid hormone levels

Serum concentration of T3, T4 and TSH were assessed in *H. fossilis* by enzyme immune assay (EIA) technique using an Enzyme Linked Immunosorbant Assay (ELISA) reader.

Statistical analysis

The data sets were presented as mean ± standard deviation (SD). The results obtained were statistically analyzed for t-test, probability value using standard statistical methods.

RESULTS AND DISCUSSION

The obtained results are summarized in Tables 1 to 6 and in the graphs of Figures 3 to 8. The analysis revealed that both chlorpyrifos and 2, 4 D were effective in increasing the serum T3 and T4 concentration up to the 20th day of treatment. However, at the terminal phase of the experimental period, that is, on the 30th day, the T3 and T4 serum level was observed to be significantly lower than that of the normal control value. In the case of T4, almost equal value was observed throughout the experimental period up to the 30th day on exposure to both the concentration of chlorpyrifos. But in the case of 2, 4 D, decreased serum T4 level was recorded on the

Table 2. Presenting the significance of differences between Group I and Group II (b) in *Heteropneustes fossilis* (Bloch.).

Groups	Days of treatment	Significance of difference	T3 (ng/ml)	T4 (nmol/l)	TSH (μ IU/ml)
Between Group I and Group II (b)	5 Days	t	-0.8841	-81.824	2.61116
		p	>0.01	>0.01	<0.01
		df	18	18	18
	10 Days	t	-3.8143	-80.744	4.77531
		p	>0.01	>0.01	<0.01
		df	18	18	18
	15 Days	t	9.4967	-98.917	18.646
		p	<0.01	>0.01	<0.01
		df	18	18	18
	20 Days	t	-2.2401	-76.751	7.30258
		p	>0.01	>0.01	<0.01
		df	18	18	18
	25 Days	t	15.6929	-71.992	-0.9681
		p	<0.01	>0.01	>0.01
		df	18	18	18
	30 Days	t	39.2866	-59.46	12.7045
		p	<0.01	>0.01	<0.01
		df	18	18	18

t = student's t-test value, p = probability value, df = degree of freedom.
If $t < 2.55$ then $p > 0.01$ which is not significant.
If $t > 2.55$ then $p < 0.01$ which is highly significant.

30th day of exposure. The TSH concentration after administration of chlorpyrifos and 2, 4 D was noticeably lower than that of the normal control group, but the impact is more pronounced in the case of 2, 4 D on the 30th day.

Application of herbicides and pesticides unknowingly threatened the non-targeted aquatic animals due to their toxic effects. These xenobiotics cause endocrine disruption by interrupting normal development, reduced fertility as well as lower hatching rates and viability of offspring. Interference with endocrine hormones affects reproduction, immune functions in several fish species (Adedeji et al., 2012).

The present study shows an initial increase of all the hormonal components of thyroid function, that is, T3, T4 and TSH and a declining trend in the terminal phase of the experiment on exposure to both the xenobiotics. This general trend was observed to be dose dependent and length of exposure time. As such, serum level of T3 increased up to the 20th day on chlorpyrifos exposure whereas in the case of 2, 4 D, it increased up to the 15th day of treatment. The T4 concentration exhibited a fluctuating trend in the middle portion of the treatment with low dose of chlorpyrifos ($1/10^{th}$ of LC_{50}), but the initial increase was observed to be sustained towards the end of the treatment with chlorpyrifos ($1/50^{th}$ of LC_{50}) exposure. On the contrary, 2, 4 D administration in both doses imparts an initial increase with steady declining trend towards the experimental period. Among the three components of the thyroid hormone profile, the test chemicals (chlorpyrifos and 2, 4 D) exhibited drastic effect on the TSH level as indicated by the significantly lower ($p<0.01$) concentration of TSH than that of the normal control group.

In comparison to chlorpyrifos, 2, 4 D was observed to be more effective in decreasing the TSH level in the experimental fishes. In the case of T4, though sustained increase from the normal control value was observed on exposure to chlorpyrifos throughout the experimental period, the intensity of increase is more pronounced in 2, 4 D treatment on the 15th and 25th day. But similar trend was noticed in the case of T3 on exposure to both the xenobiotics.

The results of the present study support the findings obtained in other studies conducted in experimental models. Haviland et al. (2010) found increased thyroid hormone levels in female mice exposed to 1 and 5 $mgkg^{-1}$ chlorpyrifos on gestation days 17-20. A decrease

Table 3. Presenting the significance of differences between Group I and Group III (a) in *Heteropneustes fossilis* (Bloch.).

Groups	Days of treatment	Significance of difference	T3 (ng/ml)	T4 (nmol/l)	TSH (μ IU/ml)
Between Group I and Group III (a)	5 Days	t	-8.3877	-83.599	12.6543
		p	>0.01	>0.01	<0.01
		df	18	18	18
	10 Days	t	0.20297	-0.7265	20.1373
		p	>0.01	>0.01	<0.01
		df	18	18	18
	15 Days	t	-4.9562	44.6885	19.5069
		p	>0.01	<0.01	<0.01
		df	18	18	18
	20 Days	t	19.7257	29.4154	23.1756
		p	<0.01	<0.01	<0.01
		df	18	18	18
	25 Days	t	18.4394	28.1876	20.4491
		p	<0.01	<0.01	<0.01
		df	18	18	18
	30 Days	t	32.6302	31.5388	24.462
		p	<0.01	<0.01	<0.01
		df	18	18	18

t = student's t-test value, p = probability value, df = degree of freedom.
If t < 2.55 then p > 0.01 which is not significant.
If t > 2.55 then p < 0.01 which is highly significant.

in serum T4 levels was also observed in CD1 mice after developmental exposure to chlorpyrifos at doses low enough to not elicit inhibition of brain acetylcholinesterase (AchE). Both sexes of CD1 mice showed reduced serum T4 levels, though a more significant effect was observed in males compared to females (De Angelis et al., 2009). Jeong et al. (2006) reported that chlorpyrifos-methyl induces hypothyroidism (decreased serum T4 and increased serum TSH) and alters thyroid and pituitary gland weights through sexual maturation and adulthood in rats after long-term *in utero* and postnatal exposure, but in the present study monitoring of the hormone profile for a duration of 30 days at five days interval showed an initial increase and final decrease of T3, sustained increase of T4 and ultimate decrease of TSH.

Certain insecticides, herbicides, and fungicides have been reported to be endocrine disruptors and, more specifically, thyroid disruptors acting through diverse mechanisms such as inhibition of thyroidal iodine uptake, interference at the thyroid hormone receptor, binding to transport proteins, interference with iodothyronine deiodinases, increased clearance of thyroid hormones, interference with cellular uptake of thyroid hormones, and interference with thyroid hormone gene expression (Rakitsky et al., 2000; Boas et al., 2006; Zoeller, 2007). Zaidi et al. (2000) assessed formulators of both organochlorines and organophosphate insecticides, and it showed an increase in TSH levels and a decrease in T3 levels in workers when compared to the control group. Malathion, an organophosphate insecticide, has been reported to affect thyroid hormone levels in freshwater catfish and bullfrog tadpoles (Sinha et al., 1992; Fordham et al., 2001), but it was not associated with any thyroid dysfunction.

Conclusion

The present investigation which compared the impact of pesticide (chlorpyrifos) and herbicide (2, 4 D) on the thyroid function indicated a clear picture of interruption in the 30 days of experimental period with the sub-lethal concentration of 2, 4 D and chlorpyrifos. However, 2, 4 D was found to be more effective in altering the hormone profile for the assessment of thyroid function.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Rita Mahanta, an Associate Professor (retired) of the Department of

Table 4. Presenting the significance of differences between Group I and Group III (b) in *Heteropneustes fossilis* (Bloch.).

Groups	Days of treatment	Significance of difference	T3 (ng/ml)	T4 (nmol/l)	TSH (μ IU/ml)
Between Group I and Group III (b)	5 Days	t	-14.754	-65.633	-1.4271
		p	>0.01	>0.01	>0.01
		df	18	18	18
	10 Days	t	-1.0304	-71.028	4.41205
		p	>0.01	>0.01	<0.01
		df	18	18	18
	15 Days	t	-2.1019	-167.14	14.1122
		p	>0.01	>0.01	<0.01
		df	18	18	18
	20 Days	t	16.57	-39.124	14.4042
		p	<0.01	>0.01	<0.01
		df	18	18	18
	25 Days	t	23.6387	-127.34	10.5917
		p	<0.01	>0.01	<0.01
		df	18	18	18
	30 Days	t	51.7044	-5.4736	25.0295
		p	<0.01	>0.01	<0.01
		df	18	18	18

t = student's t-test value, p = probability value, df = degree of freedom.
If t < 2.55 then p > 0.01 which is not significant.
If t > 2.55 then p < 0.01 which is highly significant.

Table 5. Presenting the significance of differences between Group II (a) and Group III (a) in *Heteropneustes fossilis* (Bloch.).

Groups	Days of treatment	Significance of difference	T3 (ng/ml)	T4 (nmol/l)	TSH (μ IU/ml)
Between Group II (a) and Group III (a)	5 Days	t	-30.231	-165.74	-4.429
		p	>0.01	>0.01	>0.01
		df	18	18	18
	10 Days	t	2.77717	-9.0234	0.7885
		p	<0.01	>0.01	>0.01
		df	18	18	18
	15 Days	t	-10.657	-27.683	-2.982
		p	>0.01	>0.01	>0.01
		df	18	18	18
	20 Days	t	41.1411	16.557	9.77976
		p	<0.01	<0.01	<0.01
		df	18	18	18
	25 Days	t	8.46506	-20.897	9.02305
		p	<0.01	>0.01	<0.01
		df	18	18	18
	30 Days	t	18.8292	29.4045	28.8252
		p	<0.01	<0.01	<0.01
		df	18	18	18

t = student's t-test value, p = probability value, df = degree of freedom.
If t < 2.55 then p > 0.01 which is not significant.
If t > 2.55 then p < 0.01 which is highly significant.

Table 6. Presenting the significance of differences between Group II (b) and Group III (b) in *Heteropneustes fossilis* (Bloch.).

Groups	Days of treatment	Significance of difference	T3 (ng/ml)	T4 (nmol/l)	TSH (µ IU/ml)
Between Group II (b) and Group III (b)	5 Days	t	-12.652	-9.073	-3.3759
		p	>0.01	>0.01	>0.01
		df	18	18	18
	10 Days	t	2.44892	-8.6724	1.26356
		p	>0.01	>0.01	>0.01
		df	18	18	18
	15 Days	t	-10.204	-87.94	-3.1925
		p	>0.01	>0.01	>0.01
		df	18	18	18
	20 Days	t	40.4242	27.4069	10.3053
		p	<0.01	<0.01	<0.01
		df	18	18	18
	25 Days	t	7.52592	-78.288	15.1529
		p	<0.01	>0.01	<0.01
		df	18	18	18
	30 Days	t	20.9669	28.2184	29.9693
		p	<0.01	<0.01	<0.01
		df	18	18	18

t = student's t-test value, p = probability value, df = degree of freedom.
If t < 2.55 then p > 0.01 which is not significant.
If t > 2.55 then p < 0.01 which is highly significant.

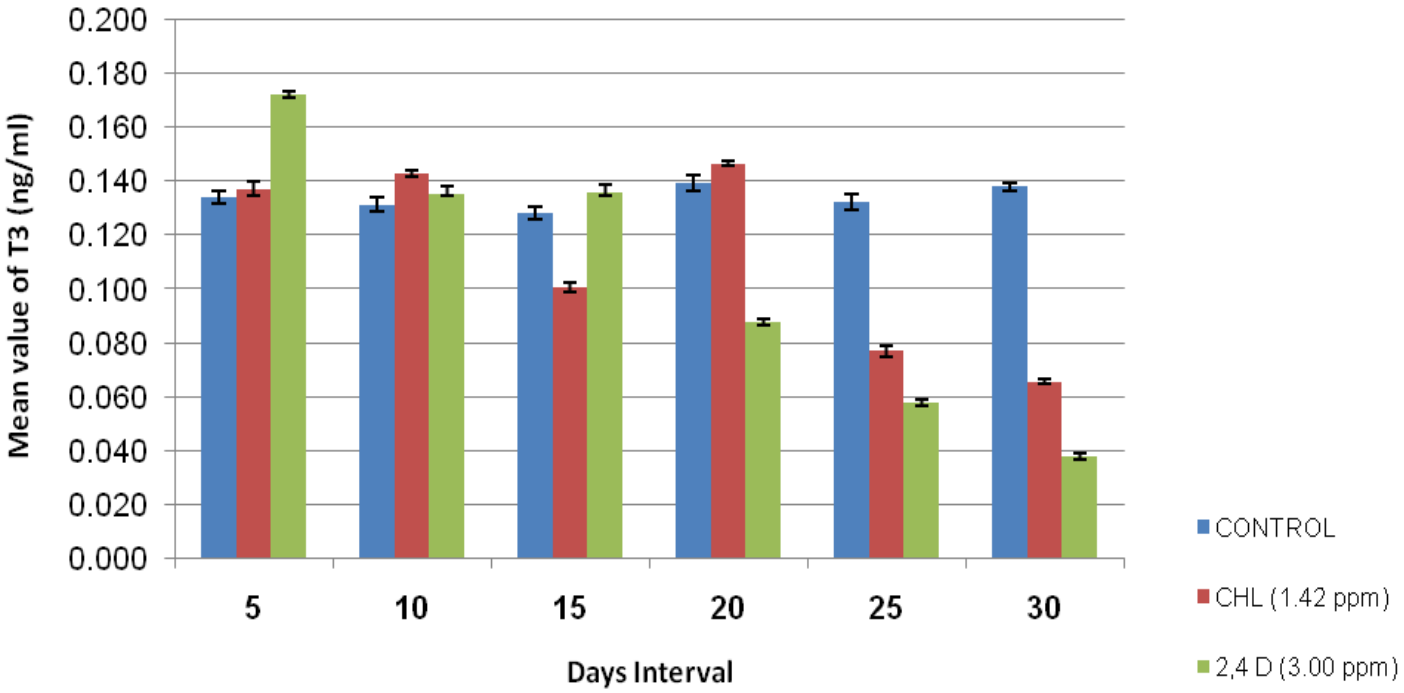

Figure 3. Presenting the mean values of T3 (ng/ml) in serum in Group I, Group II (a) and Group III (a) at different days interval.

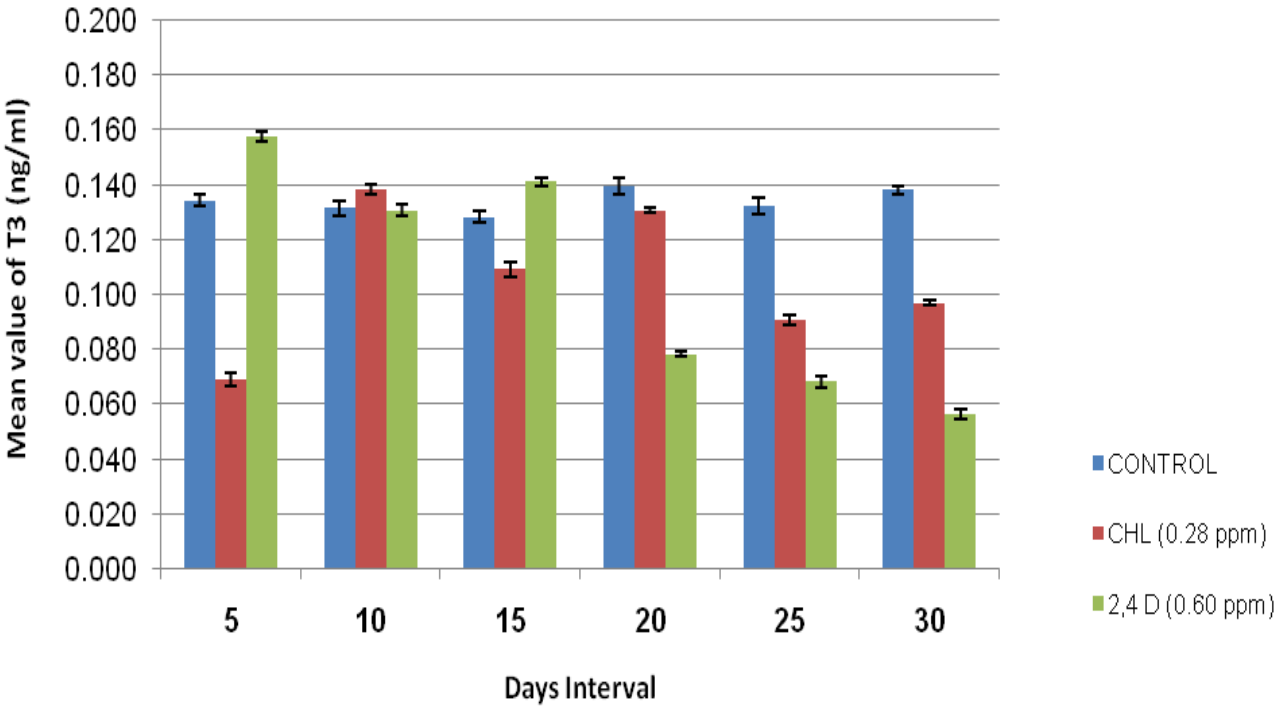

Figure 4. Presenting the mean values of T3 (ng/ml) in serum in Group I, Group II (b) and Group III (b) at different days interval.

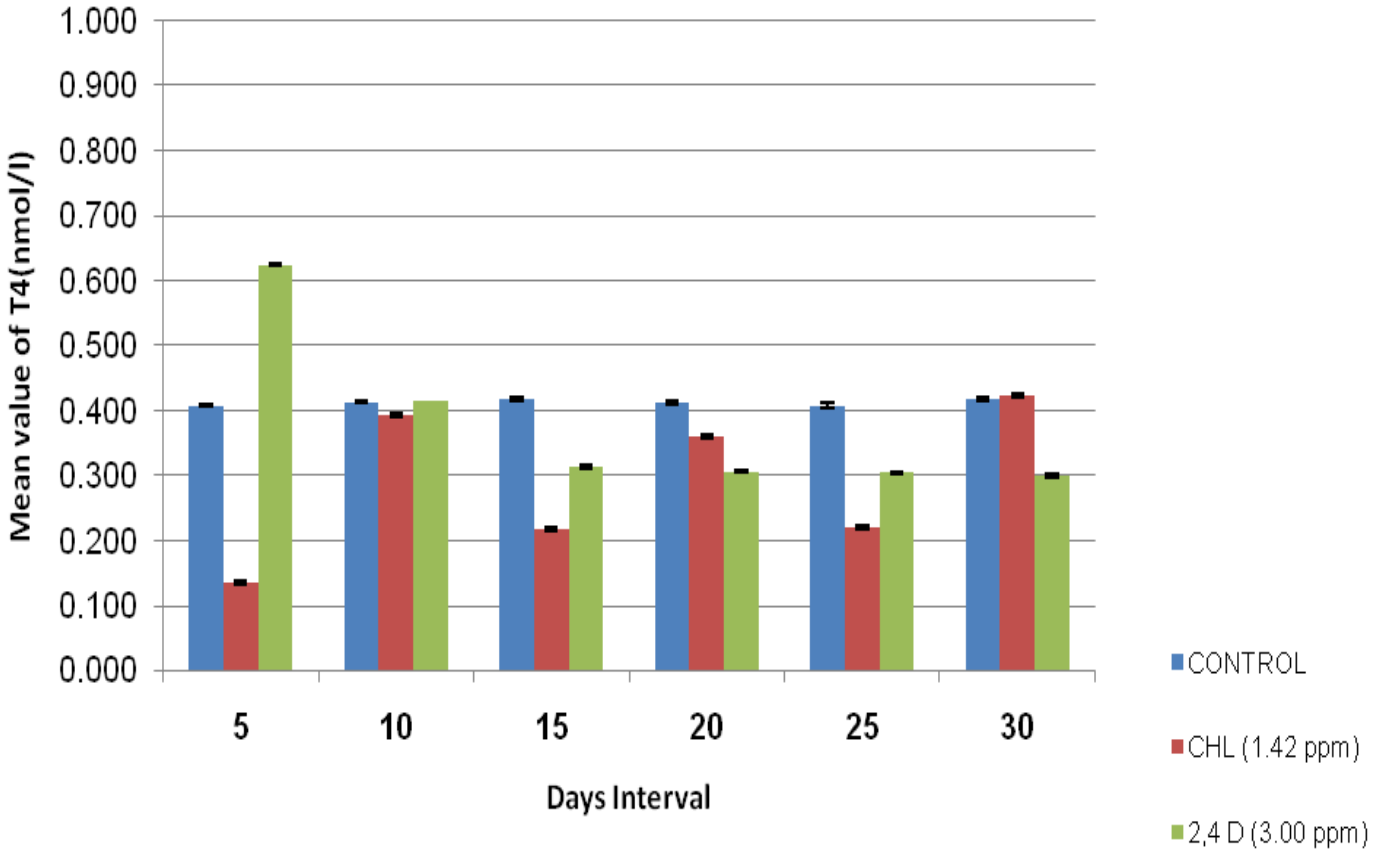

Figure 5. Presenting the mean values of T4 (nmol/l) in serum in Group I, Group II (a) and Group III (a) at different days interval.

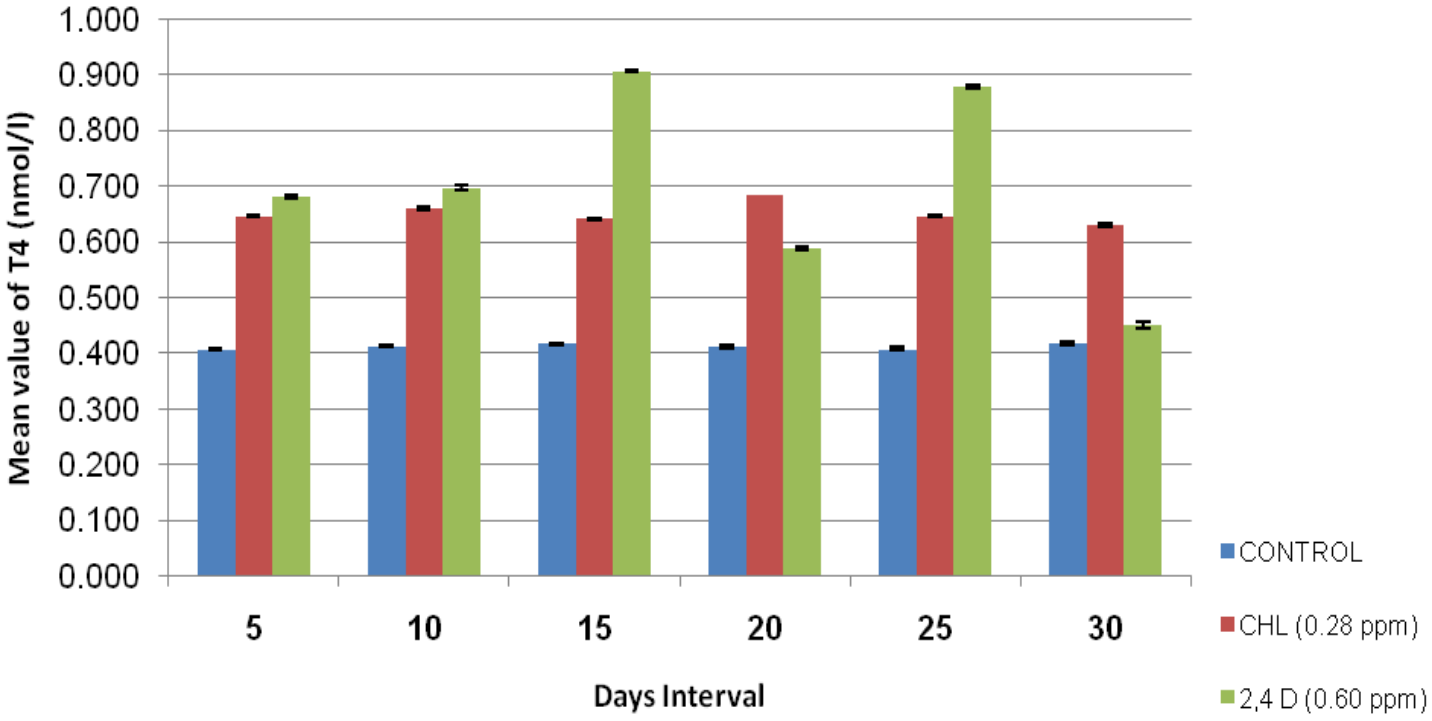

Figure 6. Presenting the mean values of T4 (nmol/l) in serum in Group I, Group II (b) and Group III (b) at different days interval.

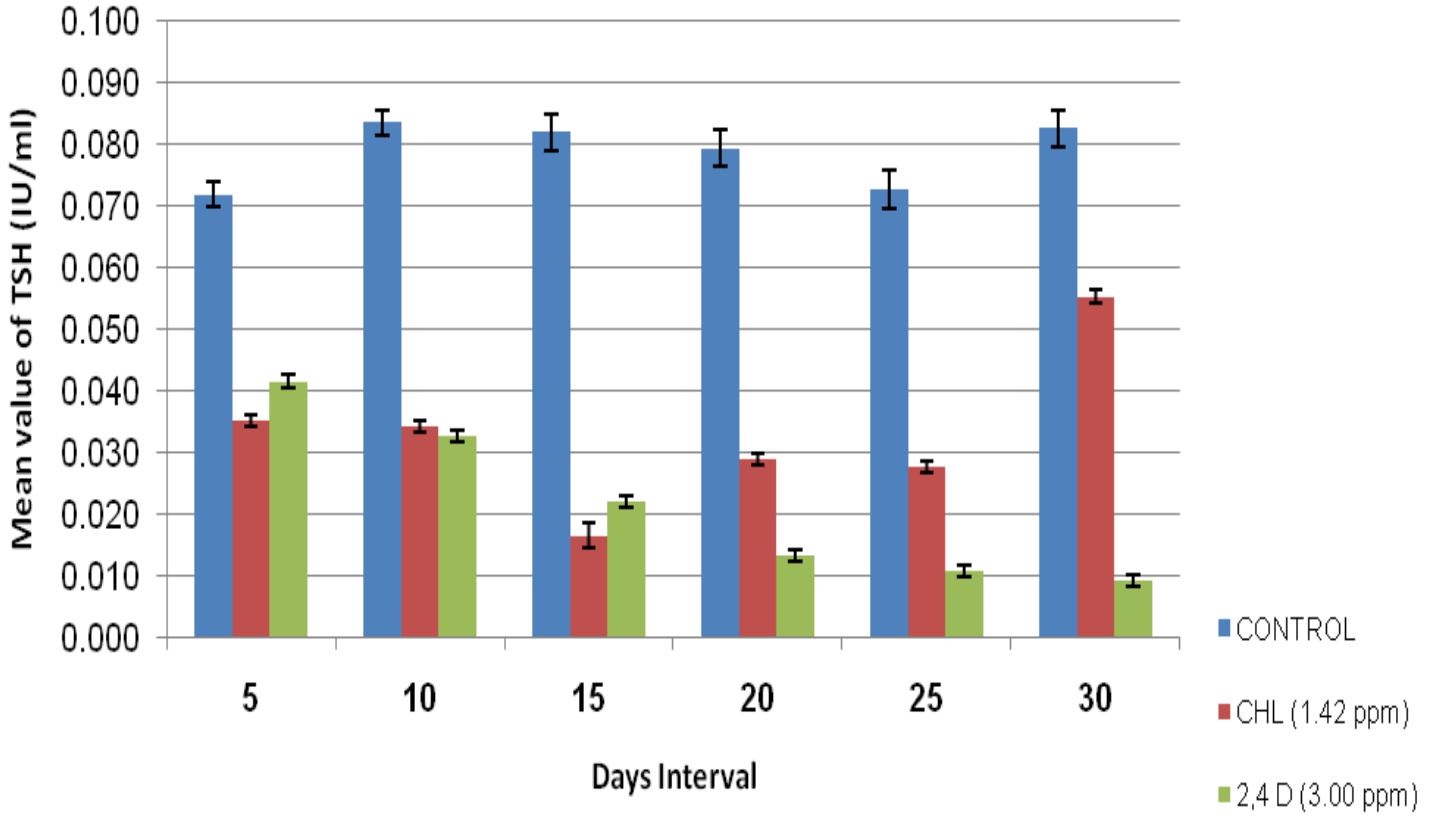

Figure 7. Presenting the mean values of TSH (IU/ml) in serum in Group I, Group II (a) and Group III (a) at different days interval.

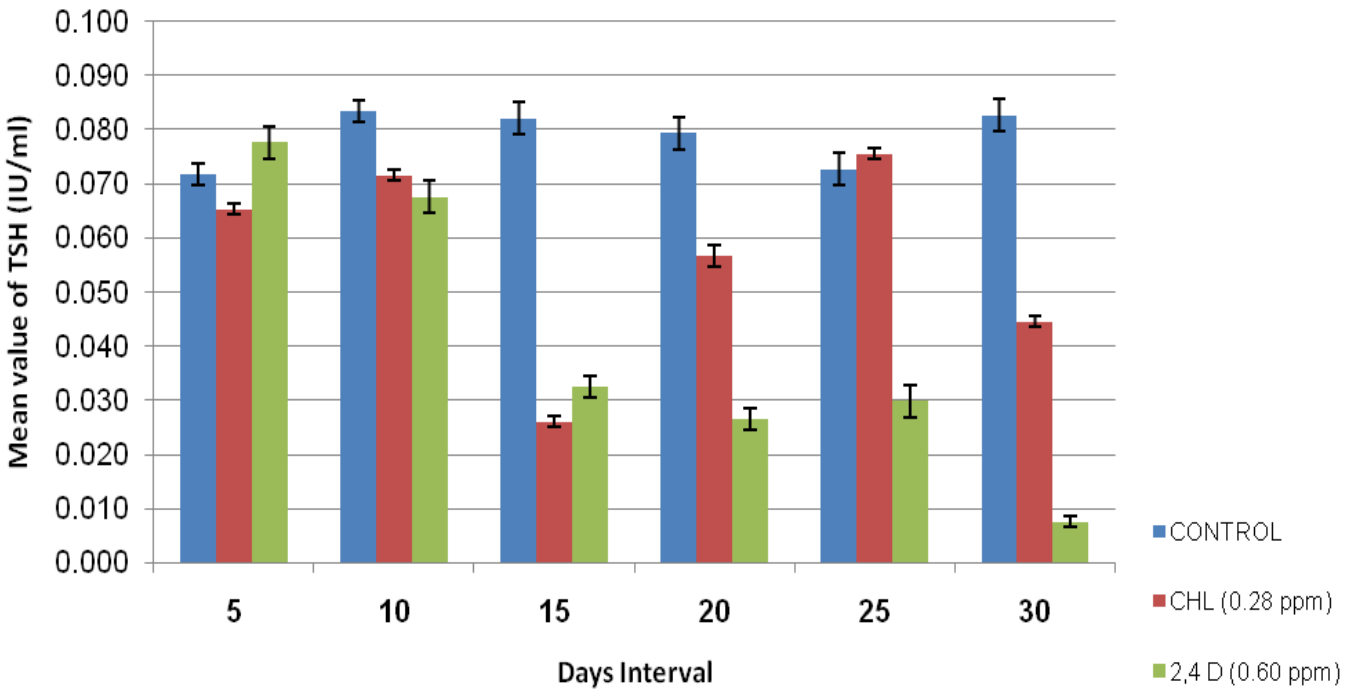

Figure 8. Presenting the mean values of TSH (IU/ml) in serum in Group I, Group II (b) and Group III (b) at different days interval.

Zoology, Cotton College, Guwahati, for her keen guidance, constructive ideas and valuable suggestions in this work.

REFERENCES

Adedeji OB, Okocha RO (2012). Overview of pesticide toxicity in fish. Advances in Environmental Biology, 6(8): 2344-2351.

Boas M, Feldt-Rasmussen U, Skakkebaek NE (2006). Environmental chemicals and thyroid function. Eur J Endocrinol. 154(5):599–611.

De Angelis S, Pricci F, Franzellin F, Olivieri A (2007). Effects of environmental chemicals on thyroid function. In The Endocrine Disruptors (Marino and Mita Eds.), pp. 53–66. Transworld Research Network, Trivandrum, Kerala, India.

De Angelis S, Tassinari R, Maranghi F, Eusepi A, Di Virgilio A, Chiarotti F, Ricceri L, Pesciolini AV, Gilardi E, Moracci G, Calamandrei G, Olivieri A, Mantovani A (2009). Developmental exposure to chlorpyrifos induces alterations in thyroid and thyroid hormone levels without other toxicity signs in Cd1 mice. Toxicol Sci., 108(2):311-319.

Finney DJ, Ed. (1952). Probit Analysis. Cambridge, England, Cambridge University Press.

Fordham CL, Tessari JD, Ramsdell HS (2001). Effects of malathion on survival, growth, development, and equilibrium posture of bullfrog tadpoles (*Rana catesbeiana*). Environ Toxicol Chem., 20(1):179–184.

Haider S, Inbaraj M (2008). Relative toxicity of technical material and commercial formulation of malathion and endosulfan to a freshwater fish, Channa punctatus (Bloch). Ecotoxicol. Environ. Saf., 11; 347-351.

Haviland JA, Butz DE, Porter WP (2010). Long-term sex selective hormonal and behaviour alterations in mice exposed to low doses of chlorpyrifos in utero. Repro Toxicol., 29(1):74-9.

Jeong SH, Kim BY, Kang HG, Ku HO, Cho JH (2006). Effect of chlorpyrifos methyl on steroid and thyroid hormones in rat F0- and F1-generations. Toxicology., 220, 189–202.

Lerda D, Rizzi R (1991). "Study of reproductive function in persons occupationally exposed to 2, 4-dichlorophenoxyacetic acid (2, 4-D)". Mutation research., **262** (1): 47–50.

Mantovani A (2006). Risk assessment of endocrine disrupters: The role of toxicological studies. Ann. N. Y. Acad. Sci., 1076, 239–252.

Maranghi F, Macrı` C, Ricciardi C, Stazi AV, Rescia M, Mantovani A (2003). Histological and histomorphometric alterations in thyroid and adrenals of CD rat pups exposed in utero to methyl thiophanate. Reprod. Toxicol., 17, 617–623.

National Pesticide Information Center (NPIC) (2009). Retrieved July 2013.Chlorpyrifos Technical Fact Sheet. ^ Chlorpyrifos Technical Fact Sheet, National Pesticide Information Center, August, 2009. Retrieved July 1, 2013.

Rakitsky VN, Koblyakov VA, Turusov VS. (2000).

Nongenotoxic (epigenetic) carcinogens: pesticides as an example. A critical review. Teratog Carcinog Mutagen., 20(4):229–240.
Sinha N, Lal B, Singh TP (1992). Thyroid physiology impairment by malathion in the freshwater catfish Clarias batrachus. Ecotoxicol Environ Saf., 24(1):17–25.
Tomlin CDS (2006). The Pesticide Manual: A World Compendium, 14th ed.; British Crop Protection Council: Surrey, UK.
U.S. Environmental Protection Agency (2005); Reregistration Eligibility Decision (RED) 2, 4-D. Office of Prevention Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC.
U.S. Environmental Protection Agency EPA (2011). [Accessed August 18, 2011]; Chlorpyrifos Facts.
WHO (1989) Environmental Health Criteria 84, Environmental Aspects - 2, 4-Dichlorophenoxyacetic acid (2, 4-D); International Programme on Chemical Safety, World Health Organization: Geneva, Switzerland.
Zaidi SS, Bhatnagar VK, Gandhi SJ, Shah MP, Kulkarni PK, Saiyed HN (2000). Assessment of thyroid function in pesticide formulators. Hum Exp Toxicol., 19(9):497–501.
Zoeller RT (2007). Environmental chemicals impacting the thyroid: targets and consequences. Thyroid., 17(9):811–817.