THIACLOPRID INDUCES THYROID DYSFUNCTION IN RATS: EVIDENCES FORM REPEATED DOSE STUDIES IN MALE SPRAGUE DAWLEY RATS

INTRODUCTION

The mammalian thyroid gland is located on each side of the trachea. It consists of two elongated oval lobes, joined by a thin isthmus crossing the trachea. It is composed of two distinct endocrine cell populations. The C-cells (or parafollicular cells) are concerned with the production of calcitonin that regulates calcium metabolism, while the follicular cells produce and secrete the thyroid hormones (Noorden et al., 1977). Microscopically the gland is made up of thyroid follicles, surrounded by connective tissue. The follicles are composed of a single layer of cuboidal cells surrounding a lumen filled with colloid (NCM, 2002; Zoeller et al., 2007, Marta, 2011). These follicles synthesize the two major thyroid hormones; 3,5,3′-triiodothyronine (T₃) and thyroxine (T₄) (Zoeller et al., 2007).

In adults, the thyroid hormones (THs) stimulate metabolic rate in the liver, kidney, heart, nervous system and skeletal muscles (Randal et al., 1997; Marta, 2011). T₃ is the more active of the two hormones, and the majority of biological actions of THs are believed to be mediated through receptors for T₃. In humans, the thyroid gland produces 100% of the T₄ found in the body, and 20% of the T₃. The other 80% is produced by peripheral conversion of T₄ to T₃ by deiodinase enzymes (Zoeller et al., 2007).

Thyroid hormones are lipid soluble and either bind to specific receptors on the inner mitochondrial membrane to activate energy metabolism, or to nuclear receptors to increase the transcription of specific genes, and ultimately alter the production of the proteins encoded by them (Marta, 2011). The thyroid hormones are cleared from the blood in the liver, following sulfonation by sulfotransferases (SULT) or glucuronidation by uridinediphosphate glucuronyltransferase (UDPGT). The modified THs are then eliminated through the bile (Zoeller et al., 2007). The synthesis of THs is regulated by the pituitary gland hormone thyrotropin also called as thyroid stimulating hormone (TSH). The levels of thyroid hormones
in the blood are regulated by a negative feedback mechanism involving the hypothalamic-pituitary-thyroid (HPT) axis (Zoeller et al., 2007).

A large number of xenobiotics are known to affect the thyroid hormone system known as thyroid disrupting chemicals (TDC). These include chemical classes like polychlorinated biphenyls (PCBs) and dioxins (PCDs), brominated flame retardants, ingredients in personal care products like UV-filters, hair dyes, antibacterial compounds and several pesticides (Brucker-Davis, 1998; Hurley et al., 1998; NCM, 2002; Crofton, 2008; Boas et al., 2009). The TDCs can perturb thyroid hormone homeostasis in a number of different ways. Some affect the thyroid gland directly, either by inhibiting the active transport of iodide into the follicular cell or by inhibiting the enzyme thyroid peroxidase, thereby affecting production of THs. Outside the thyroid, chemicals can cause transport disruption by altering binding to serum proteins.

**Figure A: Thyroid hormone control pathways and possible site for disruption (Crofton, 2008)**

Other chemicals inhibit the conversion of T₄ to T₃ by affecting peripheral deiodinases, or enhance metabolism and biliary excretion of THs from the liver, through the action of the isoenzymes UDPGT or SULT (Hurley et al., 1998; Crofton, 2008). All these possible disruption mechanisms of thyroid hormone control are shown in Figure A.

In recent years, the hazards of using pesticides have been accentuated by the sharp rise in their use in agriculture and industry (Troudi et al., 2012). Neonicotinoid pesticides are currently among the most frequently used pesticides worldwide. NRA (2001) public summary suggested
that thiacloprid is a potent toxicant to thyroid gland. EPA (2003) recorded thyroid toxicity after oral and inhalation exposure of thiacloprid in both male and female rats.

After oral exposure of thiacloprid to rats, [\(^{14}\)C]methylene- or [\(^{14}\)C]thiazolidine-labelled thiacloprid was found to be rapidly and almost completely absorbed after a single high dose (100 mg/kg body weight), with maximum plasma concentrations of radioactivity occurring at 1–3 hours. Tissue residues at 48 hours after dosing were found in liver, kidneys, lung, adrenals and thyroid being the tissues with highest residues (PMRA, 1996; PMRA, 1997). Changes in circulating hormone concentrations (e.g. \(T_4\), \(T_3\) and thyroid-stimulating hormone, TSH) and effects on the rat thyroid (e.g. increased weight, hypertrophy and increased mitotic rate of follicular cells) were observed as a consequence of the liver enzyme induction in the study conducted by Bayer AgroSciences (PMRA, 2000). Thiacloprid administration developed thyroid toxicity via elevation of TSH and suppression of \(T_3\) and \(T_4\) synthesis which developed hypothyroidism in animals (EPA, 2003). Acute and subacute exposure of thiacloprid is also known to disturb the thyroid hormone level in serum of Wistar rats (Sekeroglu, 2012).

The thyroid hormones, triiodothyronine and tetraiodothyronine are necessary for appropriate energy levels and an active life of living organisms. It has long been known that thyroid hormones are of vital importance in maintaining the initial level of phospholipids in cell membranes and fatty acid composition of the lipids (Prasad and Kumar, 2005). \(T_3\) plays a critical role in lipid metabolism by regulating genes involved in lipogenesis and lipolysis (Zhu and Chang, 2010). Hypothyroidism, characterized by low serum thyroid hormone levels, is associated with reduced metabolism, reduced lipolysis, weight gain, reduced cholesterol clearance, and elevated serum cholesterol. It is known that thyroid hormone has genomic and nongenomic effects (Davis et al., 2008). Overt hypothyroidism is associated with increased plasma cholesterol and triglyceride levels (Tulloch, 1974). Hypothyroidism may also exhibit elevated levels of high-density lipoprotein cholesterol (HDL-C), mainly due to increased concentration of cholesterol- and phospholipid-enriched HDL-2 particles (Pearce et al., 2008). Residues of thiacloprid are known to persist in thyroid gland of rat, causing thyroid gland hypertrophy by changing histoarchitecture and also increasing mitotic index of follicular cells (NRA, 2001). There is also evidence that hypothyroidism may directly affect the liver structure or function or vice versa (Van Steenbergen et al., 1989; Inkinen and Nordback, 2000). Recent studies have shown that the hepatic abnormalities associated with hypothyroidism can be
reversible over a matter of weeks with thyroxine replacement, with no residual liver damage (Huang and Liaw, 1995; Gaitan and Cooper, 1997).

From research done in animals so far, the relationship between thiacloprid induced thyroidal dysfunction and its physiological implications is still at large. A literature review has shown that no information is available concerning effect of thiacloprid on thyroid hormones. The only available information for the effect of thiacloprid on thyroid function is based on the data of the manufacturer (Bayer AgroSciences) and EPA factsheet in which evidence of oncogenicity (thyroid, ovary and uterus) in feeding studies in mice and rats has been demonstrated. Since data regarding influence on thyroid function from pesticide exposure are limited, toxicity assessment of thiacloprid in this regard deemed significant. Therefore, the specific purpose of the current study was to confirm whether the pesticide thiacloprid can disrupt thyroid function in a non-target mammalian animal system. Moreover, it would be interesting to know the effect of thyroid dysfunction on lipid metabolism and liver function since such a study can be of use in comprehending more meaningfully the general risks of exposure to thiacloprid.

To fulfill the above objective, the following parameters were assessed. 1) Evaluation of the structural and functional integrity of thyroid in SD rats after repeated thiacloprid intoxication. 2) Relevant markers were assessed to check whether altered level of thyroid hormone can interpolate lipid profile and disturb the lipid metabolism and 3) In order to understand the interplay, if any, between thyroid dysfunction and hepatic function and its implication on the behavioural pattern, efforts were made to compare the current results with that of chapter 1.

MATERIAL AND METHODS
The study was approved by the IAEC (Institutional Animal Ethical Committee) according to the norms of CPCSEA, India. A total of 30 healthy male SD rats (weight: 250–275gm; age: 7–8 weeks) were obtained from Sun Pharma Advanced Research Company (SPARC Ltd.), Baroda. Rats were housed in the departmental animal house (827/ac/04/CPCSEA) at controlled temperature (24 ± 2°C) and light-dark schedule (12:12) and were provided laboratory rat food (Pranav Agrochemicals, India) and water ad libitum. The animals were divided into three groups as control, low dose group (LTD) and high dose group (HTD), with 5 animals in each group. The commercial formulation of thiacloprid was diluted in distilled water to obtain desired dose concentrations. Rats were orally gavaged with 50mg/kg body
weight (LTD) and 100mg/kg body weight (HTD) thiacloprid every morning for duration of 28 days and 90 days. Any abnormal changes in behaviour were noticed and recorded.

The animals were sacrificed 24 hours after the last drug administration. Before sacrifice, animals were subjected to overnight fasting and blood was collected from the orbital sinus. They were sacrificed by cervical dislocation under mild diethyl ether anaesthesia. Thyroid gland was removed from the animals, washed in PBS, blotted free of fluids and weighed using a calibrated Sartorius analytical balance, following which it was fixed in 10% neutral buffered formalin for histological evaluation. Blood was kept for 2 hrs at 4ºC, and then centrifuged and serum was separated and was used for biochemical analysis.

**Protocol I: Estimation of TSH and thyroid hormone**

ELISA kit (GenWay Biotech, Inc.) of TSH, T₃ and T₄ was used for estimation of TSH, T₃ and T₄ level in rat serum. ELISA test of these hormones is based on the principle of a solid phase enzyme-linked immunosorbent assay. Mouse monoclonal anti-TSH, anti T₃ and anti T₄ antibody were used for solid phase immobilization (microtiter wells) and goat anti-TSH, anti T₃ or anti T₄ antibody was present in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the two antibodies, resulting in the TSH, T₃ and T₄ molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation at room temperature, the solid phase was washed with water to remove unbound labelled antibodies. A solution of 3,3',5,5'-Tetramethylbenzidine (TMB) was added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development was stopped with the addition of 1N HCl, and the resulting yellow colour was measured spectrophotometrically at 450nm using ELISA plate reader. The concentration of TSH, T₃ and T₄ is directly proportional to the colour intensity of the test sample.

**Protocol II: Evaluation of lipid metabolism**

1. **Lipid profile**

Cholesterol concentration in serum was estimated by the method of Allain *et al.* (1974). The yield of coloured complex at the end of procedure was read at 505nm. The intensity of colour produced is directly proportional to the concentration of total cholesterol in the sample. The HDL-C and LDL-C was assayed using a Reckon diagnostic assay kit, based
on the method described by Grundy (1993) for measuring HDL and LDL cholesterol levels from serum or plasma. Absorption of HDL-C was measured at 690nm and LDL-C at 550nm.

2. **Estimation of Triglyceride**

Fossati and Lorenzo (1982) method was adopted for triglyceride estimation in serum. Intensity of purple coloured complex was measured at 546nm which is proportional to triglyceride concentration.

**Protocol III: Histology of thyroid gland**

Thyroid gland was removed and kept for overnight fixation in 10% neutral buffered formalin. Sample was dehydrated by keeping in series of increasing grade of alcohol, and then was cleared with xylene. Paraffin wax block was prepared and sections were taken using microtome. Sections were rehydrated in a reverse grade series of alcohol and stained with haematoxylin and eosin, followed by dehydration with alcohol. Sections were mounted in DPX and then evaluated for histoarchitectural changes under the microscope (Leica DM2500).

**Statistical analysis**

All data are expressed as mean ± standard error. The statistical significance of the mean differences between control and treated groups was analyzed by ANOVA, with Bonferroni post hoc test for multiple comparisons. Statistical calculations were performed with the SPSS for windows version 12.0. A probability value of ≤ 0.05 was taken as the cut-off value to consider differences as statistically significant.

**RESULTS**

The effects of thiacloprid on TSH, free T₃ (fT₃) and free T₄ (fT₄) hormones following 28 days of oral exposure are summarized in Table 2.1. TSH level was found to be marginally increased in both the treatment groups of rats as compared to the control group, but the difference was not statistically significant. However, a significant increase was observed for the high dose treatment group. No apparent change was observed in serum level of fT₄ hormone after 28 days of thiacloprid exposure. Nevertheless, significantly depleted level of fT₃ hormone was observed in the rats treated with 100mg/kg body weight of thiacloprid (p ≤ 0.05) as compared to control animals. Value was not found significantly different for fT₃ hormone in LTD group as compared to reference animal group (Figure 2.1).
Table 2.2 summarizes the effect of commercial formulation of thiacloprid on the levels of TSH, fT<sub>3</sub> and fT<sub>4</sub> in the rat serum following 90 days of exposure. Subchronic oral treatment of thiacloprid increased the level of TSH in both the dosage groups of rats. The elevated values of TSH hormone in treated animals were found statistically significant for both LTD (p ≤ 0.05) and HTD (p ≤ 0.01) groups as compared to control value of TSH hormone. Also, significantly higher level of TSH hormone was noted in HTD group compared to LTD group of rats (p ≤ 0.05). Level of fT<sub>4</sub> in LTD group of rats was observed to be significantly lower than in control rats (p ≤ 0.05). A significantly lower value was noted in HTD group of thiacloprid-exposed rats for fT<sub>4</sub> hormone in serum, as compared to control (p ≤ 0.01). Hormone level of fT<sub>3</sub> was also found to be lower in LTD and HTD groups than the control animal group and the difference was observed to be significant for both high dose (p ≤ 0.01) and low dose groups (p ≤ 0.05) (Figure 2.2).

The data in table 2.3 summarizes values for lipid parameters in serum of male SD rats after 28 days of thiacloprid treatment. Cholesterol concentration was seen to be significantly (p ≤ 0.05) higher in 100mg/kg body weight of thiacloprid exposed rats as compared to control. Concentration of cholesterol was also observed increased in low dose group but the difference was not significant. HDL-C level was found insignificantly increased in both the treated groups of animals compared to reference group. Significantly elevated level of LDL-C in serum was observed in HTD group of treated rats when compared to control rats (p ≤ 0.05). LDL-C value of low dosage group was higher than the experimental control. Triglyceride concentration was noted to be higher in both the treatment groups of animals compared to the control animals, but value was only significant for higher dosage group (p ≤ 0.05) (Figure 2.3).

The data represented in table 2.4 display the subchronic effect of thiacloprid on serum lipid parameters. Serum cholesterol level was significantly elevated (p ≤ 0.01) for the HTD group than the control value after thiacloprid treatment for 90 days. In 50mg/kg thiacloprid treated rats, cholesterol was observed insignificantly higher than the experimental control. Both the dosage groups showed significantly higher concentration (p ≤ 0.05) of HDL-C compared to the control concentration of HDL-C. Non significant higher value of LDL-C was observed for LTD group and significantly higher (p ≤ 0.05) value was noted in HTD rats as compared to control animals. Subchronic exposure to thiacloprid led to significant increase in triglyceride concentration in both the groups of treated rats (p ≤ 0.05) (Figure 2.4).
Table 2.5 shows the weight of thyroid gland in thiacloprid intoxicated groups during both the study periods. Weight of this endocrine gland was found to be increased in both the dosage groups of subchronic and subacute thiacloprid exposed rats as compared to control, but the increase was significant only for HTD group during subchronic study (Figure 2.5). Changes such as laboured breathing, ataxia, weakness, decreased body weight, nasal secretion and tremors observed during the earlier study (Chapter 1) were again recorded in the animals during both the study periods of the current investigation.

**Histopathological changes in thyroid gland**

Histological evaluation of control thyroid gland of rats revealed uniformly pink stained colloid with irregular pattern on the borders and acini were lined with single layer of cuboidal cells. The parafollicular cells were scattered in regular patches between thyroid follicles (Figure 2.6 & 2.7). Thyroid gland of treated rats was found to be enlarged due to thiacloprid intoxication. During histopathological evaluation, variable sized irregular follicles were seen lined with single layer of cuboidal cells partially or completely occluding the lumen (Figure 2.8). Haematoxylin and eosin staining showed that the follicles contained insufficient or no colloid. Some of the follicles were smaller than the normal (microfollicular pattern) and contained small amount of colloid while others were large enough and irregular (macrofollicular pattern) in shape. The colloid in these follicles stained more basophilic than normal. Follicular cell were found to vary from low cuboidal to flattened shape (Figure 2.9). Some active follicles were dispersed among hyperplastic follicles and parafollicular hyperplasia was also seen (Figure 2.10).

**DISCUSSION**

Environmental chemicals may interfere with thyroid gland function through different mechanisms of action, for example, at the receptor level, by binding to transport proteins, by cellular uptake mechanisms or by modifying the metabolism of thyroid hormones (Boas et al., 2006; Lacasana et al., 2010; Sekeroglu et al., 2012). Data on altered reproductive or endocrine function resulting from insecticide exposure are limited, but *in vivo* and *in vitro* studies show that some insecticides or their metabolites have been assessed for potential endocrine-disrupting activity and alteration of thyroid function (Akhtar et al., 1996; Tyler et al., 2000; Hu et al., 2002; Wang et al., 2002; Liu et al., 2006; Meeker et al., 2009). A number of pesticides are also thought to possess thyroid-disrupting properties in human and experimental animals (Garry, 2004; Toft et al., 2006; Meeker et al., 2009; Du et al., 2010; Lacasana et al., 2010;
Tebourbi et al., 2010; Villanger et al., 2011) and it has been reported that pesticide exposure in agriculture workers results in disturbances of thyroid hormone levels (Zaidi et al., 2000; Garry et al., 2003; Jeong et al., 2006; Toft et al., 2006; Lacasana et al., 2010; Sekeroglu et al., 2012).

There is a single study report by Sekeroglu (2012) about the effects of thiacloprid on thyroid hormones in experimental animals. Exposure to thiacloprid at 112mg/kg body weight and 22.5mg/kg body weight doses by gavage daily for 12 hrs and 4 weeks disturbed the function of thyroid gland and lipid metabolism. In the current study too, thiacloprid exposure for 28 days caused marginal increase in serum TSH and also decreased level of fT3 hormone at the dose of 100mg/kg body weight. However, no such significant change in hormone profile was observed at the dose of 50mg/kg body weight. These results find support from similar findings reported by Sekeroglu (2012), who observed significantly increased TSH level and statistically non-significant decrease in the levels of fT3 and fT4 at 112mg/kg body weight of thiacloprid for acute exposure. Subchronic administration of thiacloprid also increased the serum TSH level and depleted serum fT3 and fT4 levels in present study indicating a state of hypothyroidism.

Recent evidence suggests that NO (Nitric oxide) participates in the regulation of thyroid function. Hah et al. (2001) reported nitroarginine-containing dipeptide amides (Huang et al., 1999) and some peptidomimetic analogues (Huang et al., 2000) as potent and selective inhibitors of neuronal nitric oxide synthase (nNOS). The most potent nNOS inhibitor among these compounds is (4S)-N-(4-amino-5-[aminoethyl]aminopentyl)-N’-nitroguanidine. Nitroguanidine is one of the metabolites of neonicotinoids and it has ability to inhibit nitric oxide synthase. The diversity in biodegradable sites of neonicotinoids and multiple pathways insures against parent compound accumulation but provides intermediates reported to be active as nicotinic agonists and inducible nitric oxide synthase inhibitors (Ford and Casida, 2006). Nitric oxide is synthesised from its precursor L-arginine by the action of NOS (Malinski et al., 1993) which inhibits NO/cGMP pathway. Long-term inhibition of the NO/cGMP pathway affects parameters of thyroid hormone biosynthesis. A novel property of NO to inhibit thyroid peroxidase (TPO) and thyroglobulin (TG) mRNA expression is reported (Ruf and Carayon. 2006). The NO/cGMP action on iodine uptake could involve cGK (cGMP protein kinase) mediation which inhibits iodine uptake (Bazzara et al., 2007). Millatt et al. (1998) suggested a possible role for NO in the regulation of TPO activity and thus thyroid hormone synthesis. TPO inhibition cannot liberate iodine for addition onto tyrosine residue on thyroglobulin for the production of T3 and T4 and inorganic iodine is not oxidized into free iodine which
increases inorganic iodine concentration and leads to NIS (Sodium-Iodide symport) blockage. Therefore it is prudent to believe that the hypothyroidism observed in the thiacloprid treated animals could be a result of its metabolic intermediary nitroguanidine mediated hampered NO pathway.

Further, it is well documented that thyroid hormone plays an important role in the metabolism of lipids (Miyamoto et al., 1997). Hypothyroidism is usually associated with an increased serum concentration of total cholesterol and lipoproteins (Pucci et al., 2000). It is known that overt hypothyroidism is associated with increased fasting plasma cholesterol and triglyceride levels (Tulloch, 1974). Results of the current investigation also showed significantly increased serum cholesterol concentration after 28 and 90 days of thiacloprid treatment at the dose of 100mg/kg body weight. In 1952, Robertson and Kirkpatrick showed very high level of cholesterol in serum of patients with prolonged hypothyroidism due to reduced cholesterol clearance.

Serum HDL-C and LDL-C were significantly increased after thiacloprid administration for 90 days and levels of both were also comparatively higher in animals dosed at 100mg/kg body weight for 28 days compared to control group. The results of the present study find support from the NRA (2001) thiacloprid public summary. Nikkila and Kekki (1972) observed a moderate increase of serum triglycerides in hypothyroid condition (in humans), associated with a decrease in efficiency of triglyceride removal from plasma, which was attributed to a low lipoprotein lipase (LPL) activity. It is now widely recognized that hypothyroidism is one of the most common causes of secondary dyslipidemia. The most common abnormalities of lipoprotein metabolism associated with hypothyroidism are elevated levels of total cholesterol and low-density-lipoprotein cholesterol (LDL-C), which are attributable to the effect of thyroid hormone on lipoprotein lipase activity (Lithell et al., 1981) and the expression of the LDL-receptor (Staels et al., 1990). Hypothyroidism may also exhibit elevated levels of high-density lipoprotein cholesterol (HDL-C), mainly due to increased concentration of cholesterol- and phospholipid-enriched HDL-2 particles (Pearce et al., 2008). A decreased HDL-2 catabolism and cholesteryl ester transfer protein activity has been observed in hypothyroidism (Andrea et al., 2004). This decrease leads to a reduced transfer of cholesteryl esters from HDL to very-low-density lipoprotein (VLDL), thus increasing HDL-C levels (Dullaart et al., 1990). Similar reports regarding thiacloprid toxicity correlated with HDL and LDL levels in any animal model is still lacking.
Increased fasting plasma triglyceride levels commonly accompany hypothyroidism (Nikkila and Kekki, 1972; Tulloch et al., 1974). There was significantly higher triglyceride level observed in rats given subchronic treatment of thiacloprid in the current study and also in animals treated with a higher dose in the subacute study as compared to control animals. Nikkila and Kekki (1972) noted that in thyrotoxicosis the average plasma triglyceride level was slightly but significantly increased above that of control subjects. This change was associated with augmented production of triglycerides whereas the mean fractional removal rate was not different from normal. EPA (2003) toxicology data have also suggested the increased level of triglyceride after intoxication of thiacloprid.

The NRA (2001) public summary documented thyrotoxicosis caused by thiacloprid oral exposure to rats and exposure resulted in increased thyroid gland weight after 13 weeks of test compound administration. The results of thyroid weight recorded in the present study are similar to the findings of NRA public summary, whereby thiacloprid exposure to SD rats for 28 and 90 days was found to increase thyroid weight.

There is also evidence that hypothyroidism may directly affect the liver structure or function and similarly chronic liver damage may develop thyroid dysfunction. Hypothyroidism has been associated with reduced bilirubin and bile excretion. In experimental hypothyroidism, the activity of bilirubin UDP-glucuronyltransferase is decreased, resulting in a reduction in bilirubin excretion (Van Steenbergen et al., 1989). The reduction in bile flow may be in part due to an increase in membrane cholesterol-phospholipid ratio and diminished membrane fluidity (Van Steenbergen et al., 1989), which may affect a number of canalicular membrane transporters and enzymes, including the Na⁺/K⁺-ATPase and alkaline phosphatase. The triad of reduced bilirubin excretion, hypercholesterolemia and hypotonia of the gall bladder seen in hypothyroidism increases the incidence of gallstones (Inkinen and Nordback, 2000; Malik and Hodgson, 2002). As observed in our previous study, thiacloprid exposure decreased bilirubin level and increased ALP activity (Chapter 1) and this development of hepatotoxicity can be correlated to the altered thyroid gland function observed in the present study. Also, several hormones are known to affect liver size in the rat. Thyroid hormones are reported to elicit hypertrophic and hyperplastic responses in the liver (Huang and Liaw, 1995). This report prompts us to correlate the hypothyroidic condition observed in the present study and liver enlargement encountered in the previous study (Chapter 1)
Histological evaluation of the thyroid gland showed that rats given 3 months oral exposure to thiacloprid developed structural malformation in the thyroid gland. This was indicated by reduction of connective tissue in interfollicular space as well as by completely absent or scanty colloids in follicles. The number of follicular epithelial cells had increased, layers of which were extremely flattened in some areas of the gland. Histopathological changes of thiacloprid-intoxicated thyroid clearly suggested follicular epithelial hyperplasia and mild to moderate parafollicular hyperplasia. NRA public summary (2001) had reported a similar kind of histopathological description of rat thyroid gland after thiacloprid administration. Summary report also concluded hypertrophy of follicular epithelium and increased mitotic rate of follicular cells in thiacloprid exposed rats. FAO (2006) has also suggested the possibility of thyroid adenoma development due to thiacloprid intoxication.

Notwithstanding the above structural and functional impairment to thyroid, during the present work, abnormal behavioural changes like tremors and ataxia were commonly noted in the thiacloprid treated animals. Aron (2005), Milanov and Sheinkova (2000) reported that tremor, muscle weakness and ataxia are well-known symptoms of thyrotoxicosis in humans. Public summary of NRA (2001) also suggested similar symptoms in rats after 3 weeks of thiacloprid exposure. Due to lack of supportive data regarding such changes in rodent or any other mammalian animals, results of the current study have not been discussed elaborately. However, comparable behaviour after thiacloprid exposure has been reported in honey bees which included changes such as shaking and tremors, uncontrolled and uncoordinated movements, staggering, inability to take up a correct position of the body, and prolonged frenetic movement of the legs and rotation when in the supine position (Daniela et al., 2011; Vidau et al., 2011). Observations of changes such as tremor and ataxia are important as they may develop due to neuronal impairment also. So these symptoms can be correlated with possible neurotoxic effects of thiacloprid on the mammalian system, which forms the mainstay of the next study.

**CONCLUSION**

Repetitive dosage of thiacloprid may disturb thyroid hormonogenesis by alteration in thyroid hormone level through possible inhibition of NO pathway by one of its intermediate metabolite in the mammalian system. This thyrotoxicosis condition may be responsible for elevation of TSH and depleted level of fT3 and fT4. Decreased level of thyroid hormone interpolated lipid metabolism and hence, cholesterol, HDL-C and LDL-C were found to be increased in serum of
thiacloprid treated rats. It has also been reported that hypothyroidism, characterized by low serum thyroid hormone levels, is associated with reduced metabolism, reduced lipolysis, reduced cholesterol clearance, and elevated serum cholesterol and triglyceride (Davis et al., 2008) which may induce pathophysiological change. Similar observations made in the current study explain the aetiology of histopathological changes observed in the thiacloprid-intoxicated thyroid gland. Disturbance in thyroid physiology can also be ably correlated to changes in liver function observed in the previous chapter. Therefore, it can be evidently concluded from the findings of present work that thiacloprid has the potency to develop thyrotoxicosis directly by down regulating various steps of thyroid biosynthesis which are under the regulating of a potentially hampered NO signalling hence, promoting hypothyroidism in rat. The later condition in turn downplay the lipid metabolism and hepatic function resulting in further structural anomalies culminating in developing abnormal behavioural response in the thiacloprid intoxicated animals.
Table 2.1. Effect of subacute thiacloprid administration on serum TSH and thyroid hormone in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>TSH µU/ml</th>
<th>fT₄ ng/ml</th>
<th>fT₃ mcg/dl</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.53±0.01*</td>
<td>12.6±0.32</td>
<td>1.3±0.037</td>
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<tr>
<td>Low Dose</td>
<td>0.56±0.07</td>
<td>11.7±0.58</td>
<td>1.15±0.042</td>
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<tr>
<td>High Dose</td>
<td>0.72±0.08</td>
<td>11.5±0.47</td>
<td>1.1±0.052↓*</td>
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</tbody>
</table>

Table 2.2. Effect of subchronic thiacloprid treatment on serum TSH and thyroid hormone in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>TSH µU/ml</th>
<th>fT₄ ng/ml</th>
<th>fT₃ mcg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.57±0.03*</td>
<td>12.2±0.47</td>
<td>1.27±0.05</td>
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<tr>
<td>Low Dose</td>
<td>2.20±0.18↑*</td>
<td>10.2±0.48↓*</td>
<td>0.96±0.07↓*</td>
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<tr>
<td>High Dose</td>
<td>3.52±0.53↑**</td>
<td>9.5±0.57↓**</td>
<td>0.8±0.08↓**</td>
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Table 2.3. Effect of thiacloprid on serum lipid parameters after 28 days of intubation

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol mg/dl</th>
<th>HDL-C mg/dl</th>
<th>LDL-C mg/dl</th>
<th>Triglyceride mg/dl</th>
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<tr>
<td>Control</td>
<td>126.6±3.9*</td>
<td>49.8±1.2</td>
<td>42.0±1.3</td>
<td>30.5±0.56</td>
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<tr>
<td>Low Dose</td>
<td>135.1±5.8</td>
<td>55.5±2.4</td>
<td>48.9±2.1</td>
<td>32.5±0.62</td>
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<tr>
<td>High Dose</td>
<td>154.8±7.8↑*</td>
<td>59.0±3.4</td>
<td>50.5±2.5↑*</td>
<td>33.7±0.71↑*</td>
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Table 2.4. Effect of thiacloprid on serum lipid parameters after 90 days of oral exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol mg/dl</th>
<th>HDL-C mg/dl</th>
<th>LDL-C mg/dl</th>
<th>Triglyceride mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>110.7±2.5*</td>
<td>44±2.7</td>
<td>45.7±2.2</td>
<td>44.5±0.96</td>
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<tr>
<td>Low Dose</td>
<td>125.4±4.4</td>
<td>58±3.1↑*</td>
<td>50.8±2.1</td>
<td>51.1±1.93↑*</td>
</tr>
<tr>
<td>High Dose</td>
<td>133.7±5.3↑**</td>
<td>61±4.5↑*</td>
<td>56.4±2.9↑*</td>
<td>53.1±1.94↑*</td>
</tr>
</tbody>
</table>

Table 1.5. Percent relative weight of thyroid gland in thiacloprid intoxicated SD rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Low dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subacute</td>
<td>3.6±0.02</td>
<td>3.7±0.06</td>
<td>3.8±0.05</td>
</tr>
<tr>
<td>Subchronic</td>
<td>3.4±0.02</td>
<td>3.6±0.07</td>
<td>3.6±0.08↓*</td>
</tr>
</tbody>
</table>

*Values are expressed as Mean±SE; n=5 for each group; * p ≤ 0.05; ** p ≤ 0.01; a significantly higher than low dose (a p ≤ 0.05)
Figure 2.1. Effect of thiacloprid on TSH and thyroid hormone after 28 days of oral exposure

Figure 2.2. Subchronic effect of thiacloprid on serum TSH and thyroid hormone of SD rats
Figure 2.3. Effect of thiacloprid on serum lipid parameters after 28 days of intubation

![Bar chart showing the effect of thiacloprid on serum lipid parameters after 28 days of intubation.](image)

Figure 2.4. Effect of thiacloprid on serum lipid parameters after 90 days of oral exposure

![Bar chart showing the effect of thiacloprid on serum lipid parameters after 90 days of oral exposure.](image)
Figure 2.5 Effect of thiacloprid on serum lipid parameters after 90 days of oral exposure: Relative weight of thyroid gland after thiacloprid intoxication 1). Subacute changes 2) Subchronic changes

1) 2)
Figure 2.6 Thyroid gland of control SD rat. Arrow showing active thyroid follicle with colloid (H&E stain, 20X).

Figure 2.7 Active follicle of thyroid gland showing high cuboidal cell (black arrow) of control rat (H&E stain, 100X).
Figure 2.8 Thiacloprid treated thyroid gland of SD rats showing variable size of thyroid follicle (asterisk) with scanty colloid (blue arrow) or no colloid (green arrow) ((H&E stain, 20X).

Figure 2.9 Thiacloprid treated rat thyroid showing hyperplasia with low cuboidal cells (green arrow) and flattened cuboidal cells of follicular epithelium (blue arrow) (H&E stain, 100X).
Figure 2.10 Thiacloprid treated rat thyroid showing parafollicular hyperplasia (green arrow) and flattened cuboidal follicular epithelial cells (H&E stain, 100X).