Abstract— The search for the toxicity of imidacloprid at the mammals was the subject of many experimental studies. The objective of our study is to evaluate the effect of 30 days oral imidacloprid toxicity at 1/85 LD50 and 1/120 LD50 on morpho-functional aspect of the thyroid in wistar female rats. Our experiment was conducted on 25 female rat distributed on three groups: group control (n=5), a group treated at 1/85 LD50 (n=10) and another group treated at 1/120 LD50 (n=10) of imidacloprid. After decapitation, the collected blood is used to thyroid hormones and thyrotropin dosage. Thyroid was fixed in aqueous boun to achieve a histopathological investigation. The treated groups present a non-significant decrease in FT3 and FT4 levels. Histopathological study shows that the thyroid parenchyma show significant alterations in all treated rats which are more pronounced with 1/85 LD50 of imidacloprid: Shrinkage of follicles, presence of collapsed follicles, loss of colloids and the presence of follicular squamous cells. Based on histo-pathological findings, we suggest that the oral toxicity of imidacloprid is dose-dependent and the no observable adverse effect level remains below 1/120 LD50 of imidacloprid.

Key-word — Female rat, Imidacloprid, DL50, Toxicity; Thyroid.

I. INTRODUCTION

IMIDACLOPRID, 1(6-chloro-3-pyridinyl) methyl-N-nitro-2-imidazolidinimine, a chloronicotyl has been a worldwide extensively used insecticide for crop protection since the last decade due to its low soil persistence and its high insectical activity at low application rate [1].Its sales as insecticide are growing very fast globally because of its low selectivity for insects and apparent safety for humans [2], [3]. Its selective toxicity was due to its high affinity to the insects nicotinic acetylcholine receptors compared to mammals [4], [2], [5], [3]. In mammals, many studies indicated that the imidacloprid toxicity can be associated with free radicals production by cell which cause an oxidative stress and tissue damages [6], [7]. These damages altered macromolecules such as nucleic acids, lipids and proteins, which caused functional changes of the target cell and accelerated it death [8]. Reference [9] shows that repeated oral administration of 20mg/kg/day imidacloprid led to significant increases in the hepatic enzymes, the acetylcholinesterase activity and the glycemia in the blood serum of rat. So far no study has been reported on the thyroid toxicity by imidacloprid. For this, we interested in research of the effects of repeated oral administration of two low doses of imidacloprid (1/85 and 1/120 of LD50 of imidacloprid).

II. MATERIAL AND METHODS

A. Chemicals

Imidacloprid technical 96 % pure, produced by the German firm Bayer, was obtained from the national institute of the agricultural research, Algeria.

B. Animals

Animals used in this study were female rats (Rattus norvigicus wistar strain) of average weight 128.16g obtained from Pasteur institute of Kouba, Algeria. Before any experiment, the animals were maintained for a period of acclimatization under controlled conditions of temperature (22 ± 2°C) and humidity (74 ± 10%) with 12h light and dark cycle. The animals were given synthetic Corks diet (industrial Society of concentrated, Bejaia, Algeria) and water ad libitum.

C. Experimental design

Female rats were given imidacloprid at a dose level of 5.18 and 3.67 mg/kg/ day which is 1/85 and 1/120 of LD50 for four weeks continuously by oral administration. Adequate dilutions were made with vegetable oil to achieve the test concentrations. Control group was given water and nutrition ad libitum. The body weights of control as well as treated rats was taken before the start of the treatment and then on the day of sacrifice. On the completion of experiment, the rats were sacrificed and thyroid was excised.

D. Thyroid hormones determination

The quantitative determination of free triiodothyronine (FT3) and free thyroxine (FT4) in the plasma were measured by E.L.I.S.A (Enzyme Linked Immuno Sorbent Assay) through fully automated biochemical analyzer (Elecsys 1010) (Sapin, 2003).

E. Histopathological studies

For light microscopic examination, the thyroid of dissected animals were cleared from adhering tissues and fixed in Bouin’s fixative for 24 h and processed for paraffin embedding. After routine processing, dehydration in several baths of ethanol in increasing degrees, paraffin sections of each tissue were cut at a thickness of 5 μm and stained with hematoxylin and eosin for microscopic examination.

III. RESULTS

A. Animals behavior

No behavior change or mortality sign were observed in female rats during 30 days of exposure to imidacloprid.

B. Body weight

The imidacloprid treatment did not disrupt the general growth of rats. A considerable increase of body weight was noticed in rats at the end of the experiment. This growth was statistically highly significant (p > 0, 05) (Table I).
EFFECT OF IMIDACLOPRID REPEATED TREATMENT ON THE RAT CORPITAL WEIGHT

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (n=5)</th>
<th>Treated with 1/85 DL50 of imidacloprid (n=10)</th>
<th>Treated with 1/120 DL50 of imidacloprid (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (Initial)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>125.7 ± 20.3</td>
<td>133.5 ± 16.1</td>
<td>141.9 ± 10.6</td>
</tr>
<tr>
<td>Weight (Final)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>157.3 ± 22.2</td>
<td>161.6 ± 10.6</td>
<td>173.3 ± 22.2</td>
</tr>
<tr>
<td>Difference</td>
<td>Highly Significant</td>
<td></td>
<td></td>
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</tbody>
</table>

**TABLE II**

EFFECT OF IMIDACLOPRID REPEATED TREATMENT ON THE THYROID HORMONE IN RATS

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (n=5)</th>
<th>Treated with 1/85 DL50 of imidacloprid (n=10)</th>
<th>Treated with 1/120 DL50 of imidacloprid (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fT3 (ng/ml)</td>
<td>6.01 ± 1.07</td>
<td>10.17 ± 4.76</td>
<td></td>
</tr>
<tr>
<td>fT4 (ng/ml)</td>
<td>24.33 ± 7.55</td>
<td>5.22 ± 1.32</td>
<td>25.33 ± 6.55</td>
</tr>
</tbody>
</table>

C. Thyroid hormones

There were no significant differences in the thyroid hormones (fT3, fT4) between control and treated rat (Table II).

D. Histopathological observations

Thyroid histological observations of control rats showed a many follicles of varying diameter; located in a stroma (Fig.1A). Each follicle is delimited by a simple epithelium composed of theoocytes with dark and ovoid nuclei. These cells surround a wide filled lumen colloid (Fig.1B). However the microscopic examination of imidacloprid (1/185 and 1/120 of LD50) treated rat thyroid revealed many degenerative changes of variable degrees in many areas of thyroid (Fig.1. C-G). Imidacloprid treatments entrain the increase in microfollicles number, loss of colloid (Fig.1. C-G) and presence of necrotic tissue (Fig.1. E). The squamous cells were observed in some follicular colloids (Fig.1.G).

IV. DISCUSSION

According to these results, neither behavior change nor mortality occurred in all rats during the experiment. Contradictory results were signaled by [9] in female rats which presented toxicity signs, diarrhea and salivation after the oral administration of 20 mg/kg/day of imidacloprid during 90 days. This study showed non mortality in rats treated by 5 mg/kg/day and 10 mg/kg/day of imidacloprid during 90 days. However, treatment of imidacloprid 1/85 and 1/120 LD 50 has no signs of toxicity on the overall growth of rats. The treated rats gained weight which is considered as a growth indication [10]. These results are inconsistent with those obtained in [9] which have noted a body weight regression in female rats treated orally with 20 mg/kg/day for 90 days imidacloprid. The histopathological examination in rats treated reveals important structural changes of the thyroid parenchyma. These changes were marked by an increase of the microfollicles number and a loss of the colloid. Comparable results were described by [11] in rats treated with 50mg/kg/day of dichlorodiphenyltrichloroethane. [12-14] noted a decrease of the thyroid follicles diameter, a size regression of the follicular cells and a colloid depletion in rats treated with 2000 mg of lithium carbonate. Concentration of 10mg/kg/day. These researchers explained the thyroid hyperplasia by follicles depletion in rats treated with 2000 mg of lithium carbonate. [15] noted a follicular hyperplasia with a thidiazole treatment at a concentration of 10mg/kg/day. These researchers explained the thyroid hyperplasia by follicles degeneration and a decrease in the colloidal volume which marks the presence of an acute inflammation. The results of T3 and T4 dosage confirmed those of [16] who registered no changes in this hormone level in treated rats with 50 mg/kg/day of dichlorodiphenyltrichloroethane.

IV. CONCLUSION

Based on the histopathology examination and the hormonal dosage results, we suggest that the oral administration of 1/85 LD50 and 1/120 LD50 of imidacloprid, during 30 days, engendered an inflammatory process in the thyroid gland which constituted a toxicity sign in female rats.

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Fig.1. Representative photomicographs of paraffin-embedded thyroid stained with hematoxylin and eosin. (A-B) Normal appearance of thyroid tissue and intact follicular structure in control rats. CC: C cells. (C-G) Micrographs of the imidacloprid treated rats. (C-D) Thyroid in a rat treated with 5.18 mg/kg/day of imidacloprid. Arrows indicate an increase in microfollicles number and loss of colloid. (E) Thyroid tissue from rats treated with 3.67 mg/kg/day of imidacloprid. (*) indicate narrowing follicular and loss of colloid. (F) Necrosis tissue (Tn). (G) Arrows indicate a presence of squamous cells. H&E ; ×100 (A, D, E) ; ×400 (B, F, G).

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