Khalij-Libya Journal of Dental and Medical Research
http://journal.khalijedental.com.ly/ eISSN:2708-888X

*Original Article*

# Effect of Pesticide Indoxacarb on the Thyroid Gland in Swiss Albino Mice

**Salma Abusrer[1]*, Zainab EL Mabrouk[1], Habiba El Jaafari[1], Naema Shibani[2], Sassia Regeai[2]**

[1]*Department of Zoology, Faculty of Science, University of Tripoli, Tripoli, Libya.*

[2]*Department of Life Sciences, School of Basic Science, Libyan Academy of Postgraduate Studies, Janzur, Libya.*

***Corresponding Email:*** *Salma_abusrer@hotmail.com*

**ABSTRACT**

Background and objectives: Pesticides play an essential role in crop protection, but their overuse caused environmental pollution and harmful effect on different animal body systems, including the endocrine system. The thyroid gland is one of the homeostatic regulators of metabolic activities, which is affected by the elements of the external environment. There are very limited studies on the effect of indoxacarb on the histological architecture and functions of thyroid gland. Therefore, this study was conducted with the aim of examining functionally and histologically of the thyroid gland exposed to indoxacarb. Method: 24 Swiss albino mice male and female were randomly divided into two groups, each group male and female; group I is a control group given orally with 1ml of distilled water and group II orally treated with 120 mg/kg Bw. of indoxacarb daily for 3 weeks. Blood samples were collected from each mouse under anesthetic to determine the thyroid-stimulating hormone (TSH), thyroxine (T4) levels. Thyroid gland histopathology was attained for the evaluation of the indoxacarb effect. Results: The treated mice showed non-significant increase in T4 levels and a significant decrease in TSH levels but there was no significant difference recorded in T4 and TSH levels between sexes. Histologically, the sections of the thyroid gland of the treated group showed empty and irregular follicles, degeneration of the follicular epithelial tissue, and hyperplasia in the lining of some follicles, the capsule with congestion blood vessels. Conclusion: This study concluded that indoxacarb may act as a thyroid gland toxicant.

**Keywords**: Thyroid gland, Indoxacarb, Histopathology, Ultrastructure.

**Citation**: Abusrer S, EL Mabrouk Z, El Jaafari H, Shibani N, Regeai S. Effect of Pesticide Indoxacarb on the Thyroid Gland in Swiss Albino Mice. Khalij-Libya J Dent Med Res. 2021;5(2):23–30. https://doi.org/10.47705/kjdmr.215204

**Received**: 14/06/21; **accepted**: 24/06/21; **published**: 25/06/21



## INTRODUCTION

The development and manufacture of pesticides is a continuous process to overwhelm the increasingly devastating effects of various plant and animal pathogens [1]. Pesticides are widely used throughout the world, in modern agriculture for crop protection to assist in the production of high yield of crops [2]. Despite the many benefits of using pesticides to control pest, there is strong evidence indicating their role in endocrine disruption [3, 4].

The oxadiazine insecticide Indoxacarb is one of the newly introduced insecticides that are considered a safe alternative for organophosphate pesticides. It is extensively used in veterinary medicine and agriculture to control sucking and biting insects [5]. Despite indoxacarb has low to moderate toxicity in mammals [6], however, it has been demonstrated that there are some functional and histological changes in some experimental animal tissues such as in the liver and kidneys [7,8]. Furthermore, some

insecticides, herbicides, and fungicides have been reported to have anti-endocrine functions such as higher incidence of subclinical hypothyroidism [9, 10, 11, 12, 13] and more specifically, thyroid disorders [14, 4]. These pesticides work through various mechanisms such as inhibiting thyroid iodine uptake [15], interference with thyroid hormone (TH) receptors [16], linked to transport proteins, iodothyronine, cellular absorption of TH and TH gene expression [17, 18, 19]. Also, it has been documented that exposure of rats to thiacloprid and to a mixture of deltamethrin and thiacloprid results in a significance increase in $T_3$ and $T_4$ level but no significant change in TSH levels [20]. In other study, diethyl phosphate caused a significant decrease in TSH level and a significant increase in the levels of $T_3$ and $T_4$ in treated rats [21]. In contrast, numerous studies have reported that mice exposed to the low doses of dichlorobiphenyl trichloroethane (DDT) and thiocarbamate led to a decrease in $T_3$, $T_4$ levels as well as an increase in TSH levels [22, 23]. Another study conducted on farmworkers exposed to pesticides organophosphates and carbamates [24] and pyrethroid [25] resulted in a significant increase in TSH levels and a significant decrease in $T_3$ and $T_4$ levels. Also, it has been shown that $T_3$ and $T_4$ significantly reduced following exposure of carp (Cyprinus carpios) to indoxacarb for 21 days [26]. Furthermore, Nassar [27] revealed that chronic exposure (60 days) to indoxacarb caused reduction in $T_3$ and $T_4$ levels of rat serum.

In addition, several animal studies have shown that exposure to pesticides can induce structural alterations of the thyroid gland [28, 29, 30], however, long-term exposure of rats to low doses of DDT led to changes in the arrangement of the follicle's epithelium [31]. Also, Chlorpyrifos caused changes in the histological structure of thyroid gland [32]. Few human studies have been conducted over the past decade on adult and children, and heterogeneity has been observed in the results of 19 different organochlorine and organophosphate pesticides been used to assess the thyroid function [14, 33], and to find the relationship between thyroid function and pesticides according to sex [34, 35]. Moreover, Lopez-Espinoza, [36] and Hernandez-Mariano, [37] revealed the relationship between the high concentration of dichlorobiphenyl chloroethylene (DDE), Dichlorodiphenyldichloroethane (p, p-DDE) and thyroid function of pregnant women. Epidemiological studies on animals are necessary to understand how chronic occupational exposures affect the thyroid gland in men and women. Studies on the effects of indoxacarb on the function and histopathology of the thyroid gland are very limited; therefore, the aim of this study was to determine the effects of indoxacarb on the function and histological architecture of thyroid gland in adult albino mice.

## MATERIALS AND METHODS

### *Reagents*

Indoxacarb insecticide ($C_{22}H_{17}ClF_3N_3O_7$), the analytical grade was purchased from Scharlab S. L, Spain. Indoxacarb 200g/L (75:25) suspension concentrate was diluted in distilled water to obtain a stock of indoxacarb solution that was prepared weekly and kept in a dark bottle at room temperature (25°C).

### *Animals*

Healthy adult male and female Swiss albino mice (6-8weeks old, weighing 28-32g) were used. These animals were inborn in the animal house of the Zoology Department, Faculty of Science, Tripoli University, Libya. Mice were house in clean polypropylene cages containing wooden flakes or ships under standard nurtured condition, and keep it at a controlled room temperature (22 ± 3°C) under 12h light/12h dark photoperiod, relative humidity 50± 5. For at least a week before the experiment and those conditions were conserved until the end of the experiment. Mice were fed a standard lab diet and given ad libitum access to food and water.

### *Experimental design*

The experiment was performed on 24 male and female adult Swiss albino mice. The mice were

randomly divided into two equal groups of 12 male and 12 female: group I: 6 male and 6 female mice served as control and orally given an equal volume of the distilled water. Group II: 6 male and 6 female received a dose of indoxacarb (120 mg/kg BW). Indoxacarb was engulfed daily for 3 consecutive weeks. At the end of the experiment, the blood was collected from jugular veins from each animal under anesthetic. The upper part of the trachea to the beginning of the tongue was removed; fixed in 10% phosphate-buffer formalin (pH-7.2), dehydrated in an ascending chain of ethanol, clearing in xylene, and impeded in paraffin wax 55 Cº to 60 Cº, for general histological analysis, 5µm thick paraffin sections stained with hematoxylin/eosin, sections taken from the anterior, medial and posterior part of the thyroid gland (three nonserial sections per each part of a lobe of the gland) were analyzed on a light microscope (Wetzlar, Germany).

### *Hormone's assay*

Blood samples were collected in non-heparinized tubes from jugular veins from each mouse. The blood samples were centrifuged at 5000 rpm for 10 min to obtain serum. Serum samples were used to estimate $T_4$ and TSH levels according to the method reported by [38] using an international immune diagnostic kit and was expressed as ng/ml from a standard curve, ElISA Cobase 411 Roche- Germany, according to manufacturer's instructions.

### *Statistical analysis*

The mean ± standard deviation (SD) values were calculated for the samples of the control and treated groups. Statistical software SPSS version 20 was used for the analysis of the data. Two-way ANOVA followed by a post hoc LSD test was used. A p-value of P<0.05 at 95% confidence interval was considered significant.

## RESULTS

### *Serology*

The current study was conducted to evaluate the effects of pesticide indoxacarb on TSH and T4 levels. The results obtained revealed that the group exposed to insecticide indoxacarb showed no significant increases in the serum T4 level (P< 0.097), while the mean value of TSH level was significantly decreased in comparison to control group (P< 0.033) and there was no significant difference between sex groups in serum T4 and TSH levels compared to the control group (P < 0.817) as shown in Table 1.

**Table 1. Effect of insecticide indoxacarb on TSH and $T_4$ levels in Swiss albino mice.**

| Mean+SD | Groups | Mean+SD |
|---|---|---|
| 6.87±0.75[a] | Female | 6.3±0.3[a] |
| 7.02±0.85[a] | Male | 7.1±0.1 [a] |
| 0.057±0.015[a] | Female | 0.067±0.012[a] |
| 0.058±0.018[a] | Male | 0.048±0.014[b] |
| Mean+SD | Groups | Mean+SD |
| 6.87±0.75[a] | Female | 6.3±0.3[a] |

*a, b: Significant differences as compared with control group (P<0.05), n=6.*

### *Histological examination of the thyroid gland*

The anatomical evaluation of the thyroid glands in the control group and the treated group found that the upper part of the trachea to the beginning of the tongue appears normal in both groups with no change in color and no edema.

Examinations of the sections that contain the thyroid gland of the treated group with indoxacarb and stained with hematoxylin and eosin showed some follicles that contain a few pallid and heterogeneous colloid (the appearance of some vacuoles in the colloid), and about 50% of follicles free of colloid (Figure 2 A and B) compared to the control sections that showed most of the cavity of the follicle filled with a rich homogeneous colloid and only about 4% of follicles that free of colloid (Fig. 1 A, B, C, and D). Also, there was a reduction in the size of most thyroid follicles and there are many follicles with irregular shape; lined with flat or squamous epithelial cells and in some other follicles revealed some vacuoles in epithelial cells (Fig. 2 D and E) compared to the control that shows round or oval

follicles, vary in size and shapes filled with dark pink homogeneous colloid. Most of the follicles are lined by a single layer of low columnar to cuboidal epithelial tissue (Fig. 1 A, B, C, and D).

Furthermore, the treated sections showed hyperplasia in the lining of some follicles and the dissociation of epithelial cells of others (Fig. 2 E and F), also there was degenerated and necrosis in the intercellular connective tissue and blood vessels dilation and congested (Fig. 2 E), there was an expansion in the interstitial connective tissue observed between lobules follicles and the presence of thick collagen fibers (Fig. 2 E and F) compared to the control that shows numerous capillaries in the areolar connective tissue cluster or individual parafollicular cells, polygonal in shape, light-colored or low-density cytoplasm next to or around the intrafollicular thyroid follicles (Fig. 1 D).

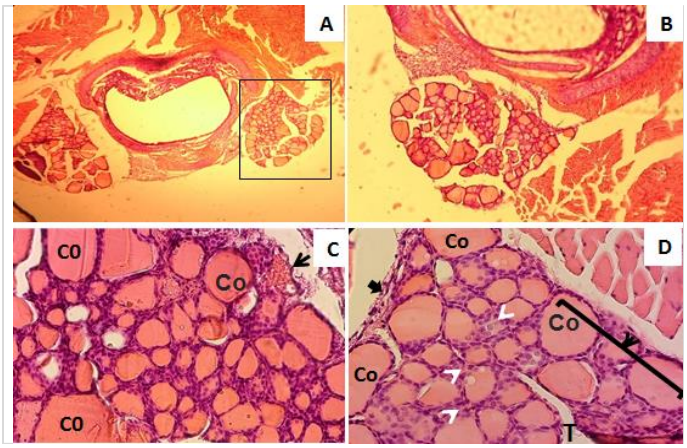


Figure 1: Shows pictures of thyroid sections of control animals, (A) Shows two lobes of the thyroid gland, (B) the square shows one lobe of the thyroid gland, surrounded by thin capsule large arrow and containing blood sinuses thin arrow (D). C & D shows Lobes divided in to small lobules by trabeculae (T), the follicles between brackets are round or oval in shape, Parafollicular cells (white arrow), and Colloid (Co). (H & E respectively 5X, 10X, 40 X).

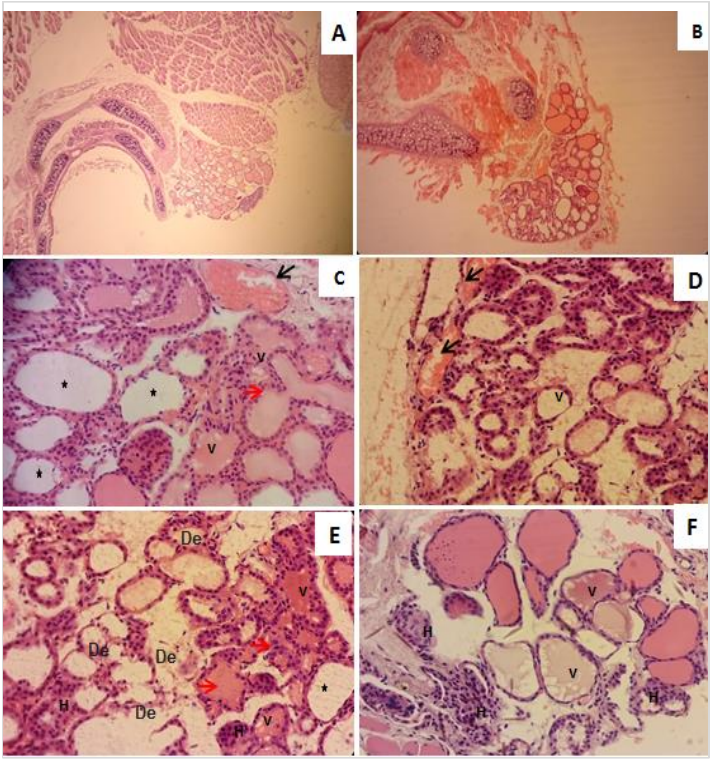


Figure 2: Sections of the thyroid gland of indoxacarb-treated group of mice show: (A&B) the entire lobe with empty follicles. (C & D) shows the capsule contains congestion in blood vessels (thin black arrows). (C, D & E) many empty follicles of colloid [*]. and the others containing small cavities in the colloid (Vacuoles (V) .(E) shows the irregular follicles (red arrow) and appearance of the degeneration (De) of the follicular epithelial tissue, (F) the appearance of hyperplasia (H) in the lining of some follicles, H & E respectively 40 X, 10X, 5X.

## DISSCUSION

The incorrect belief that says the thyroid gland function is only to homeostasis metabolic processes, but it seems to be associated with several other health issues, such as diabetes [39], cardiac disease [40, 41], the reproductive system [42], Lupus disease [43] and rheumatoid arthritis [44]. The thyroid gland is intensely affected by internal factors and highly sensitive to the toxicity of many external environmental factors, including herbicides, fungicides, and insecticides. However, the current

results evaluate the effect of indoxacarb on the thyroid gland of albino mice treated with indoxacarb (120mg/kg daily) of for 21 days showed that indoxacarb affect the thyroid gland function with non- significant increase in the T4 level and a significant decrease in TSH level in both sexes compared to control group. This study is in agreement with other studies that illustrated exposure to thiacloprid and deltamethrin [20] led to an increase in the T4 level. While Kongtip et al. [45] reported that T4 significantly elevated in 78 farmers exposed to several pesticides. Also, the present results are in consistent) with the study of [21] and [32] who revealed that diethyl phosphate and chlorpyrifos considerably decreased TSH level in rats respectively. Moreover [12] that revealed a significant decrease in the TSH level in people who were exposed to pesticides such as, organic chlorine and DDE. In contrast, these results disagreed with the effects of chlorpyrifos and deltamethrin on the thyroid toxicity on albino mice and rats [46, 47], that reported a significant decrease in T3, T4 levels and a significant increase in the TSH level. Also, this study contradicted with other studies that showed the exposure to pesticides such as indoxacarb, DDT, polychlorinated biphenyls, organophosphorus, organochlorine, cause a decrease in the T3, T4 levels and a significant increase in the TSH level [22, 35, 27, 24]. This controversy in the results between the different studies may be attributed to the dosage of the pesticide, the method of administration, and the period of treatment.

The mechanism by which indoxacarb cause change in the thyroid hormones levels and structure is not known. However, since the pesticide indoxacarb used in this study affects the nervous system of insects [48], it may also, has a significant effect on the pituitary gland of mammals, as it caused a significant decrease of TSH level, which led to an increase in the T4 production and thus led to thyroid stress. This result conflicted with the negative feedback mechanism as reported by [49].

Furthermore, the histopathological results of this study also showed shrinkage in the size of some follicles, a decrease in the amount of colloid, and the evacuated colloid in 50% of the follicles of the thyroid gland. Also, the epithelial cells lining the thyroid follicles were transformed into a squamous epithelium and some of them replaced entirely by fibrous tissue. Besides, some sections that treated with indoxacarb showed thick collagen fibers compared to the control group, this result is in accordance with the data observed by [31] who reported the prolonged exposure to very low doses of DDT led to severe changes in the thyroid gland tissue of rats, as well as confirmed by [47, 32] studies showed the exposure to insecticide deltamethrin and Chloroperofus respectively, led to an effect on the histological morphology of the thyroid gland tissues in albino rats. Also, this study agreed with another study on rats which reported that sodium fluoride caused a histopathological alteration of thyroid gland such as follicular cystic dilatation with flattened lining epithelium and eosinophilic colloid with congestion in blood vessels and functional changes [50].

The distortion of the architecture and the disruption of the function of thyroid may be caused by a variation of mechanisms; many environmental elements interfere with the hypothalamic-pituitary-thyroid axis at altered levels. Mechanisms of action may include the receptors for T4, T3 or TSH and thyroid peroxidase enzymes. Also, may involve sodium–iodide symporter, transport proteins, or cellular uptake mechanisms. The circumferential metabolism of the THs can be affected through effects on iodothyronine deiodinases or hepatic enzymes. Any small changes in thyroid homeostasis may adversely influence human health. Therefore, it is urgent to clarify whether the animal data showing effects of indoxacarb on thyroid function can be extended to humans.

## CONCLUSION

In summary, the previous study revealed that pesticide indoxacarb has a significant toxic effect on the function and ultrastructure of the thyroid gland, which are of great importance for most of the physiological processes of the various body systems, For the environment safety, men must use the adequate quantities of pesticides and using them with caution.

### *Disclaimer*

The article has not been previously presented or published, and is not part of a thesis project.

### *Conflict of Interest*

There are no financial, personal, or professional conflicts of interest to declare.

## REFERENCES


1. Abdelrasoul AM. Modulation of Abamectin and Indoxacarb -Induced Toxicity on Male Albino Rats by *Moringa oleifera*. ASEJ. 2018; 39 (2):232-243. doi:10.21608/asejaiqjsae.2018.6831
2. Popp J, Pető K, and Nagy J. Pesticide productivity and food security. A review J Agron Sustain Dev. 2013; 33 (1): 243-255. doi:10.1007/s13593-012-0105-x
3. Vidau C, Brunet J, Badiou A. and Belzunces LP. Phenylpyrazole insecticides induce cytotoxicity by altering mechanisms involved in cellular energy supply in the human epithelial cell model Caco-2. Toxicol. In-Vitro. 2009; 23: 589-597. doi: 10.1016/j.tiv.2009.01.017
4. Leemans M, Couderq S, Demeneix B. and Fini JB. Pesticides With Potential Thyroid Hormone-Disrupting Effects: A Review of Recent Data. Front Endocrinol (Lausanne). 2019; 10: 743. doi: 10.3389/fendo. 00743.
5. Dias JL. Environmental fate of indoxacarb. Environmental Monitoring Branch Department of Pesticide Regulation Sacramento, 2006; CA95812-4015.
6. EPA: Factsheet, Indoxacarb. Office of Prevention, Pesticides and Toxic Substances, Washington D.C. 2000.
7. EL Mabrouk Z, Abusrer S, Shibani N. and El Jaafari H. The effect of Indoxacarb on blood parameters and liver tissues in Mice. Liby. J. Vet. Med. Sci. 2016; 2 (2): 23-30.
8. Abusrer S, Shibani N, El Jaafari H, Regeai S. and EL Mabrouk Z. Pathological Effects of Pesticide Indoxacarb (Avaunt®) on Kidney of Female Mice Libyan bulletin for studies. Dar Azzawyah Lelketab. 2017; 1 (13): 220-230.
9. Steenland K, Cedillo L, Tucker J, Hines C, Sorensen K, Deddens J. et al. Thyroid hormones and cytogenetic outcomes in backpack sprayers using ethylene bis (dithiocarbamate) (EBDC) fungicides in Mexico. Environ Health Perspect. 1997; 105 (10): 1126–1130. doi: 10.1289/ehp.971051126.
10. Garry VF. Biomarkers of thyroid function, genotoxicity, and agricultural fungicide use. J Bioch. Mol. Toxi. 2005; 19 (3): 175. doi: 10.1002/jbt.20073
11. Toft G, Flyvbjerg A, Bonde JP. Thyroid function in Danish greenhouse workers [electronic article]. Environ Health. 2006; 5 (32):1-8.
12. Quraishi R, Akbar F, Karim S, Neelab, Ahmad S, Ullah S. Effects of Pesticides on Haematology, Thyroid Stimulating Hormone (TSH) and Tri-iodothyronine (T3) Hormones of Agricultural Workers in Swat, Pakistan. J. Bio. Life Sci. 2015; 6 (1): 96-107. ISSN 2157-6076
13. Shrestha S, Parks CG, Goldner WS, Kamel F, Umbach DM, Ward M, et al. Incident thyroid disease in female spouses of private pesticide applicators. J. Environ Int. 2018; 118: 282–292. doi: 10.1016/05.041.
14. Lerro C, Beane Freeman L, DellaValle C, Kibriya M, Aschebrook-Kilfoy B, Jasmine F, et al. Occupational pesticide exposure and subclinical hypothyroidism among male pesticide applicators. Occup Environ Med. 2017; 0:1–11. doi:10.1136/oemed-2017-104431.
15. Hernández AF, Bennekou SH, Hart A, Mohimont L. and Wolterink G. Mechanisms underlying disruptive effects of pesticides on the thyroid function. Toxic. 2020; 19: 34–41.
16. Montanelli L, Benvenga S, Hegedüs L, Vitti P, Latrofa F, Duntas LH. Drugs and Other Substances Interfering with Thyroid Function.

Thyroid Diseases pp 733-761| Part of the Endocrinology book series (ENDOCR) First Online: 31 May 2018.

17. Akhtar N, Kayani SA, Ahmad MM, Shahab M. Insecticide-induced changes in secretory activity of the thyroid gland in rats. J Appl Toxicol. 1996 Sep-Oct;16(5):397-400. doi: 10.1002/(SICI)1099-1263(199609)16:5<397::AID-JAT362>3.0.CO;2-Y.
18. Crump D, Werry K, Veldhoen N, Van Aggelen G. and Helbing C. Exposure to the Herbicide Acetochlor Alters Thyroid hormone-dependent Gene Expression and Metamorphosis in Xenopus Laevis. Env. Health Pers. 2002; 110 (12):1199-1205. https://doi.org/10.1289/ehp.021101199
19. Zoeller RT. Environmental chemicals impacting the thyroid: targets and consequences. Thyroid. 2007; 17 (9): 811–817 https://doi.org/10.1089/thy.2007.0107.
20. Sekeroglu V, Sekeroglu ZA. and Demirhan ES. Effects of commercial formulations of deltamethrin and/or thiacloprid on thyroid hormone levels in rat serum. Toxic. Indust. Health. 2014; 30 (1): 40–46. https://doi.org/10.1177/0748233712448114
21. **21**-Yang FW, Zhao GP, Ren FZ, Pang G, Li YX. Assessment of the endocrine-disrupting effects of diethyl phosphate, anonspecific metabolite of organophosphorus pesticides, by in vivo and in silico approaches. Environ. Int. 2020; 135, 105383. https://doi.org/10.1016/j.envint.2019.105383.
22. **22**-Boas M, Feldt-Rasmussen U, Skakkebaek NE. and Main MK. Environmental chemicals and thyroid function. Eur J Endocrinol. 2006;154 (5): 599–611. https://doi.org/10.1530/eje.1.02128
23. **23**-Farokhi F, Taravati A. Pesticide exposure and thyroid function in adult male sprayers. Int J Med Invest. 2014; 3 (4): 127-132.
24. Ibrahim BA, Amer SA, Younis SE, Kofi MF. and Abdel-Halim AW. Chronic Pesticides Exposure and Thyroid Functions among Farmers in Almnaif District - Ismailia Governorate, Egypt. Suez Canal Univ. Medi. J. 2020; 23 (2): 117-128. DOI: 10.21608/scumj.2020.123149
25. Abou El-magd SA, Sabik ME, Shoukry A. Pyrethroid Toxic Effects on some Hormonal Profile and Biochemicl Markers among Workers in Pyrethroid Insecticides Company. Life Sci J. 2011; 8 (1): 311–322. (ISSN: 1097–8135).
26. Ghelichpour M, Mirghaed AT, Mirzargar SS, Joshaghani H, Mousavi HE. Plasma proteins, hepatic enzymes, thyroid hormones and liver histopathology of Cyprinus carpio (Linnaeus, 1758) exposed to an oxadiazin pesticide, indoxacarb. Aquaculture Research. 2017; 1-11. https://doi.org/10.1111/are.13390
27. Nassar MK. Comparative endocrine-disrupting effects of abamectin and indoxacarb insecticides. International Journal of Pharmacology and Toxicology, 2016; 4 (1): 89-92. doi: 10.14419/ijpt.v4i1.6125.
28. Ram RN. Carbofuran-induced histophysiological changes in thyroid of the teleost fish, Channa punctatus (Bloch). Ecotoxicol Environ Saf. 1988; 16 (2): 106-113.doi: 10.1016/0147-6513(88)90023-1.
29. Hadie SN, Mansor O, Shariff SE. Carbofuran on Thyroid Gland of Male Rats. International Medical Journal. 2012; 19 (1): 16 – 20.
30. European Food Safety Authoeit**y** (EFSA), Federica Crivellente, Andy Hart, Antonio F. Hernandez-Jerez, Susanne Hougaard Bennekou, Ragnor Pedersen, Andrea Terron, Gernit Wolterink, Luc Mohimont. 2019 Establishment of cumulative assement groups of pesticides for their effects on the thyroid.
. https://doi.org/10.2903/j.efsa.2019.5801.
31. Yaglova NV, Yaglov VV. Cytophysiological Changes in the Follicular Epithelium of the Thyroid Gland after Long-Term Exposure to Low Doses of Dichlorodiphenyltrichloroethane (DDT). Bull. Exp. Biol. Med. 2017; 162, 699–702.
32. Lamfon H. Effect of Selenium on Chlorpyrifos-Induced Thyroid Toxicity in Albino Rats. Resea. in Endocri. 2014; Article ID 751500, 11. doi: 10.5171/2014.751500.
33. Suhartono S, Kartini A, Subagio HW, BudiyonoUtari A, Suratman S, Sakundarno M. Pesticide Exposure and Thyroid Function in Elementary School Children Living in an Agricultural Area, Brebes District, Indonesia. Int J Occup Environ Med. 2018; 9:137-144. doi:10.15171/ijoem.2018.1207.
34. Freire C, Lopez-Espinosa MJ, Fernandez M, Molina-Molina JM, Prada R, Olea N. Prenatal

exposure to organochlorine pesticides and TSH status in newborns from Southern Spain. Sci. Total Environ. 2011; 409, 3281–3287.

**35.** Freire C, Koifman RJ, Sarcinelli PN, Simoes Rosa AC, Clapauch R, Koifman S. Long-term exposure to organochlorine pesticides and thyroid status in adults in a heavily contaminated area in Brazil. Environ. Res. 2013; 127, 7–15.

**36.** Lopez-Espinosa MJ, Vizcaino E, Murcia M, Llop S, Espada M, Seco V, et al. Association between thyroid hormone levels and 4,40 -DDE concentrations in pregnant women (Valencia, Spain). Environ. Res. 2009; 109, 479–485.

**37.** Hernandez-Mariano JA, Torres-Sanchez L, Bassol-Mayagoitia S, Escamilla-Nunez MC, Cebrian ME, Villeda-Gutierrez EA, et al. Effect of exposure to p,p-DDE during the first half of pregnancy in the maternal thyroid profile of female residents in a Mexican floriculture area. Environ. Res. 2017; 156, 597–604.

**38.** Nelson JC. and Wilcox RB. Analytical performance of free and total thyroxine assays. Clin. Chem. 1996; 42 (1): 146-154. http://dx.doi.org/10.1093/ PMid:8565218.

**39.** Duntas LH, Brabant G. The interface between thyroid and diabetes mellitus. Clin. Endocr. 2011; 75, 1-9. https://doi.org/10.1111/j.1365-2265.2011.04029.

**40.** Osman F, Gammage MD. and Franklyn JA. Thyroid disease and its treatment: short-term and long-term cardiovascular consequences. Curr Opin Pharmacol. 2001; 1 (6): 626-31. doi: 10.1016/s1471-4892(01)00107-2. PMID: 11757819.

**41.** Klein, I. and Danzi, S. Thyroid disease and the heart. Circulation. 2007.116, 1725-1735. https://doi.org/10.1161/CIRCULATIONAHA.106.678326

**42.** Krassus GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. Endocr. Rev. 2010; 31, 702-755. https://doi.org/10.1210/er.2009-0041

**43.** Klionsky Y. and Antonelli M. Thyroid Disease in Lupus: An Updated Review. ACR Open Rheumatol. 2020; 2 (2): 74–78. doi 10.1002/acr2.11105.

**44.** Sivridis E, Kouroupi M, Koukourakis MI, Arelaki S, Lyratzopoulos N. and Giatromanolaki A. Rheumatoid nodules in thyroid gland parenchyma as an expression of rheumatoid arthritis: a case report. J. Medi. Case Repo. 2019; 13: 159. 2461. https://doi.org/10.1186/s13256-019-2113-9.

**45.** Kongtip P, Nankongnab N, Pundee R, Kallayanatham N, Pengpumkiat S, Chungcharoen J. et al. Acute Changes in Thyroid Hormone Levels among Thai Pesticide Sprayers. Toxics. 2021; 9, 16. https://doi.org/10.3390/toxics9010016

**46.** De Angelis S, Tassinari R, and Maranghi F. Developmental exposure to chlorpyrifos induces alterations in thyroid and thyroid hormone levels without other toxicity signs in CD-1 mice. Toxi. Sci. 2009; 108: 311- 319. https://doi.org/10.1093/toxsci/kfp017

**47.** Abdul-Hamid M, Salah M. Lycopene reduces deltamethrin effects induced thyroid toxicity and DNA damage in albino rats. J. Basic. Appli. Zoo. 2013; 66, (4), 155-163. https://doi.org/10.1016/j.jobaz.2013.08.001

**48.** Lapied B, Grolleau F, Sattelle DB. Indoxacarb, an oxadiazine insecticide, blocks insect neuronal sodium channels. GJP. 2001;132, 587–595; doi:10.1038/sj.bjp.0703853

**49.** Belchetz PE, Gredley G, Bird D, Himsworth RL. Regulation of thyrotropin secretion by the negative feedback of tri-iodothyronine on the hypothalamus. J. Endo. 1978; 76, 439-448.

**50.** Foda DS, and Shams SG. A trial for improving thyroid gland dysfunction in rats by using a marine organism extract. Braz. J. Biol. 2021; 81(3): 592-600