Toxicity of Chlorpyrifos Evidenced by Alterations in Thyroid Function Tests: Thyroxine T4, Triiodothyronine T3, and Thyroid Stimulating Hormone TSH in Juvenile *Clarias gariepinus*(Burchell, 1822)

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March, 2024

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY, FACULTY OF LIFE SCIENCES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELORS OF SCIENCE, B.Sc (HONS) IN ANIMAL AND ENVIRONMENTAL BIOLOGY, UNIVERSITY OF BENIN, BENIN CITY, EDO, STATE NIGERIA.

March, 2024

CERTIFICATION

This is to certify that this project work was carried out by **OBIUWEVBI Ekugbe Sarah** with the matriculation of LSC19063633 in the department of Animal and Environmental Biology, University of Benin, Benin City, Edo State, Nigeria.

Dr P. AOpute **DATE**
(Project Supervisor)

PROF. M.O. Omoigberale **DATE**

DEDICATION

This work is dedicated to the Almighty God for his grace and to my family for their support. God bless you all richly.

ACKNOWLEDGEMENT

Gratitude flows from the depths of my heart as I acknowledge the divine presence of God Almighty, whose unwavering protection, grace, and blessings have illuminated every step of my journey. To my exceptional lecturers, your guidance, mentorship, and supervision have been the compass guiding me through the compilation of this report.

My heartfelt appreciation extends to my dear Parents, Mr. and Mrs. OBIUWEVBI, and my siblings, whose unyielding support has been the cornerstone of my educational pursuits. Their unwavering encouragement has propelled me forward. I also want to acknowledge the unwavering love from my siblings:Obiuwevbi Gideon, Obiuwevbi Godfrey and Obiuwevbi Gospel.

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ABSTRACT

Pesticides, though not intended for aquatic environments, often contaminate water bodies, impacting water quality and aquatic organisms. Chlorpyrifos, a commonly used organophosphate insecticide, poses significant risks to freshwater ecosystems due to its potential endocrine disruptiveeffects on aquatic organisms like *Clarias gariepinus*. This study aims to explore the correlation between chlorpyrifos exposure and thyroid hormone parameters in *Clarias gariepinus*. Eight hundred(800)Juvenile *Clarias gariepinus* were acquired and acclimatized before exposure to controlled concentrations of chlorpyrifos. Muscle tissue samples were collected for analysis of thyroid hormones, including Thyroxine(Free T4), Triiodothyronine(Free T3), and Thyroid Stimulating Hormone(FT3), using standard techniques. Chlorpyrifos exposure significantly affected T3 levels in *C.gariepinus* ($p<0.05$). The control group exhibited the highest T3 level (0.90 ± 0.20), differing significantly from treated groups (CHL 1: 0.72 ± 0.11, CHL 2: 0.74 ± 0.12, CHL 3: 0.66 ± 0.11). Thyroid Stimulating Hormonelevels also showed a significant difference between control (0.70 ± 0.01) and treated groups (CHL1: 0.80 ± 0.10, CHL2: 15.76 ± 7.56). Additionally, T4 levels varied significantly across groups, with the highest in CHL2 (5.9 ± 3.73) and lowest in CHL1 (4.53 ± 1.22).Chlorpyrifos exposure alters thyroid hormone levels in *Clarias gariepinus*, indicating potential disruption of thyroid function. These findings underscore the ecological risks associated with chlorpyrifos contamination in aquatic ecosystems, warranting further research for informed mitigation strategies to safeguard both aquatic organisms and human health.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Pesticides are not designed for aqueous systems, but they may enter water bodies through run-off and spray drift applications, leading to deteriorated water quality and affecting aquatic organism (Guo *et al*., 2020). Mostly due to the extensive use pesticides, such as insecticides, herbicides, fungicides, and acaricides, has led to widespread contamination of the aquatic environment (Ortiz-Delgado *et al*., 2019). Many pesticides are endocrine disrupting chemicals (EDCs) or have been proven to be such chemicals, which might affect the reproductive and neuroendocrine systems of humans and wildlife, especially those of aquatic organisms directly exposed to environmental pollutants, causing aging and even carcinogenesis (Rhind, 2005; Patrick, 2009; Kabir, *et al*. 2015). One of the most serious consequences of the increasing use of pesticides is the chemical pollution of freshwater and estuarine ecosystems, which are the ultimate storehouse of their residues (Lal, 2012: Nugegoda *et al*., 2017). Pesticide residues in natural waters do not exist separately, but exist in mixtures in most cases (Runnalls *et al*., 2015; Hua *et al*., 2016).

Among the pesticides, chlorpyrifos stands out as an organophosphate insecticide extensively utilized in agriculture for pest control (Wołejko*et al*., 2022). It is very toxic to freshwater fish as well as estuarine and marine organisms (Woke *et al*., 2009). Chlorpyrifos is known to be directly toxic to the nervous system and it is transformed inside animals to chlorpyrifos-oxon and 3,5,6-trichloro 2pyridinol (TCP), both of which are more toxic to the nervous system than chlorpyrifos ethyl itself (Woke and Aleleye-Wokoma, 2009; foodsafety, 2024). Aquatic pollution of pesticides results from atmospheric deposit and or run off from agricultural land during rainfall (Tudi *et al*., 2021). Some pesticides are employed to kill pests and insect vectors. The mode of action of chlorpyrifos and their metabolites is the inhibition of the enzyme acetyl cholinesterase (AchE) (Greer*et al*., 2019). Chlorpyrifos ethyl is more persistent than other organophosphorus pesticides because of its lipophilic character (Woke and Aleleye-Wokoma, 2009). The active site of the enzyme reacts with acetylchlorine and hence chlorpyrifos ethyl pholphorylates the enzyme (Woke and Aleleye-Wokoma, 2009). Its persistence and bioaccumulative properties contribute to its potential to disrupt behavioral and physiological systems of aquatic inhabitants, including fish like the African mud catfish (*Clarias gariepinus*) (Malik, 2016; Loganathan *et al*., 2020 Maurya and; Ismanto*et al*., 2022). Exposure to chlorpyrifos, even at low concentrations, can induce hazardous impacts on fish metabolism, leading to alterations in growth, development, reproduction, and overall physiological processes (Singh *et al*., 2004; Sabra *et al*., 2015; Opute and Isibor, 2024).

Thyroid hormones such as thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine (FT4), play a crucial role in the growth and development of fish, and any disruption to their function can have significant consequences on their health and well-being (Shahid *et al*., 2018). Thyroid-stimulating hormone (TSH) is a hormone produced by the pituitary gland that stimulates the thyroid gland to produce thyroid hormones. Free triiodothyronine (FT3) and FT4 are the active forms of thyroid hormones that play essential roles in various physiological processes, including growth, development, and metabolism (Deal and Volkoff, 2020). Exposure to pesticides can alter the thyroid hormone levels in catfish (Lal, Sarang and Kumar, 2013), leading to disruptions in their reproductive and neuroendocrine functions. Pesticides can affect thyroid signalling by blocking, mimicking, or synergizing the endogenous hormones, through direct thyroid receptor interactions, and indirectly via upstream signalling pathways, by inhibiting hormonal synthesis (Ortiz-Delgado *et al*., 2019)

Understanding the dose-response relationship between thyroid hormone parameters and Chlorpyrifos in catfish is crucial for several reasons. First, it can help identify the most sensitive species and life stages to pesticide exposure, which can inform risk assessment and management strategies. Second, it can provide insights into the mechanisms of thyroid hormone disruption by pesticides, which can guide the development of mitigation strategies. Lastly, it can contribute to the overall understanding of the effects of environmental toxicants on fish health and the broader ecosystem.

1.2 Aim and Objectives of the study

To investigate the potential correlation between thyroid hormone parameters, specifically free T4, free T3, and FT3, and exposure to chlorpyrifos in *Clarias gariepinus.*

The specific objectives of the study are:

1. To determine the baseline levels of thyroid hormones, including free T4, free T3, and FT3, in Clarias gariepinus specimens.
2. To assess the potential impact of environmental factors, such as exposure to chlorpyrifos, on thyroid hormone levels in Clarias gariepinus specimens.
3. To investigate any correlations between chlorpyrifos exposure levels and alterations in thyroid hormone concentrations, specifically free T4, free T3, and FT3, in Clarias gariepinus specimens.

CHAPTER TWO

LITERATURE REVIEW

2.1 CHLORPYRIFOS

Chlorpyrifos, also known as chlorpyrifos ethyl, is an organophosphate pesticide widely used to control insects in various settings, including agriculture, residential areas, and commercial spaces. It acts on the nervous systems of insects by inhibiting the acetylcholinesterase enzyme (Ncbi, 2024). Chlorpyrifos is classified as moderately hazardous to humans and animals by the World Health Organization (WHO, 2019). Exposure exceeding recommended levels has been linked to neurological effects, developmental disorders, and autoimmune disorders (Israel, 2024). Exposure during pregnancy can harm the mental development of children (Israel, 2024)

The use of chlorpyrifos has been banned in several countries and jurisdictions due to its health risks. In the United States, the EPA announced a ban on chlorpyrifos on food crops in 2021 (Beyound, 2024). Most home uses of chlorpyrifos had already been banned in the U.S. and Canada since 2001 (Beyound, 2024). In Nigeria, The National Agency for Food and Drug Administration and Control (NAFDAC) has taken steps to address these concerns by initiating a ban and phase-out of 12 pesticide and agrochemical active ingredients, including chlorpyrifos, in the country. This action is in line with efforts to safeguard health and regulate the importation, distribution, and use of pesticides to mitigate the risks associated with their toxicity (Abdullahi 2023; Ihejirika, 2023). Chlorpyrifos poses ecological risks, leading to concerns about its impact on the environment. The European Union decided not to renew approvals for chlorpyrifos and chlorpyrifos-methyl in what year? due to human health concerns related to genotoxicity and developmental neurotoxicity (foodsafety, 2024). Acute exposure to chlorpyrifos can lead to a range of neuromuscular symptoms due to interference with acetylcholine neurotransmission. Symptoms may include headaches, blurred vision, excessive salivation, muscle weakness, nausea, and more (foodsafety, 2024).

2.2 EFFECTS OF PESTICIDES ON FISH HEALTH

Pesticides have significant impacts on fish health, affecting various aspects of their physiology and behaviour (Rohani, 2023). Conducting a comparative study on the effects of pesticides like chlorpyrifos, freshwater fish can provide valuable insights into the chronic toxicity and potential disruptions to the hypothalamic-pituitary-thyroid axis in these aquatic organisms (Ghayyur*et al*., 2021). Pesticides can directly harm fish by causing toxicities that lead to behavioural changes, haematological disturbances, histopathological alterations, enzyme changes, and genotoxicity (Ray and Shaju, 2023). Additionally, pesticides indirectly affect fish by limiting food organisms in aquatic ecosystems, altering water body environments, and making fish more vulnerable to predators (Ray and Shaju, 2023). These indirect impacts can be critical for fish survival and ecosystem health (Ray and Shaju, 2023).

Pesticides bioaccumulate in fish bodies, leading to oxidative stress and various toxicities that can affect not only fish but also human health through the consumption of contaminated fish. The presence of pesticide residues in aquatic environments poses risks to both aquatic organisms and humans along the food chain (Ray and Shaju, 2023). Sublethal exposure to pesticides can induce physiological and biochemical changes in fish even at concentrations below lethal levels. These changes may include alterations in respiratory function, histology, haematology, defence mechanisms, behaviour, reproduction, growth, and other physiological functions (Ogueji and Auta, 2007; Korkmaz and Örün, 2022). Understanding these sublethal effects is crucial for assessing the overall impact of pesticides on fish populations (Ogueji and Auta, 2007; Korkmaz and Örün, 2022). A study comparing the effects of chlorpyrifos, Dimethoate, and Acetamiprid on *Cirrhinusmrigala* fish demonstrated time-dependent negative effects on various health biomarkers during chronic toxicity exposure. The study highlighted the importance of evaluating how these pesticides impact different physiological parameters in freshwater fish over time to assess their potential long-term effects on fish health (Ghayyur*et al*., 2021).

2.3 THYROID HORMONE

Thyroid hormone is a crucial hormone that controls the body's metabolism, which is the process of converting food into energy. It consists of two main hormones released by the thyroid gland: thyroxine (T4) and triiodothyronine (T3) (Shahid *et al*., 2018; Cleveland, 2024). These hormones play a vital role in regulating metabolism, impacting every cell and organ in the body (Shahid *et al*., 2018). The production and release of thyroid hormone are controlled by a feedback loop involving the hypothalamus, pituitary gland, and thyroid gland (Shahid *et al*., 2018). Thyroid hormone affects various bodily functions, including heart, muscle, and digestive function, brain development, and bone maintenance (Yourhormones, 2024). The thyroid gland also produces calcitonin to regulate calcium levels in the blood (Yourhormones, 2024). In cases where the thyroid is dysfunctional, it can lead to imbalances in T3 and T4 levels, affecting metabolism and overall health (Shahid *et al*., 2018).

2.4 THE ROLE OF THYROID HORMONES IN FISH

The role of thyroid hormones in fish is crucial for various aspects of their growth and development. Thyroid hormones, including thyroxine (T4) and triiodothyronine (T3), play essential roles in early development, metamorphosis, reproduction, osmoregulation, and metabolism in fish species (Power *et al*., 2001; Deal and Volkoff, 2020). Research has shown that thyroid hormones are present in high quantities in fish eggs, with concentrations decreasing during embryogenesis until endogenous production begins (Power *et al*., 2001). Thyroid hormone receptors (TR) have been identified in various teleost, with two receptor isoforms, TR alpha and TR beta, expressed during early embryos and larvae stages of fish like the Japanese flounder, zebrafish, and seabream (Power *et al*., 2001). The thyroid axis in fish is an endocrine feedback system that influences growth, differentiation, and reproduction by maintaining homeostasis (Deal and Volkoff, 2020). Fish rely on this system for somatic growth, metamorphosis, reproductive events, and adaptation to changing environments (Deal and Volkoff, 2020). While significant progress has been made in understanding the thyroid axis in mammals like rodents, there are still gaps in knowledge regarding its role in non-mammalian vertebrates such as fish. Understanding the thyroid axis in fish is essential for aquaculture practices and addressing potential threats to the thyroid system from environmental factors (Deal and Volkoff, 2020). Thyroid hormones have been found to be involved in larval development and metamorphosis in teleost fishes. Studies have shown that enhancing eggs with thyroid hormones can lead to improved survival and growth of embryos and larvae (Darras, 2019). Additionally, thyroid hormones play a critical role in promoting larva-juvenile transition or metamorphosis in teleost fish (Campinho, 2019). Disruption of proper thyroid hormone function can result in deformities in juvenile fish, which is a common issue observed in hatcheries (Yamano, 2005). Environmental chemicals also have the potential to disrupt thyroid hormone function during the development of fish embryos and juveniles (Yamano, 2005).

2.5 IMPACT OF PESTICIDES ON THYROID FUNCTION

Fish thyroid function can be disrupted by environmental toxicants, such as pesticides, which can have negative health impacts and possibly ecological ramifications (Nugegoda and Kibria, 2017).

Disruption of fish thyroid function by environmental stressors has the potential to result in deleterious effects including the inhibition of sperm production, reduction in egg production, gonad development, ovarian growth, swimming activity, fertilisation and increase in larval mortality (Nugegoda and Kibria, 2017). Fish thyroid function has been found to be disrupted by a number of environmental toxicants, including phthalates, metals, pesticides, PBDE, PCBs, PCDDs, PCDFs, PAHs/oil, and PCBs. These compounds have the ability to disrupt aquatic organisms' thyroid hormone synthesis, secretion, transport, metabolism, and action function (Brown, 2004; Nugegoda and Kibria, 2017). Evaluating these toxicants' effects on fish health and ecosystem dynamics requires an understanding of the mechanisms by which they alter thyroid function (Brown, 2004; Nugegoda and Kibria, 2017). Exposure to high concentrations of organochlorine pesticides has been shown to significantly alter thyroid activity in fish, both in short-term and long-term scenarios (Jayaraj, 2016). Pesticides like Chlorpyrifos-Ethyl have been linked to disruptions in the hypothalamic-pituitary-thyroid (HPT) axis in fish species. Exposure to this pesticide can interfere with the synthesis, secretion, and regulation of thyroid hormones, leading to imbalances in thyroid function and potential physiological disturbances in aquatic organisms like Clarias gariepinus and rainbow trout (Brown *et al*., 2004; Adewumi *et al*., 2018). These chemicals have the potential to disrupt the normal function of fish thyroid glands, underlining the importance of evaluating the effects of specific pesticides, such as chlorpyrifos, at safe concentrations.

CHAPTER THREE

MATERIALS AND METHODS

3.1 EXPERIMENTAL FISH

Eight hundred (800) *Clarias gariepinus* juveniles were bought from the Department of Fisheries, Faculty of Agriculture, University of Benin, Benin City, Edo state, Nigeria. The juveniles were transferred from the pond using a perforated gallon down to the animal unit, where they were transferred to an already washed holding tank, where they had access to air, and dechlorinated tap water. Appropriate care was taken to mitigate stress such as pouring in water from the side of the tank to prevent excessive turbulent.

3.2 ACCLIMATIZATION

The juveniles were kept in a big holding tank for a period of seven days to ensure acclimatization. During this period, they were fed with 2mm fish feed from [insert producer], and the water in the tank was changed every two days (48 hours) to ensure constant oxygen availability, given that the water quality may be negatively affected both by their feed and waste products. The acclimation period allows organisms to gradually adapt, enhancing their chances of survival in altered conditions.

3.3 MORPHOMETRY

The length and weightof the fishes were measured using a metre rule and a digital scale respectively. The standard length, total length and wet weight of the fishes were measured prior to the exposure and after exposure to the single chemicals. Before exposure, the juveniles had an average length of 10.6 ± 0.66 cm and an average weight of 11.14 ± 1.97 g (plate 1 and 2).

Plate 1: measurement of standard and total length

Plate 2: weighing of test organism.

3.4 ACQUISITION OF THE TEST CHEMICAL

The commercial formulation of chlorpyrifos (200g/l chlorpyrifos 20EC), was purchased from Lagos Street, Ring Road, Benin City, Edo State, Nigeria. It bears the commercial name chlorpyrifos, produced by Food View Nig LTD, located at No 5F Gasharin Road, Bamawa Kaduna, Kaduna State. The formulation comes in a 1-litre volume.

3.5 EXPERIMENTAL SETUP

The juvenile *Clarias gariepinus*were randomly divided into different treatment groups in triplicates. The fish were exposed to chlorpyrifos at controlled concentrations, simulating realistic environmental conditions. This exposure is a crucial aspect of the study to observe the effects of chlorpyrifos on thyroid function. A control group was established where fishes were maintained under identical conditions but without exposure to chlorpyrifos. This allows for comparison and assessment of the specific effects of chlorpyrifos exposure on thyroid function. The fish were closely monitored throughout the experiment for any behavioural changes, mortality rates, and physical abnormalities. The quality of the test water was carefully monitored and maintained within optimal levels throughout the experiment. This ensures that any observed effects on the fish are not due to changes in water quality parameters. The fish were fed a standard diet to meet their nutritional requirements.

Muscle tissue samples were collected from the experimental fish at predetermined intervals to assess the localized effects of chlorpyrifos on thyroid function. The tissue collection process involved carefully excising small portions of muscle tissue from each fish under sterile conditions to prevent contamination.

The collected muscle tissue samples underwent analysis to evaluate various parameters related to thyroid function. These parameters included the quantification of thyroid hormones, such as free thyroxine (free T4), free triiodothyronine (free T3), and FT3 levels. Additionally, other relevant markers indicative of thyroid function and potential impacts of chlorpyrifos exposure were also investigated within the muscle tissue samples.

Plate 3: experimental setup

Plate 4: Harvesting of liver and muscle tissue of Juvenile *Clarias gaariepinus*.

3.6 ANALYSIS OF THYROID HORMONES

Thyroxine (Free T4)

Free T4 represents the unbound fraction of thyroxine circulating in the bloodstream. It serves as a crucial marker of thyroid function, as it is the precursor to the biologically active form of thyroid hormone, triiodothyronine (T3). Alterations in Free T4 levels can indicate disruptions in thyroid hormone synthesis or metabolism, potentially reflecting the impact of chlorpyrifos exposure on thyroid function in *Clarias gariepinus.*

Triiodothyronine (Free T3)

Free T3 refers to the unbound fraction of triiodothyronine, the biologically active form of thyroid hormone. Free T3 plays a pivotal role in regulating metabolism, growth, and development. Like Free T4, deviations in Free T3 levels may signify disturbances in thyroid function induced by environmental stressors such as chlorpyrifos exposure. Monitoring Free T3 levels provides valuable insights into the physiological effects of chlorpyrifos on *Clarias gariepinus.*

Thyroid Stimulating Hormone (TSH)

Thyroid Stimulating Hormone (TSH), also known as thyrotropin, acts as the conductor of the thyroid orchestra. Produced by the pituitary gland, it orchestrates the thyroid's hormone production by stimulating the synthesis and release of thyroxine (T4) and triiodothyronine (T3).

TSH operates within a finely tuned feedback loop. When thyroid hormone levels dip, the hypothalamus releases thyrotropin-releasing hormone (TRH), prompting the pituitary gland to release TSH. Elevated TSH levels then signal the thyroid gland to boost T4 and T3 production, restoring balance.

3.7 STATISTICAL ANALYSIS

The statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS version 21). Treatment means ± standard deviation of the mean (SD) was calculated and presented for all data sets.To determine the significance among exposure chemicals and concentrations, a one-way Analysis of Variance (ANOVA) was performed, with significance set at $P < 0.05$. Tukey's posthoc test was then employed to compare the means of all treatments and identify any significant differences.

3.8 DETERMINATION OF PHYSICOCHEMICAL PARAMETERS OF WATERS

3.8.1 Temperature

The temperature of the water used for the experiment was recorded prior to the exposure of the fishes to the chemicals, using a mercury in glass thermometer.

3.8.2 Hydrogen Ion Concentration (Ph)

The hydrogen ion concentration (Ph) was measured using a Ph meter (Mettler Toledo Ph meter). This was done by dipping the end of the electrode ion into the test solution and the appropriate mode selected to give the reading.

3.8.3 Conductivity

Both the control and test solutions were tested for conductivity. Conductivity is a measure of the ions in a water body, it was measured using a conductivity meter (Mettle Toledo conductivity/TDS meter). Samples from each tank was collected and the probe was dipped inside, and the readings were recorded in Us/cm.

3.8.4 Dissolved Oxygen

Samples from the tanks were collected and taken to the laboratory.

3.8.5. Total Alkalinity of Test Water

The total alkalinity of both the control and treatment group were measured by titrating 0.02N aqueous hydrogen tetraoxosulphate (IV) solution against 25ml of control and treatment solutions using two drops of methyl orange as indicator. The alkalinity values were then calculated from the equation below.

$$\text{Total alkalinity} = \frac{\text{Total volume (ml) x 50 = 1000}}{\text{25ml (volume of test solution used)}}$$

3.8.6. Turbidity

Turbidity of water samples in control and treatment tanks were measured using a hand held turbidimeter. Measurements were taken in nepholeometric turbidity unit (NTU).

CHAPTER FOUR

RESULTS

The potential effects of the organophosphate insecticide chlorpyrifos on thyroid hormones, thyroid-stimulating hormone (TSH), levels of triiodothyronine (T3) and thyroxine (T4)were evaluated in *Clarias gariepinus*. The results of this investigation are presented below.

4.1: Effect of Chlorpyrifos on Triiodothyronine(T3) level in *C. gariepinus*

Results for the effect of chlorpyrifos on *C. gariepinus* are represented in Figure 4.1. There was a significant ($p<0.05$) effect of chlorpyrifos treatment on T3 levels in *C. gariepinus*. The control sample group had the highest T3 level (0.90 ± 0.20) which was significantly ($p< 0.05$) different from all chlorpyrifos-treated groups. There was no significant difference ($p>0.05$) between the treatment groups, CHL 1 (0.72 ± 0.11), CHL 2 (0.74 ± 0.12), and CHL 3 (0.66 ± 0.11).

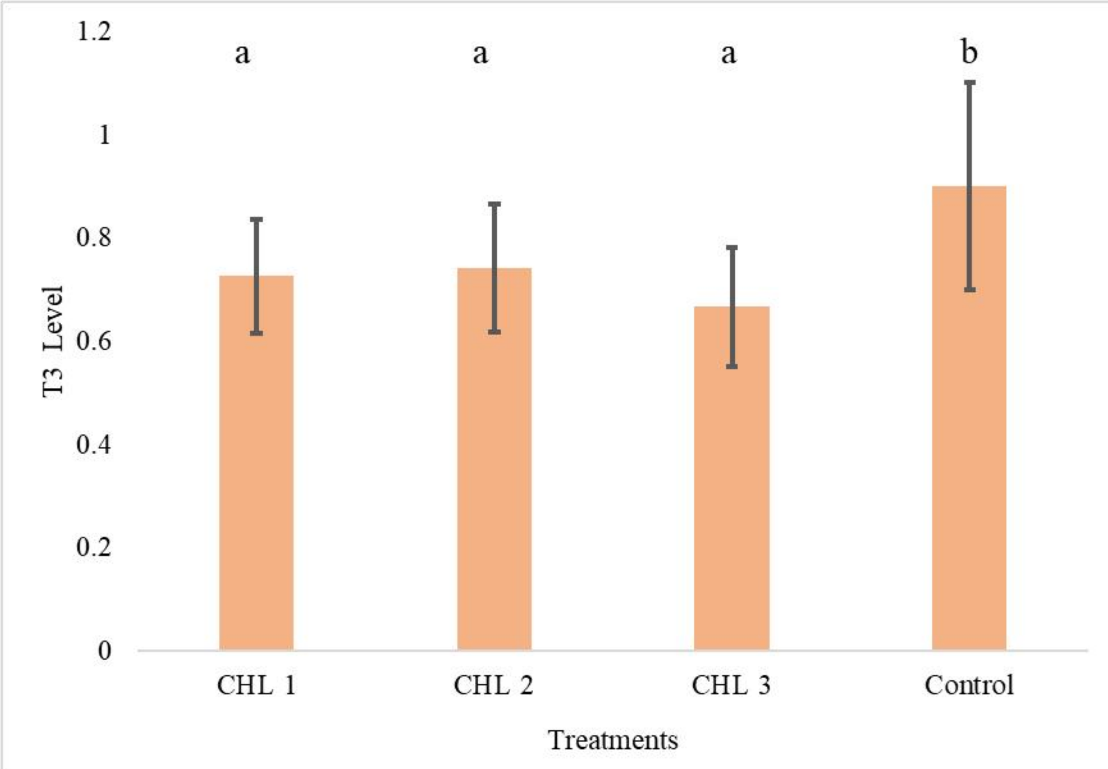

Figure 4.1: Effect of Chlorpyrifos on T3 level in *C. gariepinus*

4.2. Effect of Chlorpyrifos on TSH level in *C. gariepinus*

The effect of chlorpyrifos on TSH levels in *C. gariepinus* is represented in Figure 4.2. There was a significant effect ($p<0.05$) between the treatment groups and control. This is evidenced by the significant difference ($P< 0.05$) in TSH levels in control samples compared to CHL1 (0.70 ±0.01), and CHL2 (0.80 ±0.10). The control group had the highest TSH level but was not ($p>0.05$) significantly different from the CHL3 group (15.76 ± 7.56). The lowest TSH level was observed in CHL1 (0.70 ±0.01), which was not significantly ($p>0.05$) different from CHL2 (0.80 ±0.10). In summary, there was a dose-dependent decrease in the level of TSH across the treatment groups.

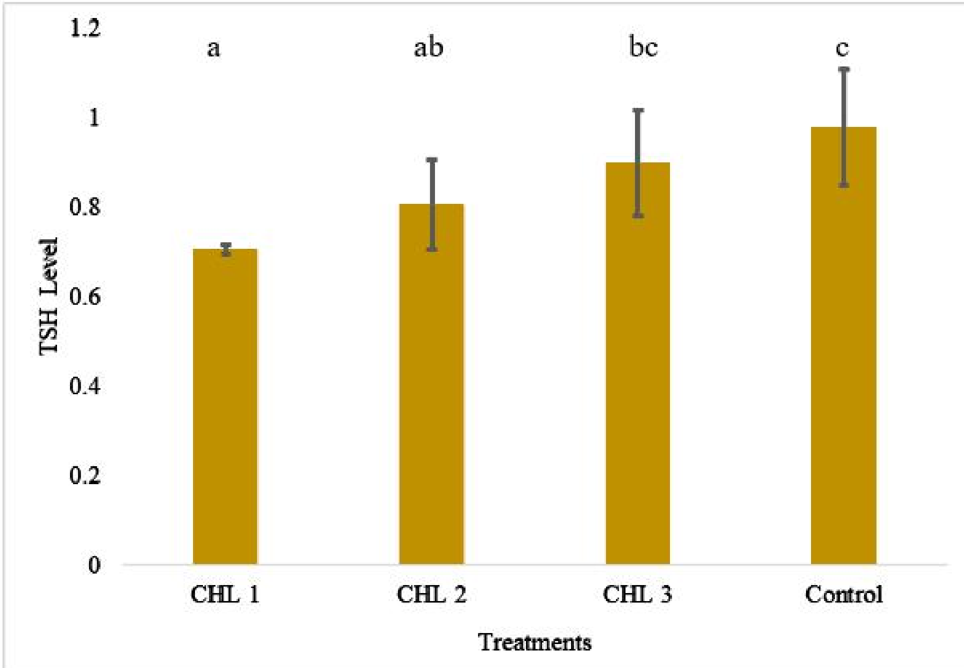

Figure 4.2: Effect of Chlorpyrifos on TSH level in *C. gariepinus*

4.3. Effect of Chlorpyrifos on T4 level in *C. gariepinus*

The effect of chlorpyrifos on T4 levels in *C. gariepinus* is represented in Figure 4.3. There was a significant difference ($p<0.05$) in the overall T4 levels in the examined *C. gariepinus* across all chlorpyrifos treatment groups. The lowest T4 levels were found in the CHI (4.53±1.22), while the highest T4 was seen in CHL2 (5.9 ±3.73) and was significantly different ($P<0.05$) from the control. Notably, there was no statistical difference ($P<0.05$) between the control samples (4.67±0.50) and the CHL1 (4.53±1.22) and CHL3 (4.93 ±1.36) treatment groups.

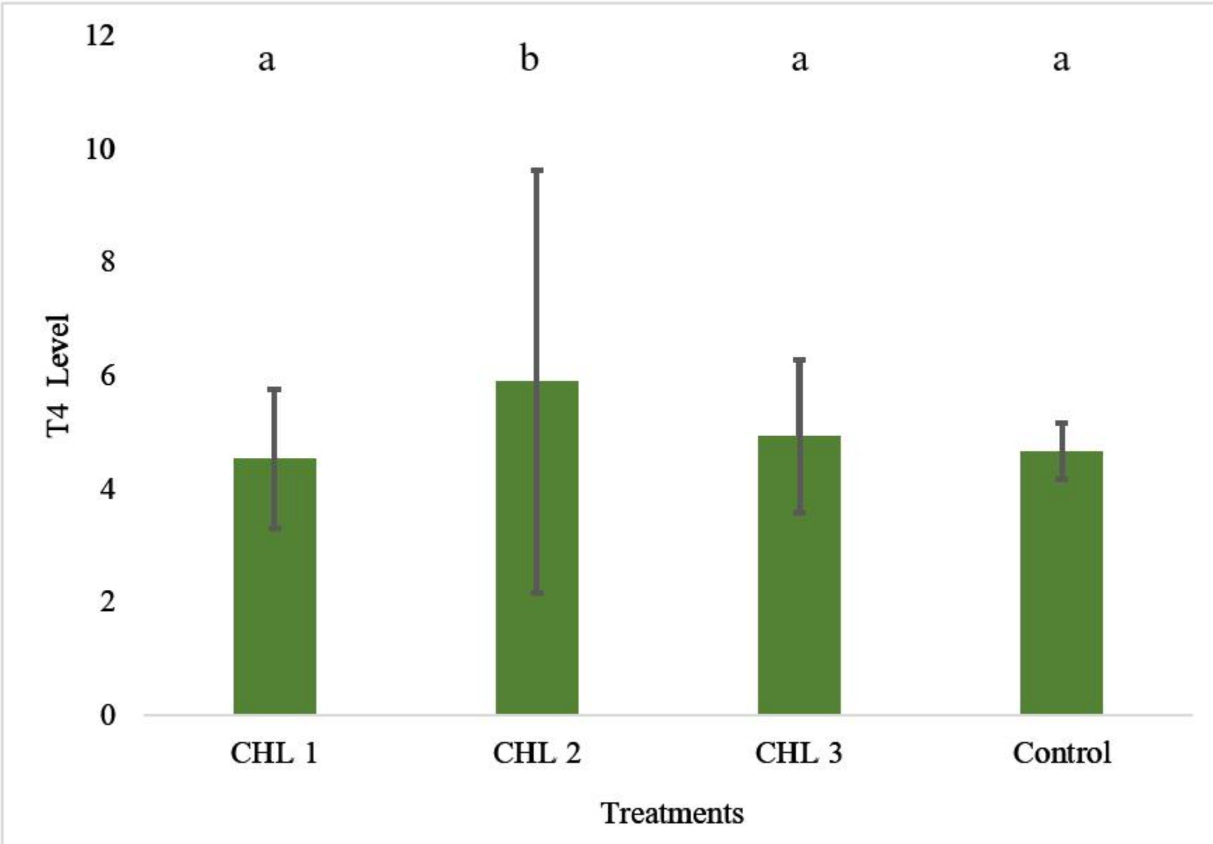

Figure 4.3: Effect of Chlorpyrifos on T4 level in *C. gariepinus*

CHAPTER 5

DISCUSSIONS

The resultsindicate that exposure to chlorpyrifos, an organophosphate insecticide, had a significant effect on the levels of triiodothyronine (T3), a thyroid hormone, in*Clarias gariepinus*. The control group, which was not exposed to chlorpyrifos, had the highest T3 levels. In contrast, the groups exposed to different concentrations of chlorpyrifosshowed significantly lower T3 levels compared to the control. However, there was no significant difference in T3 levels among the chlorpyrifos-treated groups.These findings are consistent with a study by Adedeji et al. (2009) who reportedthat exposure to diazinon, another organophosphate insecticide, caused a significant decrease in T3 levels in *C. gariepinus.*

Exposure to chlorpyrifos had a significant effect on the levels of thyroid-stimulating hormone (TSH) in *C. gariepinus*.The control group, had the highest TSH levels, indicating normal thyroid function. The groups exposed to lower concentrations of chlorpyrifos (CHL1 and CHL2) showed significantly lower TSH levels compared to the control group, suggesting disruption of the hypothalamic-pituitary-thyroid (HPT) axis (Peluso et al., 2023).Interestingly, the group exposed to the highest concentration of chlorpyrifos (CHL3) did not show a significant difference in TSH levels compared to the control group. This could potentially be due to a compensatory mechanism at higher exposure levels or other factors that need further investigation.However, it is important to note that chlorpyrifos exposure can lead to a range of toxic effects, including haematological and metabolic disorders (Elsharkawy, 2013), and that the route and vehicle of administration can impact the body burden and toxic responses (Smith, 2009). Further investigation is needed to fully understand the potential compensatory mechanisms and other factors at play in chlorpyrifos exposure.

Chlorpyrifos has been found to have a significant impact on the binding capacity of triiodothyronine (T3) to serum proteins (Mogensen, 1963). This effect may be further exacerbated by the presence of other chemicals, such as atrazine and cyanazine, which can enhance the toxicity of chlorpyrifos (Wacksman*et al*., 2016). However, the specific mechanism through which chlorpyrifos affects T3 levels is not fully understood and requires further investigation(Juberg et al., 2013).

The results from this study alsoindicatedthat chlorpyrifos exposure caused a significant change in T4 levels in *C. gariepinus*at different treatment levels. Specifically, the lowest T4 levels were observed in the highest chlorpyrifos concentration group (CHI), while the second-highest chlorpyrifos concentration group (CHL2) had the highest T4 levels, which were significantly different from the control. Interestingly, the control group and the lower chlorpyrifos concentration groups (CHL1 and CHL3) did not show a statistically significant difference in T4 levels.The complex response observed in thyroxine (T4) levels, with the highest chlorpyrifos concentration resulting in the lowest T4 levels and the intermediate concentration exhibiting the highest T4 levels, is consistent with the biphasic response reported in Sharma and Ansari (2013). This pattern suggests potential compensatory mechanisms or other factors influencing the hormonal response at different exposure levels.Sharma and Ansari (2013) observed a biphasic response on *Cyprinus carpio* (common carp) in T4 levels upon chlorpyrifos exposure, with an initial increase at lower concentrations followed by a decrease at higher concentrations. This pattern is somewhat similar to the results of this study, where the intermediate concentration exhibited the highest T4 levels, while the highest concentration was associated with lowest T4 levels.

CONCLUSION

This study highlights the significant impact of chlorpyrifos,on the thyroid function of *C. gariepinus*. Exposure to chlorpyrifos resulted in notable alterations in the levels of thyroid hormones, including triiodothyronine (T3) and thyroxine (T4), as well as thyroid-stimulating hormone (TSH).The observed decrease in T3 levels across all chlorpyrifos-exposed groups, along with disruptions in TSH levels, suggests a disruption of the hypothalamic-pituitary-thyroid (HPT) axis, indicating potential thyroid dysfunction. Interestingly, the highest concentration of chlorpyrifos did not show a significant difference in TSH levels compared to the control group, indicating a complex response that may involve compensatory mechanisms or other factors.Moreover, the study suggests that chlorpyrifos exposure may impact the binding capacity of T3 to serum proteins, further exacerbating its effects on thyroid function.

The observed effects of chlorpyrifos on thyroid function of *C. gariepinus*raises concerns about potential adverse impacts on aquatic ecosystems where this insecticide is in high concentration.While the present study provides valuable insights into the endocrine-disrupting effects of chlorpyrifos in *C. gariepinus*, further research is needed to elucidate the underlying mechanisms and potential long-term implications of exposure to this pesticide. Additionally, investigating the interactive effects of these compounds with other environmental stressors would contribute to a more comprehensive understanding of their ecological impacts on aquatic organisms.Understanding these mechanisms is crucial for assessing the environmental risks associated with chlorpyrifos exposure and developing appropriate mitigation strategies to protect both aquatic ecosystems and human health.

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