

Impact of Insecticide, Chlorpyrifos on Protein and Amino Acid Contents in Liver, Kidney and Brain of Exotic Teleost Fish, *Channa punctatus* (Bloch, 1973)

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ABSTRACT

Pesticides are one of the most potentially harmful toxic chemicals introduced into the environment and human ambient. These pesticides are utilized widely to ensure agricultural harvests against the harm brought about by different sorts of irritations. Anyway, these chemicals may reach non focused on biological systems like lakes and waterways through rain and wind, influencing numerous different organisms. Biochemical factors show explicit reactions to specific types of ecological pressure. The methodology of present work was to assess toxic effects of an insecticide, chlorpyrifos on biochemical constituents like protein, and Amino acid in liver, kidney and Brain of freshwater fish, *Channa punctatus*. The fish exposed to chlorpyrifos showed a decrease the protein and increase the amino acid levels for 15 and 45 days in liver, kidney and brain compared to control. The objectives of the present work was to observe the effect of chlorpyrifos on protein and amino acid levels in the liver, kidney and brain of teleost fish *Channa punctatus*.

Key words: Chlorpyrifos, Biochemical constituents, Liver, Kidney, Brain, *Channa punctatus*.

INTRODUCTION

Pollution problem is currently encountered everywhere the globe, with a rise in manufacture, urbanization, surplus use of agrochemicals like pesticides, herbicides, fungicides etc. To enhance yield of agricultural merchandise. Among the various kinds of pesticides, organophosphates have gotten one of the generally utilized classes of pesticides. The impacts of introduction of aquatic environment to these pesticides are hard to survey in light of their short steady in water section because of low solvency and quick debasement. Hence, monitoring of these insecticides is important.^{1,2}

Occupational exposure to pesticides is a common and alarming worldwide phenomenon. Around, 3 million instances of intense harming and 0.22 million passages from pesticide presentation have been accounted for yearly.³⁻⁶ In India, pesticides set up a critical section in cultivating headway and affirmation of general prosperity since the tropical environment is useful for bothering reproducing.⁷

Contamination by pesticides in aquatic ecosystem is a serious problem and fishes are more frequently exposed to these pollutants and may be taken in through gills, skin and contaminated foods.⁸ Fish are broadly used to assess the strength of aquatic biological systems since poisons develop in the natural pecking order and are liable for unfriendly impacts and demise in the sea-going frameworks.⁹

Synthetic Organophosphates [OP] Chlorpyrifos [CPF] is a pharmaceutical product, widely used to control various pests of agriculture and veterinary for public health protection.¹⁰ According to a recent report, children exposed to chlorpyrifos while in

the womb have an increased risk of delays in mental and motor development at age 3 and an increased occurrence attention-deficit hyperactivity disorder.¹¹ There are some reports shows toxic potentials of OP pesticides on tadpoles.¹²

Chlorpyrifos (O, O-diethyl-O-3, 5, 6-trichlor-2-pyridyl phosphorothioate; CPF) (Figure 1) is a wide range organophosphate bug spray (OP) which is industrially utilized for over 10 years to control creepy crawly bug. Chemical formula C₉H₁₁Cl₃NO₃PS. Since, it was first introduced into the market place in 1965. It is the second biggest selling OP and saw as more lethal to angle than organochlorine compounds. CPF passes through air float or surface spillover into regular waters, where it is collected in various life forms living in water, particularly in fish, consequently making it powerless against a few noticeable impacts. CPF is known to inhibit acetylcholinesterase, cause behavioural, neurological, oxidative, histopathological, endocrine and other effects at low doses. CPF is also known by its trade names Dursban and Lorsban.¹³

CPF (Chlorpyrifos) is also widely used in agriculture as the substitutes for methamidophos and parathion in China, and has become one of the major pesticides detected in farm products.^{14,15} Since farming uses

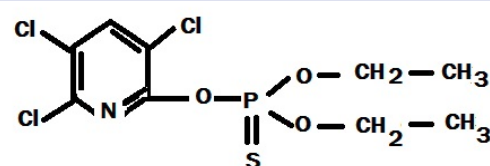


Figure 1: Structure of Chlorpyrifos.

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on plantations and column crops persevere, CPF has been every now and again identified in air, nourishment and water. Although various standards exist to minimize its exposure in food and water, CPF is frequently used and bio-accumulates in certain scenarios.^{16,17} Fish are presumably the most significant non-target casualties of pesticide over presentation as they have a significant job in natural way of life.

The present study, above the reports on the effects of low dose of Chlorpyrifos in fresh water teleost fish *Channa punctatus* are still scanty. In the light of above information and ideas, present investigation is aimed to study the effect of Chlorpyrifos on liver kidney and brain of the fresh water teleost fish *Channa punctatus*.

MATERIALS AND METHODS

Procurement of test fish

The fresh water fish, *Channa punctatus* were collected from kollidam river around sirkali city with the help of fisherman. The fishes having average length 13-16 cm and weight about 20-25 gm were brought to the laboratory and transferred to aerated aquarium for acclimatization. The fishes were fed daily on commercial fish feed.

Pesticide

Organophosphate pesticide chlorpyrifos purchased from local agro chemist shop was used for the present study.

Plan of experiment

A total of 40 fishes (10 fishes per aquarium) were separated as four groups.

The following experimental groups were conducted in the freshwater fish *Channa punctatus* for the period of 45 days.

- Group 1 *C. punctatus*, without any pesticide exposure (control)
- Group 2 *C. punctatus*, on exposure to 5 ppm chlorpyrifos for a period of 15 and 45 days
- Group 3 *C. punctatus*, on exposure to 6 ppm chlorpyrifos for a period of 15 and 45 days
- Group 4 *C. punctatus*, on exposure to 7 ppm chlorpyrifos for a period of 15 and 45 days

Biochemical methods

The protein and amino acid content in Liver, kidney and brain of *Channa punctatus* were estimated by the method of Lowry et al., 1951 and Moore and Stein (1954) respectively.^{18,19}

STATISTICAL ANALYSIS

The data obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance.²⁰

RESULT

The protein and amino acid contents in Liver, kidney and brain of *Channa punctatus* exposed to low, medium and high sublethal concentrations of chlorpyrifos showed significant decrease in the level of protein whereas increase the levels of amino acids when compared to control fish. The decrease the level of protein and increase the level of amino acid in liver, kidney and brain of *Channa punctatus* were more pronounced at 15 and 45 days of exposure period (Tables 1 and 2).

DISCUSSION

Various aquatic scavengers have been utilized to assess ecotoxicological dangers; nonetheless, little data about them by and by contemplated

Table 1: Protein level changes (mg/g) in Liver, kidney and brain of *Channa punctatus* exposed to sublethal concentrations of chlorpyrifos.

| Treatment groups | 15 days | 45 days |
|----------------------|----------------------------|----------------------------|
| Liver | | |
| Control | 141.39 ± 1.67 ^a | 142.78 ± 2.85 ^a |
| Low concentration | 136.94 ± 4.62 ^b | 134.84 ± 1.49 ^b |
| Medium concentration | 128.52 ± 8.27 ^c | 125.76 ± 2.75 ^c |
| High concentration | 120.92 ± 7.30 ^d | 117.53 ± 1.56 ^d |
| Kidney | | |
| Control | 92.39 ± 2.67 ^a | 93.67 ± 2.49 ^a |
| Low concentration | 84.75 ± 6.39 ^b | 83.57 ± 1.84 ^b |
| Medium concentration | 76.36 ± 9.47 ^c | 72.64 ± 1.59 ^c |
| High concentration | 68.58 ± 7.89 ^d | 70.93 ± 2.50 ^d |
| Brain | | |
| Control | 87.39 ± 2.98 ^a | 88.98 ± 1.47 ^a |
| Low concentration | 81.48 ± 8.45 ^b | 79.48 ± 1.39 ^b |
| Medium concentration | 74.69 ± 7.25 ^c | 72.85 ± 2.05 ^c |
| High concentration | 68.31 ± 4.62 ^d | 63.65 ± 1.83 ^d |

All the values mean SD of six observations; Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$) [Duncan multiple range test (DMRT)].

Table 2: Amino acid (mg/g) in Liver, kidney and brain of *Channa punctatus* exposed to sublethal concentrations of chlorpyrifos.

| Treatment groups | 15 days | 45 days |
|----------------------|---------------------------|---------------------------|
| Liver | | |
| Control | 7.03 ± 0.29 ^a | 7.78 ± 0.32 ^a |
| Low concentration | 7.98 ± 0.47 ^b | 8.02 ± 0.24 ^{ab} |
| Medium concentration | 8.38 ± 0.18 ^{bc} | 8.67 ± 0.32 ^c |
| High concentration | 9.42 ± 0.57 ^d | 9.84 ± 0.54 ^d |
| Kidney | | |
| Control | 4.29 ± 0.19 ^a | 4.37 ± 0.38 ^a |
| Low concentration | 4.88 ± 0.38 ^b | 4.92 ± 0.10 ^b |
| Medium concentration | 5.59 ± 0.31 ^c | 5.89 ± 0.39 ^c |
| High concentration | 6.32 ± 0.19 ^d | 7.14 ± 0.42 ^d |
| Brain | | |
| Control | 3.67 ± 0.28 ^a | 3.89 ± 0.29 ^a |
| Low concentration | 3.98 ± 0.36 ^b | 4.02 ± 0.21 ^{ab} |
| Medium concentration | 4.67 ± 0.48 ^c | 4.98 ± 0.54 ^c |
| High concentration | 5.10 ± 0.27 ^d | 5.67 ± 0.37 ^d |

All the values mean SD of six observations; Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$) [Duncan multiple range test (DMRT)].

bug sprays have been accounted for in these creatures. In such a manner to intense poisonous quality testing, different pesticides have been contemplated. Permethrin, for instance, demonstrated a mean static intense 96-h LC₅₀ relating to 0.81mg/L in red bog crawfish *Procambarus clarkii*; in a similar creature, a differentiating LC₅₀ estimation of 951 mg/L was resolved for the bug spray chlorantraniliprole, while for the neonicotinoids mixes thiametoxan and dinotefuran, creators detailed estimations of 967 mg/L and 2032 mg/L, separately; which are altogether different from the saw in the pyrethroids clotianidin, lambda-cyhalothrin, and etofenprox: 59, 0.16 and 0.29 mg/L, individually.²¹⁻²³

Chlorpyrifos poisonous quality has been the subject of numerous toxicological examinations, and much information has been accumulated up to this point, indicating its principle components of activity and different unfavorable impacts, both in creatures and people. Nonetheless, what has not been replied at this point, and our investigation attempted to clarify, is whether a rehashed 15 and 45 - day presentation to low, medium and High "practical" convergences of chlorpyrifos produce any indications of inebriation. Our outcomes

could reveal new insight into chlorpyrifos' harmfulness profile and add new discoveries important to human hazard appraisal.

Fish represents the higher trophic level in the amphibian natural pecking order. Hence, the steadiness of lethal compound amasses to the most extreme fixation in their body when contrasted with dissimilar living beings in the sea-going condition.²⁴ Fish is generally devoured in numerous pieces of the world since it has high protein content, low soaked fat and furthermore contains omega unsaturated fats known to help great wellbeing.²⁵ Proteins are significant natural substances vital for living beings in tissue building and assume a critical job in energy metabolism.²⁶

The kidney, which is an important organ of excretion and osmoregulation, is indirectly affected by pollutants through blood circulation.²⁷ The brain is highly vulnerable to oxidative stress due to its high metabolic rate, the reduced capacity for cellular regeneration, and numerous cellular oxidative stress targets like lipids, nucleic acids, and proteins. Generally, most molecules cannot cross the blood-brain barrier (BBB). But, due to large surface area, the NPs made of certain materials and with varying particle sizes can overcome this physical barrier and enter into the brain.²⁸

Pesticides can make genuine disability the physiological and wellbeing status of fish. Accordingly, biochemical tests are normal research center tests valuable in perceiving intense or constant harmfulness of bug sprays and can be a commonsense device to analyze danger impacts in target organs and to decide the physiological status in fish. Chlorpyrifos prompted biochemical adjustments in fish detailed by different analysts proposed they are great parameters that help to see the impacts of toxicants on the digestion of fish.^{29,30}

In the present investigation freshwater fish, *Channa punctatus* exposed to sublethal concentrations of chlorpyrifos for the periods of 15 and 45 days shows decrease the levels of protein and elevated levels of amino acid in liver, kidney and brain. The present work agrees with Tulasi and Jayantha Rao (2013)³¹ addressed that total protein content is decreased and it may be due to breakdown of proteins in the fabrication of some amount of energy for organism.

In an organism toxic substances brings about a kind of stress and an organism responds to that by developing necessary potential. During stress, organism needs sufficient energy which is supplied from reserve food material *i.e.* protein, glycogen and lipid.³² Protein plays a crucial role in virtually all biological processes. Under extreme stress, protein supply energy in metabolic pathways and biochemical reactions.³³ Decrease in protein might be due to the impairment of protein synthesis or increase in the rate of its degradation to amino acid. This might be fed to TCA cycle aminotransferase to cope with high energy demands to meet the stress condition. Decreased protein content observed in the present investigation was might be due to these reasons.

Lakshmanan *et al.* (2013)³⁴ examined the significant reduced in glycogen, protein and albumin in fish *Oreochromis mossambicus* during treatment of dichlorvos. Nagraju and Venkata Rathnamma (2013)³⁵ researched the effect of profenofos and carbosulphan on biochemical characteristics of the fresh water fish *Labeo rohita* and reported decreased glycogen content in gill, liver, kidney, brain and muscle.

Padmini and Rajaram (2016)³⁶ addressed the effect of different concentrations of chlorpyrifos viz. 0.04, 0.045, 0.05, 0.055, 0.06 and 0.065 ml/L on protein, glycogen and lipid in liver and kidney of *Channa gachua* for 96h and reported decreased levels in both the tissues in comparison with control. The decreased protein and increased amino acid contents in the gill, liver, kidney, heart and muscle of *Catla catla* exposed to cadmium chloride.³⁷

Reddy *et al.* (2011)³⁸ studied the effect of sublethal concentrations of chlorpyrifos on protein metabolism in gills, kidney, liver, and muscle of *Clarias batrachus* exposed to 0.825 mg/L and 1.65 mg/L for 7, 14, 21, and 28 days. Total protein, amino acid, and ammonia contents were decreased in all tissues for 28 days. Urea and glutamine levels were elevated, except in kidneys. The activities of protease, alanine, and aspartate aminotransferases, and acid and alkaline phosphatases were elevated in the tissues for 28 days exposure at both concentrations.

It is apparent that proteins are corrupted to meet the vitality prerequisites during pesticide CPF presentation. It very well may be reasoned that in *Channa punctatus* presented to sublethal groupings of Chlorpyrifos causes vitality emergency and change protein digestion.

CONCLUSION

In view of higher than results and discussion, gift study showed organophosphate treated fishes changes in macromolecule, and organic compound content in bony fish of *Channa punctatus*, that indicates organic chemistry manifestation great to the toxic action of toxicants. Toxic induces its impact at cellular or maybe at molecular level and ultimately causes organic chemistry alterations. The changes in organic chemistry composition of fishes can naturally have an effect on the nutritious price of aquatic fauna and deteriorating the worth of fish and it'll conjointly nice danger to individual great to continuous consumption of such fish.

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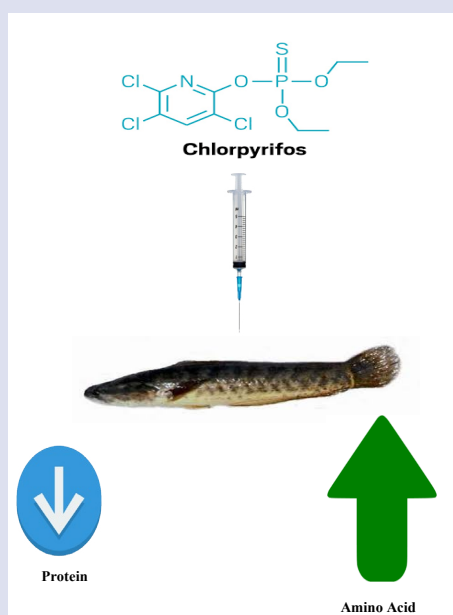
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GRAPHICAL ABSTRACT



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