



Sub-Lethal Exposure To Chlorpyrifos: Implications For Aquatic Ecosystems And Human Health

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ABSTRACT

Sub-lethal exposure encompasses a range of negative effects that may disrupt ecological balance and food web dynamics beyond mere mortality. Even non-fatal injuries may have long-term consequences for a species' fitness and survival if they lead to changes in behavior, decreased reproductive success, or heightened vulnerability to predators. Stress certainly contributed to a decrease in locomotor activity, as evaluated by both distance traveled and swimming speed, in fish exposed to 60 Ag/L (1/5 of LC50). Accumulation of acetylcholine (ACh), a neurotransmitter at synaptic synapses, and the subsequent alteration in locomotor behavior may result from either the decrease of AChE enzyme activity (40-55% in the brain) or the bioaccumulation of the toxicant in other parts of fish. Chlorpyrifos has the highest bioaccumulation values in the viscera, followed by the brain and the remainder of the body. Chlorpyrifos residues in food and water sources increase worries about long-term, low-dose exposure and its potential health consequences on humans.

Keywords: Chlorpyrifos, Sub-lethal, Locomotor, Acetylcholine, Enzyme.

I. INTRODUCTION

In the realm of modern agriculture, the use of pesticides has become an indispensable tool to safeguard crop yields and ensure global food security. Chlorpyrifos, a widely employed organophosphate pesticide, has played a pivotal role in pest management for decades. Its effectiveness in targeting a broad spectrum of agricultural pests, coupled with its relatively low cost, has made it a staple in the agricultural industry. However, the ubiquity of chlorpyrifos comes at a significant cost to environmental health. While the acute toxicity of chlorpyrifos has been widely studied and documented, the insidious and often overlooked threat lies in its sub-lethal effects on non-target organisms and ecosystems.

Sub-lethal exposure refers to situations in which organisms survive pesticide exposure but endure adverse physiological, behavioral, or biochemical changes, which can have far-reaching ecological consequences. Chlorpyrifos, with its neurotoxic mode of action,

poses a particular threat to non-target species, disrupting neural signaling pathways and inducing a cascade of unintended effects. Understanding the intricacies of sub-lethal exposure to chlorpyrifos is not only vital for safeguarding the health of our ecosystems but also for addressing the potential risks it poses to human populations through food and water contamination.

Chlorpyrifos, or O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate to give it its chemical name, is a pesticide, has earned its place in the agricultural arsenal due to its broad-spectrum insecticidal properties. Introduced in the mid-20th century, this organophosphate pesticide quickly gained popularity among farmers and pest control professionals for its ability to combat a wide range of agricultural pests, from aphids and leafhoppers to soil-dwelling nematodes. Its effectiveness lies in its interference with the nervous systems of these pests, leading to paralysis and eventual death.

In response to mounting concerns over the environmental and human health risks associated with chlorpyrifos exposure, regulatory bodies in various countries have taken steps to restrict its use. These actions include banning or phasing out the pesticide in agricultural applications, especially in residential and recreational areas. Despite such measures, chlorpyrifos remains prevalent in the environment, raising questions about its sub-lethal impacts on non-target organisms.

At the heart of chlorpyrifos' neurotoxicity lies its mechanism of action. This pesticide disrupts neural signaling by inhibiting the enzyme acetylcholinesterase (AChE), which regulates the neurotransmitter acetylcholine (ACh) in the nervous system. In normal synaptic transmission, AChE rapidly breaks down ACh, terminating the signal and allowing the neuron to reset for subsequent impulses. However, when chlorpyrifos inhibits AChE, ACh accumulates at synapses, leading to continuous nerve stimulation and the overactivation of muscles and nerves.

II. REVIEW OF LITERATURE

McClelland, Sara et al., (2018) The widespread use of pesticides raises the risk of contamination to species that are not intended targets. Even at low concentrations, pesticides may have direct and indirect effects on aquatic environments. Mesocosm studies demonstrated that very low concentrations of the herbicide chlorpyrifos affected tadpole shape and neurological development. That's significant because it shows that chlorpyrifos' effects on the trophic community were distinct from its effects on brain development. Metamorphs' body form did not alter when they were grown in mesocosms with chlorpyrifos-sensitive zooplankton, but the metamorphs' sensitivity to zooplankton population did change when chlorpyrifos was removed. Finally, a vertebrate model suggests that organophosphorous pesticides may have direct effects on neurodevelopment even at low, ecologically relevant concentrations.

Bernal-Rey, Daissy et al., (2017) The organophosphate pesticide chlorpyrifos (CPF) has seen extensive use. Two species of freshwater fish were tested to see how chlorpyrifos affected their acetylcholinesterase activity (AChE, a biomarker of neurotoxicity). Both the *Cnesterodon decemmaculatus* and the *Gambusia affinis* were among these fish. Animals drank water with CPF (1 and 5 g L⁻¹) at sublethal dosages for 96 hours. At 1 and 5 g CPF L⁻¹, *C. decemmaculatus* AChE activity was lowered by 48% and 69%, whereas *G. affinis* AChE activity was reduced by 27% and 36%. No species-specific variations in the enzyme's sensitivity to CPF were observed in in vitro testing.

Sunanda, M., et al., (2016). The primary function of agrochemicals is to repel pests and insects that may otherwise damage agricultural crops. They are an essential resource for protecting crops from a wide range of pests without endangering humans or other beneficial organisms. However, due to their chemical composition, inappropriate handling, use, and storage might result in severe environmental damage. Pesticide residues get up in aquatic habitats via a variety of pathways, including leaching, agricultural runoff, air transfer, spray drift, and incorrect disposal. When pollutants are introduced into an aquatic environment, they accumulate in the tissues of fish and other aquatic organisms, causing biochemical and physiological changes that may be either adaptive or harmful. Keeping fish populations healthy is crucial since they provide food for such a large proportion of the world's population. As the second most used pesticide in India, the organophosphorus chemical chlorpyrifos has been the subject of this paper's examination of its hazardous consequences. There is substantial concern for the safety of aquatic species and human people due to its acute toxicity and deleterious consequences, which include behavioral, morphological, and other abnormalities in fish. This literature review establishes a foundation for future research on the impact of numerous hazardous chemicals, with the goals of elucidating the mechanisms of their pollutant action, identifying strategies for mitigating their negative impacts, and planning for their control..

Vinod Verma and Anita Saxena (2012) In order to determine the potential dangers of chlorpyrifos pesticide, its effects on the behavior of the freshwater fish *Puntius chola* (Hamilton-Buchanan) were studied. We conducted many toxicology tests to determine risk. The half-life measured in the 96-hour experiment was 0.219 ppm, which is fatal. Chlorpyrifos elicited an abrupt and immediate behavioral reaction, indicating its high toxicity. As an aquatic creature, fish may serve as a useful indication of the toxicity of pesticides and other pollutants in the aquatic environment.

Narra, Madhusudan Reddy, et al., (2011) Protein synthesis and breakdown in the gills, kidneys, liver, and muscles of the freshwater fish. *Clarias batrachus* was studied to determine the effects of sublethal doses of chlorpyrifos. Exposure periods of 7, 14, 21, and 28 days were used, with fish being dosed at 1/20th and 1/10th of the 96 h LC50. Fish were placed in salt water for 28 days and then returned to fresh water for another 21 days to observe the adaption process. At certain time intervals, fish were slaughtered and analyzed for total protein, amino acids, ammonia, urea, glutamine, protease,

transaminases, and phosphatases in their gills, kidneys, livers, and muscles. After 28 days, total protein, amino acid, and ammonia levels all decreased, pointing to progress. Normal urea and glutamine levels were slow to return in the kidneys, even after the body had finished recovering. Proteases, alanine and aspartate aminotransferases, and acid and alkaline phosphatases were all more active in tissues that had been treated with either dosage for 28 days. The purifying procedure restored normal enzyme activity.

Ramesh Halappa and Muniswamy David (2009) For 96 hours, common carp fingerlings were exposed to chlorpyrifos, an organophosphate pesticide with a 20% effective concentration (EC), at dosages ranging from 0.120 to 0.200 mg/L. The median lethal concentration (LC50) of chlorpyrifos in a semi-static bioassay was 0.160 mg/L. One-seventh and one-fourteenth of the 96 h LC50, respectively, were employed in the subacute trials (0.0224 and 0.0112 mg/L). One, seven, and fourteen days of sublethal concentrations were utilized, followed by seven days of recovery in toxicant-free media. We kept an eye out for any abnormalities in behavior or appearance throughout the trial stages. Fish exposed to toxic media swam in a disordered, erratic, and darting pattern; they were also hyperexcitable, disoriented, and eventually sank to the bottom. The carp were stressed, although death was low at the two non-lethal doses. The primary morphological change that occurred throughout the test periods was a caudal bending. Acetylcholinesterase (AChE) inhibition may be at the root of the observed behavioral and morphological alterations. Hyperstimulation and the stoppage of neuronal transmission (paralysis) arise from an insufficient amount of acetylcholine (ACh) being degraded at cholinergic synapses. Even during remission times, impaired behavioral reactions and physical malformations were identified.

III. RESEARCH METHODOLOGY

There was no further purification of the reagents used in this assay, all of which were of analytical quality. The chlorpyrifos used in the study was synthesized at the Indian Institute of Chemical Technology and was characterized as having a purity of 99%. Following transport from farm to lab in oxygenated polythene bags, the *Gambusia affinis* (Order: Cyprinodontiformes, Family: Poeciliidae) were released into glass aquaria containing 100 L of well-aerated, unchlorinated ground water. The 1255mg fish were fed commercial dry meal pellets while they spent seven days in a 40 L glass tank (60 x 30 x 30 cm). An Indian-made Jumbo-Jet aquarium air pump (Super- 8300) was used for daily water changes and aeration. The local standard of 13 hours of daylight and 11 hours of darkness (L:D) was adhered to. Temperature was maintained at 26.2°C, pH was maintained at 7.100.05, and dissolved oxygen was maintained at 8.15554 mg/L for both acclimation and testing.

Determination of Median Lethal Concentration (LC50)

The chlorpyrifos acute LC50 was calculated using a semi-static technique in a controlled laboratory setting. The experimental concentration ranges were derived from 95-hour

lethal concentration (LC50) tests. By introducing the toxicant dissolved in 2 ml of acetone and replenishing the solution daily without aeration, we were able to maintain concentrations of 200, 250, 300, 400, and 500 g/L in 40 L of water. After two days of fasting, ten male and female fish were randomly assigned to each tank and subjected to one of five concentrations twice. Simultaneously, a series of tests using just a carrier solvent served as a control. Fifty male and female fish fasted for two days, and then the sub lethal test dose of 60 g/L (1/5 of LC50) was introduced to each tank. To maintain the correct concentration, the toxicant was dissolved in 2 ml of acetone and the solution was changed out everyday (for the whole 20-day exposure period) without aeration. There was no limit on the number of pellets the fish could consume. The control tank was the one that wasn't treated. The solvent utilized as the "carrier" was acetone. Computer-aided electronics, video-camera tracking was used to observe the fish's changed locomotor activity on days 6, 12, 18, 24 and 30 of the exposure period.

Statistical Analysis

Ten fish were selected at random from each period, and their locomotor behavior was analyzed for each. Student's t test was used to establish statistical significance, and a value of P 0.05 was regarded to indicate a significant difference from the control group.

IV. DATA ANALYSIS AND INTERPRETATION

G. affinis displayed aberrant behaviors such as erratic swimming with jerky movements, loss of balance, and secretion of copious amounts of mucus from entire body when exposed to higher concentrations (400 and 500 Ag/L) of chlorpyrifos, used to determine the fatal concentration. The fish were listless, and just before they died, they showed brief signs of excitement. Fish exposed to OP insecticides have been observed to have similarly altered behavior. There was no discernible change in opercular activity between control and treatment fish at 60 Ag/L.

Since chlorpyrifos tends to build up in organisms over time, it's possible that estimations of tissue concentrations might be more useful for evaluating circumstances in the wild. Therefore, HPLC technique was used to evaluate the buildup of the toxicant in various regions of the test organism.

On day 6, bioaccumulation was highest in the brain, the body, and the viscera, compared to all other time points. The data show that chlorpyrifos accumulates most heavily in the viscera, followed by the head and the rest of the body. Viscera, the brain, and the rest of the body had average bioconcentration factors (Ag/g) of 0.113, 0.015, and 0.006 (table 1).

Table 1: Bio-concentration factors of chlorpyrifos in viscera, head and body of fish *Gambusia affinis* during sub-lethal exposure

| Exposure Period (Days) | Time After Initial Value (Hours) | Bio-Concentration Factors* in Different Parts of Fish | | |
|---|----------------------------------|---|-------------------|-------------------|
| | | Viscera | Head | Body |
| 6 | 0 | 0.201 (0.01) | 0.013 (0.00) | 0.007 (0.00) |
| 12 | 95 | 0.156 (22.82) | 0.008 (22.44) | 0.004 (0.00) |
| 18 | 191 | 0.108 (48.23) | 0.006 (34.68) | 0.005 (20.01) |
| 24 | 287 | 0.048 (71.58) | 0.008 (56.17) | 0.002 (39.94) |
| 30 | 383 | 0.042 (80.70) | 0.005 (78.61) | 0.002 (60.04) |
| Average bio concentration values \pm SE | | 0.113 \pm 0.031 | 0.015 \pm 0.004 | 0.006 \pm 0.002 |

*Bio-concentration factor = accumulated concentration in tissue (Ag/g)/concentration in media (Ag/L).

V. CONCLUSION

Sub-lethal exposure to chlorpyrifos is a pressing concern with far-reaching implications for environmental health, biodiversity, and human safety. This exploration has highlighted the need for continued research and informed decision-making to mitigate the hidden threats posed by pesticides like chlorpyrifos. By understanding the sub-lethal impacts of chlorpyrifos, we can move towards more sustainable pest management practices, protect non-target organisms, and safeguard the ecological balance upon which our ecosystems and societies depend. The journey into the world of sub-lethal pesticide exposure is ongoing, and its lessons are vital for the future of our planet and all its inhabitants.

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