



Determination of LC₅₀ and behavioral modifications in *Cyprinus carpio* L. exposed to halosulfuron-methyl herbicide

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Abstract

Halosulfuron-methyl, is a sulfonylurea-based post emergence herbicide. It has gained wider application in agricultural management especially for Rice and Sugar cane, due to its high efficacy activity at low doses. In spite of its agricultural advantage, unintended release of herbicides into different water bodies by agricultural runoffs would definitely harm aquatic species, especially fish, which are primary serves as bioindicators of environmental health. The present study is conducted to evaluate acute toxicity of Halosulfuron-methyl herbicide on freshwater fish *Cyprinus carpio* L., additionally to assess behavioral responses in exposure conditions. Laboratory assays are performed using 10 fish per group in test chemical, which are under static conditions, for about 96 hour of exposure period. The median lethal concentration (LC₅₀) has been determined in addition to mortality rate. Abnormal behavioral responses are also observed, depending on dose response manner. Lateral swimming, loss of equilibrium with sinking, repeated schooling behavior, fright response, upward and backward swimming, dashing and burst movements, whirling and corkscrew motion, excessive opercular activity and rapid fin beating were observed in behavioral studies. Mucus is secreted in this period. These responses are due to suggestive neuro muscular distress, respiratory dysfunction and physiological stress targeted by herbicide.

Keywords: *Cyprinus carpio*, halosulfuron-methyl, LC₅₀, acute toxicity, behavioral responses

Introduction

Herbicides are extensively used in agriculture to control unwanted vegetation and improve crop yields. However, their extensive application causes contamination in aquatic ecosystem. Herbicides enter into aquatic life through surface runoff, leaching and drift during exposure to agriculture crops. Herbicides pose significant risks to aquatic organisms, particularly freshwater fish. Fish play an important role in maintaining aquatic ecosystem and it acts as a bioindicators of water quality. Aquatic organisms shows sensitivity to Herbicide exposure. Accumulated herbicides show severe changes in internal organs. They affect enzymes, proteins and molecular mechanisms that causes genetical changes, results mutation and develops abnormal development of organs leads to death. Herbicides are emerged as knight armours for crops (Srivastava and Singh, 2014) [18]. Halosulfuron-methyl is a sulfonylurea based selective and systemic herbicide for post emergence control of sedges and other weeds. Sulfonylurea herbicides are special class of chemicals extensively used in agriculture fields to control weed. They chiefly inhibit acetolactate synthase enzyme (ALS), it is crucial for synthesis of essential amino acids in plants (Mousdale and Coggins 1991; Zhou *et al.*, 2007; Liu *et al.*, 2017) [12, 23, 9]. Acetolactate synthase inhibition dislocates molecular signals in growth of plants that effective control weeds. Sulfonylureas are largely recognized in agriculture (Aubert *et al.*, 1997) [1]. It is absorbed by the root system and leaf surface, transported to meristem tissues in plants as postemergence herbicide. It has been widely used in agricultural practices, particularly in cultivation of agricultural crops like rice and sugarcane. Halosulfuron-methyl gives high effectiveness at lower application in weed control. The extensive use of halosulfuron-methyl causes ecosystem imbalance. Accidental release into aquatic

ecosystems through agricultural runoff causes accumulation of this herbicide. This accumulation postures hazardous bell to aquatic organisms, particularly fish species that play an essential role in maintaining aquatic environmental health get affected (Darshan and Parimala, 2025) [4]. Herbicides exposure shows toxic effects to fish, such as neurotoxicity, which is evidenced by acetylcholinesterase inhibition and tissue damage with organ level alteration. Although Herbicide causes some effect on fish behavior and physiological stress responses such as loss of equilibrium, erratic swimming and respiratory distress underlying neuro muscular and respiratory disfunctions that require comprehensive evaluation (Shinn *et al.*, 2015; Sani and Idris, 2016; Bojarski *et al.*, 2018) [16, 15, 2].

Cyprinus carpio is an ecologically significant freshwater fish commonly used model organism in toxicological studies. This species extremely sensitive to environmental pollutants, several researchers uses this fish to assess the impact of contaminants in aquatic ecosystems (David, 2021; Yalsuyi *et al.*, 2021; Khoshnood, 2024) [5, 22, 8]. Assessing the acute toxicity of halosulfuron-methyl herbicide on *C. carpio* provides important understandings of potential environmental risk caused by this herbicide. Toxicological evaluations are very much crucial and important for the benefit of aquatic life.

Materials and Methods

Healthy and active *Cyprinus carpio* L. fingerlings (n = 1024), weighing 4 to 6 ± 0.3 g with an average length of 7 ± 0.5 cm, have been procured from the State Fisheries Department, Turuvekere, Karnataka, India. The fishes are transported to the laboratory in large aerated containers and are acclimatized for 30 days in large tanks. During acclimatization, the fish were fed with commercial dry feed pellets (Optimum, Aquatic P. Feed).

The acute toxicity experiments has been conducted in aquaria containing 10 L of tap water. The water quality parameters are analyzed according to standard APHA (2023) and it found to have following physiochemical parameters such as temperature $24 \pm 2^\circ\text{C}$, pH 7.1 ± 0.2 at 24°C , dissolved oxygen 9.6 ± 0.8 mg/L, carbon dioxide 6.3 ± 0.4 mg/L, total hardness 23.4 ± 3.4 mg, specific gravity 1.001, conductivity less than $10 \mu\text{S/cm}$, particulate matter below 20 mg/L, total organic carbon less than 2 mg/L and unionized ammonia below $0.87 \mu\text{g/L}$. Water has been renewed daily. A photoperiod of 12 h light and 12 h dark is maintained throughout acclimatization and testing periods. Feeding continued regularly during acclimatization but was ceased for two days prior to exposure to the test medium (Patil and David 2008, 2010; Ramesh and Muniswamy, 2009; Marigoudar *et al.*, 2009; Chebbi and David, 2010; Singh and Zahra, 2017; Vandana and Kaippallil, 2021) [13, 14, 10, 3, 17, 20].

Halosulfuron-methyl is procured from local market at Tumkur, Karnataka, India. The expiry date has been verified and considered suitable prior to experiments. The required quantity of Halosulfuron-methyl is weighed using a spatula as per the experimental requirements. Experiments were done as per OECD guidelines.

In the range finding test, fishes have been exposed to varying herbicide concentrations ranging from 350 to 750 mg/L. In batches of ten per 10 L test medium. Six replicates were maintained per concentration alongside control groups. The test medium was renewed every 24 hours and the desired concentration of the test compound was replenished after water renewal. Concentration selections for the definitive acute toxicity tests are based on the range between the highest concentration causing 100% mortality and the lowest concentration causing zero mortality (Table 1). Mortality observations are recorded every 24 hours for up to 96 hours. Dead fish have been removed immediately to maintain water quality. Behavioral abnormalities are simultaneously monitored and documented.

Mortality data is analyzed using Duncan's multiple range test (Duncan, 1955) [6]. Residual variance has been estimated

through repeated measures ANOVA (Winner, 1971) [21]. Time of exposure has been treated as the repeated measure factor, while treatment (concentration and control) as the second factor. Median lethal concentration (LC_{50}) values with 95% confidence limits has been estimated at 96 hours using Probit analysis following Finney (1971) [7] and APHA (2023) [19] protocols.

Behavioral Study of the Control and Exposed Fish

In the present study, control *C. carpio* were active during feeding and responsive to the slightest disturbances. Their movements were well coordinated and did not vary significantly among control groups. These consistent behaviors have been used as standards throughout the experiment. Fish exposed to Halosulfuron-methyl exhibited a range of altered behaviors. In the observation lateral swimming, sinking phenomena, disrupted schooling behavior, fright responses, backward swimming, dashing movements, upward swimming, whirling, corkscrew movements and burst swimming recorded. Additionally, changes in fin beat frequency and opercular beat frequency were also recorded (Table 2).

Halosulfuron-methyl is classified as sulfonylurea herbicide. It acts by inhibiting the acetolactate synthase enzyme (ALS), it is crucial for synthesis of essential amino acids. Halosulfuron-methyl disrupts metabolic processes rather than immediate neuro muscular transmission. The fish exhibited fright responses and disruptions in shoaling behavior, which increases vulnerability to predation. Excess mucus secretion was sometimes observed, which is a nonspecific protective mechanism to reduce contact with the toxic medium. Mucus layer act as a barrier to minimize irritation to harmful compounds through epidermal secretions.

The observed behavioral changes clearly shows significant physiological stress and functional damages induced by Halosulfuron-methyl. These findings clearly explains that exposure to this herbicide can evidently affect normal locomotor activities and respiratory mechanisms in *C. carpio* potentially affecting survival in natural habitats.

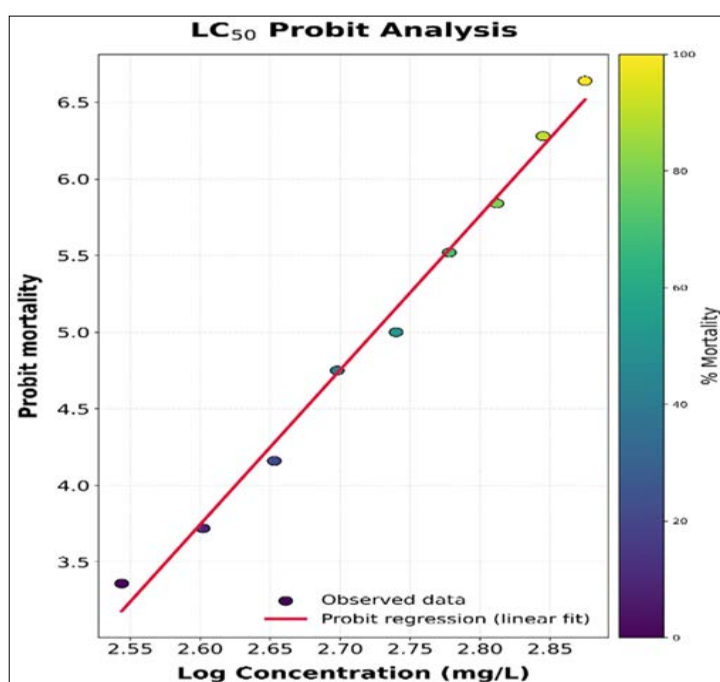


Fig 1: Probit analysis Graph – Probit Kill vs Log Concentration.

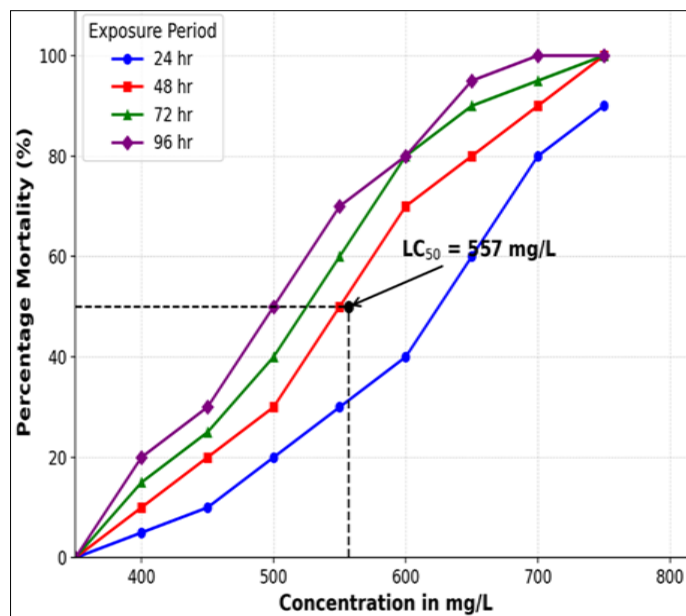


Fig 2: Percentage Mortality Responses at Various concentration for Different Exposure Durations and Estimation of LC₅₀ Value.

Table 1: Mortality rate of *C. carpio* following 96 - hour exposure to different concentrations of Halosulfuron-methyl.

Sl. No	Concentration (mg/L)	Log(conc)	No. of Fish Exposed	No. of Fish Alive	No. of Fish Dead	% Kill	Probit Kill
1	350	2.544	10	10	0	0	3.36
2	400	2.602	10	9	1	10	3.72
3	450	2.653	10	8	2	20	4.16
4	500	2.698	10	7	4	40	4.75
5	550	2.740	10	5	5	50	5.00
6	600	2.778	10	3	7	70	5.52
7	650	2.812	10	2	8	80	5.84
8	700	2.845	10	1	9	90	6.28
9	750	2.875	10	0	10	100	6.64

Table 2: Behavioral responses of *C. carpio* following 96-hour exposure to different concentrations of Halosulfuron-methyl.

Sl. No	Observed Behaviours	Control	2 Hours	24 Hours	48 Hours	72 Hours	96 Hours
1	Lateral swimming	Normal	None	++	+++	+++	++++
2	Sinking phenomenon	Absent	None	++	+++	+++	++++
3	Schooling behaviour	Normal	None	++	+++	+++	++++
4	Fright response	None	None	++	+++	+++	++++
5	Backward swimming	None	None	++	+++	+++	++++
6	Dashing movement	None	None	++	+++	+++	++++
7	Upward swimming	Normal	None	++	+++	+++	++++
8	Whirling and cork movement	None	None	++	+++	+++	++++
9	Burst swimming	None	None	++	+++	+++	++++
10	Fin beat frequency	Normal	None	++	+++	+++	++++
11	Opercular beat frequency	Normal	None	++	+++	+++	++++

Legends: = Moderate, ++ = Frequent, +++ = Strong, ++++ = Persistent

Results

Acute toxicity test of Halosulfuron-methyl on *C. carpio* confirmed a concentration dependent increase in mortality over the 96 hour exposure period. The median lethal concentration (LC₅₀) calculated through probit analysis found to be 557 mg/L. Mortality was minimum at lower concentrations but increased sharply at higher dosage as shown in table 1, this clearly depicts the toxicity of Halosulfuron-methyl herbicide to freshwater fish *C. carpio*. Behavioral observations revealed significant modifications by exposure. Early signs included increased lateral swimming and occasional dashing movements. As concentration increased, fish exhibited irregular swimming patterns such as whirling and burst swimming. Sinking behavior and disrupted schooling were noted, indicating

compromised buoyancy control and social interaction. The fish also displayed fright responses and backward swimming. Respiratory distress was evident from increased opercular beat frequency and fin beat frequency compared to controls. Mucus secretion on body surface was regularly observed, this is because of activation of defense mechanism in fish. These behavioral studies observed during 2hrs, 24hrs, 48hrs, 72hrs and 96hrs exposure and persistently as shown in table 2. All these are evidences for physiological stress caused to fish by exposed to Halosulfuron-methyl herbicide.

The findings are notable evidences that Halosulfuron-methyl influences both survival and behavior of *C. carpio* through disruption of normal neuro muscular junctions and metabolic functions in a physiological manner.

Statistical Analysis

Mortality data of *C. carpio* exposed to different concentrations of Halosulfuron-methyl was analyzed using probit analysis. Probit analysis is used to determine the median lethal concentration (LC₅₀) at 96 hours. The concentration responses shows a clear dose dependent increase in fish mortalities, with no mortality at 350 mg/L and 100% mortality at 750 mg/L. The Probit values increased correspondingly with concentration, confirming the suitability of the dataset for Probit and linear regression as shown in fig 1. The LC₅₀ estimated from the Probit mortality linear curve was 537 mg/L, indicating the concentration at which 50% mortality is expected. This value aligns closely with the LC₅₀ calculated by the Percent Mortality Sigmoid Curve method (568 mg/L) and Dragsted and Behrens' Equation (565 mg/L). The mean LC₅₀ across method was 556.66 mg/L, with a standard deviation of 0.017, reflecting high precision and consistency across statistical methods. The high coefficient of determination ($R^2 = 0.9909$) from the Probit confirms that excellent fit between observed mortality and the logarithmic transformed concentrations. Acute toxicity of Halosulfuron-methyl to freshwater fish, dose response relationship has been confirmed by plotting mortality percentage against concentrations. The data show that there is a clear increasing tendency in mortality with rising concentrations with respect to durations as shown in fig 2. Statistical analysis clearly shows that there is a significant effect of both concentration and exposure time on fish mortalities. The dose response curve shifted with improved exposure time, this indicating enhanced sensitivity of freshwater fish *C. carpio* to Halosulfuron-methyl over continued period.

All these strong linear relationships support the reliability of the LC₅₀. Such analysis encounters OECD guidelines for fish acute toxicity test and provides critical baseline data for LC₅₀ assessment of Halosulfuron-methyl. Probit analysis is an effective tool for calculating toxic effect. The consistent LC₅₀ value obtained from several methods strengthens assurance in these findings and highlight the importance of controlled exposure experiments combined with statistical assessment in ecotoxicology.

Conclusion

Study determine acute toxicity LC₅₀ and evaluated behavioral changes in *Cyprinus carpio* L. exposed to Halosulfuron-methyl herbicide over a period of 96. The median lethal concentration (LC₅₀) values calculated by probit analysis. It provides a critical baseline toxicity data for this sulfonylurea herbicide in a freshwater fish model. Behavioral observations showing significant disruptions such as altered swimming patterns, lateral swimming, sinking, schooling disturbances, fright responses, whirling movements and changes in fin and opercular beat frequencies. These behavioral responses clearly specify physiological stress and impaired neuro muscular functions in *Cyprinus carpio* L. related to metabolic disruption caused by Halosulfuron-methyl herbicide. This herbicide activates toxicity in fish through different biochemical pathways and survival mechanisms. The excess mucus secretion observed is consistent with a nonspecific defense response justifying direct contact with the toxicant. Statistical analysis shows a strong correlation between concentration and mortality, with LC₅₀ values consistently estimated around 557 mg/L, confirming acute toxicity. Overall, this research shows

important toxicological knowledge regarding the effects of Halosulfuron-methyl on aquatic organisms, highlighting the need for careful environmental discharge.

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