

Influence of Lethal and Sublethal Toxicity of Rogor on Blood Parameters of Fresh Water Fish *Heteropneustes fossilis*

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ABSTRACT

This study examines lethal (acute) and sublethal effects of Rogor® (commercial formulation of dimethoate 30% EC) on hematological parameters of the freshwater stinging catfish *Heteropneustes fossilis*. Following OECD-guided acute toxicity assays, the 96-h LC₅₀ was estimated and behavioural responses recorded. For sublethal exposures, fish were maintained at selected fractions of the LC₅₀ for 7, 14 and 28 days and blood samples were analysed for erythrocyte count (RBC), haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC) and differential leukocyte counts (DLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and serum biochemical markers. Rogor/dimethoate caused concentration- and time-dependent reductions in RBC, Hb and PCV and increases in TLC and neutrophil proportions, consistent with hypochromic anaemia and inflammatory stress. Results underscore that both lethal and environmentally realistic sublethal concentrations of Rogor produce significant haematological disturbances in *H. fossilis*, supporting the use of blood parameters as sensitive biomarkers of organophosphate contamination in freshwater ecosystems.

Keywords- Rogor, dimethoate, *Heteropneustes fossilis*, hematology, LC₅₀.

I. INTRODUCTION

Rogor® is a widely used commercial organophosphate insecticide whose active ingredient is dimethoate, commonly applied in agricultural fields to control a broad spectrum of insect pests. Due to its extensive use in crop protection, Rogor frequently enters nearby aquatic ecosystems through agricultural runoff, leaching and spray drift, particularly during monsoon seasons in tropical regions. Once introduced into freshwater bodies, dimethoate poses a significant threat to non-target organisms, especially fish, owing to its high solubility in water and moderate persistence in the environment (FAO, 2012; WHO, 2019).

Organophosphate insecticides exert their toxic action primarily through inhibition of acetylcholinesterase, leading to accumulation of acetylcholine at synaptic junctions and continuous nerve impulse transmission. In fish, this neurotoxic mechanism manifests as abnormal swimming behaviour, loss of

equilibrium, respiratory distress and, at higher concentrations, mortality. Apart from neurotoxicity, organophosphates are also known to induce oxidative stress, metabolic disruption and tissue damage, which collectively impair physiological homeostasis (Fulton and Key, 2001; Gupta et al., 2018).

Fish are considered sensitive bioindicators of aquatic pollution because they occupy higher trophic levels and respond rapidly to changes in water quality. Among the various biomarkers used in ecotoxicological studies, haematological parameters are particularly valuable as they reflect the internal physiological condition of the organism and provide early warning signals of environmental stress. Alterations in blood parameters such as red blood cell count, haemoglobin concentration and leukocyte profiles are commonly associated with exposure to pesticides and other toxicants (Adhikari et al., 2004; Banaee et al., 2013).

Haematological indices are directly related to oxygen transport, immune competence and metabolic activity in fish. A decline in erythrocyte count,

haemoglobin and packed cell volume is often interpreted as anaemia or haemodilution, while changes in leukocyte count may indicate immune stimulation or suppression. These changes may compromise the ability of fish to survive under natural conditions by reducing growth, resistance to disease and reproductive success (Wedemeyer and Yasutake, 1977; Hrubec et al., 2000).

The freshwater stinging catfish *Heteropneustes fossilis* is a commercially important air-breathing fish species widely distributed in rivers, ponds, canals and wetlands of the Indian subcontinent. It is an important source of animal protein and is well adapted to hypoxic environments, making it a suitable model organism for toxicological studies. Its ecological relevance and physiological sensitivity to pollutants have led to its extensive use in studies evaluating the effects of pesticides and heavy metals (Saxena and Seth, 2002; Sinha et al., 2017).

Acute toxicity tests, particularly the determination of median lethal concentration (LC_{50}), are widely employed to assess the immediate toxic potential of chemicals to aquatic organisms. The 96-hour LC_{50} value provides a quantitative measure of toxicity and serves as a basis for selecting sublethal concentrations for chronic and subchronic studies. International guidelines such as those issued by the OECD recommend standardized methods for conducting fish acute toxicity tests to ensure reproducibility and comparability of results (OECD, 2019).

While acute toxicity studies provide information on lethal thresholds, sublethal exposure studies are ecologically more relevant because environmental concentrations of pesticides are often below lethal levels. Sublethal concentrations may not cause immediate mortality but can induce significant physiological and biochemical disturbances over time. Such chronic stress can lead to long-term population declines even in the absence of visible fish kills (DeLorenzo et al., 2001; Cengiz, 2006).

Several studies have reported that dimethoate exposure leads to significant haematological alterations in freshwater fish species, including reductions in erythrocyte count, haemoglobin and packed cell volume, along with increases in leukocyte count. These changes have been attributed to haemolysis, inhibition of erythropoiesis, oxidative damage to blood cells and stress-induced immune responses (Tilak et al., 2007; Narra et al., 2017).

The magnitude and nature of haematological responses to pesticides depend on species, age, size, exposure duration, concentration and environmental conditions such as temperature and dissolved oxygen. Therefore, species-specific studies are essential to accurately assess ecological risk and to develop regionally relevant water quality guidelines. Data generated from commonly consumed fish species such as *Heteropneustes fossilis* are particularly important from both ecological and human health perspectives (Begum, 2004; Mishra and Mohanty, 2008).

Despite the widespread use of Rogor and the known toxicity of dimethoate, comprehensive studies combining acute lethal toxicity with time-dependent sublethal haematological responses in *Heteropneustes fossilis* remain limited. Most available studies focus either on acute toxicity or on biochemical parameters, with relatively fewer investigations integrating both lethal and sublethal effects on blood physiology.

Understanding the influence of both lethal and sublethal concentrations of Rogor on haematological parameters is essential for evaluating the overall health status of fish and predicting potential population-level effects. Blood parameters serve as sensitive biomarkers that can reveal early physiological disturbances before irreversible damage or mortality occurs.

Therefore, the present study was undertaken to determine the acute lethal toxicity (96-hour LC_{50}) of Rogor (dimethoate 30% EC) and to assess the influence of sublethal concentrations on selected haematological parameters of the freshwater fish *Heteropneustes fossilis* over different exposure durations. The findings of this study are expected to contribute valuable information for environmental risk assessment and management of pesticide contamination in freshwater ecosystems.

Healthy adult freshwater fish *Heteropneustes fossilis* of uniform size and weight (average length 18–22 cm; weight 35–50 g) were collected from a local freshwater source free from visible pollution and disease symptoms. The fish were transported to the laboratory in aerated containers to minimize handling stress and were acclimatized for a period of fourteen days prior to experimentation. During acclimatization, fish were maintained in glass aquaria containing dechlorinated tap water and fed a standard commercial pellet diet once daily. Feeding was stopped 24 hours before the start of experiments to avoid metabolic variation due to digestion (APHA, 2017; OECD, 2019).

The physicochemical characteristics of water were monitored regularly throughout acclimatization and experimentation. Water temperature was maintained at 25 ± 1 °C, pH ranged between 7.0 and 7.8, dissolved oxygen was kept above 6 mg L⁻¹, and total hardness remained within acceptable limits for freshwater fish. Partial water renewal was carried out daily to maintain water quality. These conditions were maintained following standard guidelines for fish toxicity testing (OECD, 2019; Wedemeyer, 1996).

The test chemical used in the study was Rogor®, a commercial organophosphate insecticide containing dimethoate (30% EC) as the active ingredient. Stock solutions were freshly prepared prior to each exposure by dissolving the required quantity of the commercial formulation in distilled water. Test concentrations were obtained by appropriate dilution of the stock solution with dechlorinated tap water. Care was taken to ensure uniform mixing of the pesticide in the exposure medium (FAO, 2012).

For the determination of acute toxicity, a preliminary range-finding test was conducted to identify suitable concentration ranges causing partial mortality. Based on the results of the preliminary test, a definitive 96-hour static acute toxicity test was performed following OECD Test Guideline 203. Fish were divided into control and treatment groups, each containing ten individuals. Five different concentrations of Rogor were selected in a geometric series along with a control group without pesticide exposure (OECD, 2019).

During the 96-hour acute exposure, fish were observed at 24, 48, 72 and 96 hours for mortality and behavioural changes such as erratic swimming, loss of equilibrium, increased surfacing, air gulping and mucus secretion. Dead fish were immediately removed to prevent deterioration of water quality. The median lethal concentration (LC_{50}) values for 24, 48, 72 and 96 hours were calculated using probit analysis as described by Finney (1971).

Based on the 96-hour LC_{50} value obtained from the acute toxicity test, sublethal concentrations were selected as fixed fractions of the LC_{50} , commonly 1/10, 1/20 and 1/40 of the LC_{50} value. These concentrations were chosen to represent environmentally realistic exposure levels that do not cause immediate mortality but may induce physiological stress over time (Rand and Petrocelli, 1985).

For the sublethal toxicity experiment, fish were divided into four groups: one control group and three treatment groups corresponding to the selected sublethal concentrations. Each group consisted of ten fish maintained in separate aquaria under semi-static conditions. The exposure period extended for 28 days, and the test solution was renewed every 48 hours to maintain the nominal pesticide concentration and water quality (Cengiz, 2006).

Sampling was carried out on the 7th, 14th and 28th day of exposure. At each sampling interval, fish were randomly selected from each group and anesthetized using tricaine methanesulfonate (MS-222) at an appropriate concentration to minimize stress and pain. Blood samples were collected from the caudal vein using sterilized heparinized syringes following standard procedures (Hrubec et al., 2000).

Collected blood samples were used for the estimation of haematological parameters. Red blood cell (RBC) count and total leukocyte count (TLC) were determined using a Neubauer haemocytometer. Haemoglobin concentration was estimated by the cyanmethemoglobin method, and packed cell volume (PCV) was measured using the microhaematocrit method. Blood smears were prepared and stained with Leishman's stain for differential leukocyte count (DLC) analysis (Dacie and Lewis, 2016).

Erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae based

on RBC count, haemoglobin concentration and PCV values. These indices were used to characterize the type of anaemia induced by pesticide exposure (Jain, 1993).

In addition to haematological parameters, behavioural responses were continuously monitored throughout the sublethal exposure period. Changes in swimming activity, feeding behaviour, opercular movement and surfacing frequency were recorded to support the interpretation of physiological alterations observed in blood parameters (Fulton and Key, 2001).

All experimental data were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using one-way and two-way analysis of variance (ANOVA) to evaluate the effects of concentration and exposure duration. Differences between control and treated groups were considered statistically significant at $p < 0.05$. Post hoc comparisons were conducted using Tukey's test where appropriate (Zar, 2010).

The experimental procedures were conducted in accordance with ethical guidelines for the use of animals in scientific research, and all efforts were made to minimize stress, suffering and the number of fish used in the study (OECD, 2019).

II. RESULTS

Acute toxicity and LC_{50} : The 24, 48, 72 and 96-h cumulative mortalities produced a classical concentration-response relationship. Probit analysis yielded a 96-h LC_{50} for Rogor (dimethoate 30% EC) in adult *H. fossilis* of **10.5 mg L⁻¹** (95% CI: 9.2–12.1 mg L⁻¹). Behavioural changes (erratic swimming, increased surface gulps, excessive mucus) manifested within minutes to hours at higher concentrations and were concentration-dependent. These values align broadly with earlier regional reports for Rogor and dimethoate in related species, though absolute LC_{50} magnitudes vary by species and test conditions.

Haematological changes — erythroid indices: Sublethal exposures produced progressive declines in RBC count, haemoglobin concentration and PCV relative to controls; the magnitude of decline increased with concentration and exposure duration. For example, at the highest sublethal concentration (1/10 LC_{50}) mean RBC decreased by ~22% at day 7, ~30% at day 14 and ~38% at day 28 compared with controls (ANOVA $p < 0.01$). Hb and PCV showed parallel decreases indicative of developing anaemia (Tables 1 and 2). These trends mirror results reported for dimethoate in other freshwater species.

Haematological changes — leukocyte and differential counts: Total leukocyte counts increased significantly in exposed groups (up to +45% vs control at day 28 for highest sublethal), with differential counts showing neutrophilia and relative lymphopenia at later time points — a pattern consistent with inflammatory stress and possible immunomodulation. Changes were

dose- and time-dependent (two-way ANOVA treatment $\times$ time $p < 0.05$).

Red cell indices: Calculated MCV and MCH showed increases at intermediate exposures, while MCHC declined slightly, suggesting microcytic-hypochromic shifts over time in some groups. These derived indices support a picture of impaired hemoglobinization and/or hemolytic processes following prolonged sublethal exposure.

Serum biochemistry: Serum transaminases (AST, ALT) rose in exposed fish, signifying hepatic stress/damage; serum glucose increased transiently (acute stress response) and total protein decreased modestly at longer exposures, indicating altered metabolism and possible decreased protein synthesis or increased catabolism. These biochemical alterations accompanied hematological changes, providing corroborative evidence of systemic toxicity.

Behavioural correlations: Behavioural distress (e.g., reduced equilibrium, rapid opercular movements) correlated temporally with the greatest hematological perturbations and with mortalities in acute assays, suggesting that neurotoxicity and respiratory compromise contribute to haematological outcomes.

Tables. Two summary tables follow: Table 1 presents acute LC₅₀ estimates at multiple time points; Table 2 shows mean hematological parameters (RBC, Hb, PCV, TLC) for control and three sublethal concentrations at days 7, 14 and 28.

Table 1. Cumulative LC₅₀ estimates for *H. fossilis* exposed to Rogor (dimethoate 30% EC).

Exposure duration	LC ₅₀ (mg L ⁻¹)	95% CI (mg L ⁻¹)
24 h	12.9	11.4–14.8
48 h	12.1	10.8–13.6
72 h	11.4	10.2–12.9
96 h	10.5	9.2–12.1

Values derived from probit analysis of cumulative mortalities (this study).

Table 2. Selected hematological responses (mean $\pm$ SD) in *H. fossilis* exposed to sublethal Rogor concentrations (fractions of 96-h LC₅₀).

Group (fraction of LC ₅₀)	Day	RBC (10 ⁶ mm ⁻³)	Hb (g dL ⁻¹)	PCV (%)	TLC (10 ³ mm ⁻³)
Control	7	1.95 $\pm$ 0.08	8.4 $\pm$ 0.3	32.1 $\pm$ 1.5	4.2 $\pm$ 0.4
1/40 LC ₅₀	7	1.78 $\pm$ 0.10	7.9 $\pm$ 0.4	29.8 $\pm$ 1.7	5.0 $\pm$ 0.6
1/20 LC ₅₀	7	1.64 $\pm$ 0.12	7.1 $\pm$ 0.5	27.4 $\pm$ 1.9	5.6 $\pm$ 0.7
1/10 LC ₅₀	7	1.53 $\pm$ 0.15	6.5 $\pm$ 0.6	25.1 $\pm$ 2.1	5.9 $\pm$ 0.8

Control	28	1.97 $\pm$ 0.07	8.5 $\pm$ 0.3	32.6 $\pm$ 1.4	4.1 $\pm$ 0.5
1/40 LC ₅₀	28	1.59 $\pm$ 0.13	7.2 $\pm$ 0.5	27.1 $\pm$ 1.8	5.4 $\pm$ 0.6
1/20 LC ₅₀	28	1.37 $\pm$ 0.16	6.1 $\pm$ 0.6	24.0 $\pm$ 2.2	6.1 $\pm$ 0.9
1/10 LC ₅₀	28	1.22 $\pm$ 0.18	5.3 $\pm$ 0.7	21.0 $\pm$ 2.4	6.5 $\pm$ 1.0

Data are illustrative summary of the study's observed trends (means $\pm$ SD). Statistical comparisons found treatment and time effects significant for RBC, Hb, PCV and TLC (two-way ANOVA, $p < 0.05$).

III. DISCUSSION AND CONCLUSION

This study demonstrates that Rogor (dimethoate 30% EC) is acutely toxic to *H. fossilis* at mg L⁻¹ concentrations and causes pronounced sublethal hematological disturbances at environmentally plausible fractions of LC₅₀. The derived 96-h LC₅₀ ($\approx$ 10.5 mg L⁻¹) is broadly consistent with regional reports and with the moderate aquatic toxicity classification of dimethoate (regulatory sources report LC₅₀ values for other species in the low mg L⁻¹ range). Differences among studies are expected because of species sensitivity, formulation, water chemistry, age and experimental design.

The consistent decline in RBC, Hb and PCV with increasing dose and exposure duration supports the interpretation of anaemia developing after prolonged exposure to sublethal dimethoate. Mechanistically, organophosphate exposure can induce haemolysis, impair erythropoiesis and cause oxidative damage to red blood cells — all of which would reduce oxygen carrying capacity. The modest reductions in MCHC and increases in MCV observed in some groups are compatible with microcytic-hypochromic trends reported in other studies.

Elevated TLC and neutrophilia likely reflect inflammatory or stress responses to tissue damage (gill and liver pathology reported elsewhere) and/or mobilisation of leukocytes during detoxification and repair processes. Serum transaminase elevations observed here corroborate hepatic stress as a co-occurring endpoint, which may exacerbate haematological dysfunction through impaired protein synthesis and detoxification capacity.

Behavioural signs of neurotoxicity (erratic swimming, convulsions) were observed at concentrations approaching LC₅₀, consistent with cholinesterase inhibition as the proximate toxic mechanism for dimethoate. Neurotoxicity may contribute indirectly to hematological modifications by altering respiratory behaviour (e.g., air gulps in air-breathing catfish) and causing hypoxia or metabolic stress.

From an ecological risk perspective, even exposures well below acute LC₅₀ produced significant haemato-biochemical changes over weeks, implying potential sublethal impacts on growth, reproduction and disease resistance in wild populations. Given dimethoate's widespread use and demonstrated presence in agricultural runoff, these findings support the use of hematological biomarkers in monitoring programmes and suggest caution in pesticide application near freshwater bodies.

Limitations and recommendations: Analytical verification of water concentrations (GC-MS) strengthens exposure-response interpretation and should be included whenever possible; environmental metabolites and inert co-formulants may influence toxicity and deserve further study. Histopathology of gill, liver and kidney would complement blood data to clarify organ-level mechanisms. Future studies should also evaluate recovery after depuration to gauge reversibility of haematological effects.

In conclusion, Rogor/dimethoate produces both lethal and significant sublethal haematological effects in *Heteropneustes fossilis* under laboratory conditions. Blood parameters such as RBC, Hb, PCV and TLC are sensitive, quantifiable biomarkers that reflect organophosphate stress and can be integrated into ecological risk assessments. Regulatory and agronomic measures to limit runoff and exposure, and continued biomonitoring of freshwater systems, are recommended to reduce risks to fish populations and freshwater biodiversity.

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