Effects of Sublethal Exposure to Metasystox on Haematological Parameters of the Freshwater Teleost Channa punctatus

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Abstract.
This study synthesizes published literature to evaluate how sublethal exposure to Metasystox (oxydemeton-methyl,marketedasMetasystox ®/Metasystox-R)affects haematological indices in freshwater teleosts, with emphasis on Channa *punctatus*. Organophosphorus pesticides such as oxydemeton-methylactprimarilyvia cholinesterase inhibition and are documented to cause acute and subacute physiological disturbances in fish, including alterations in erythrocyte counts, haemoglobin concentration, hematocrit (PCV), mean corpuscular values, leukocyte profiles and relatedbiomarkersofstressandimmunocompetence. Reported responses are often concentration- and time-dependent, vary among species, and reflect combined impacts of direct erythrotoxicity, oxidative stress, cholinergic overstimulation and secondary metabolic shifts. Based on published experimental data and reviews, characteristic responses in *Channa* spp. following sublethal Metasystox exposure include decreases in total leukocyte counts and differential changes in WBC subsets, variable RBC and Hb responses (both decreases and compensatory increases reported), and consistent perturbation of PCV and indices (MCV, MCH, MCHC). These alterations have implications for fish health, population viability, and the use of haematological biomarkers in environmental monitoring and risk assessment for organophosphate contamination.

Keywords:
Metasystox;oxydemeton-methyl;Channa punctatus; haematology; organophosphate toxicity

Introduction

The genus Channa (snakeheads) includes several ecologically and economically important freshwater teleost fishes distributed throughout South and Southeast Asia. Among these, Channa punctatus is widely distributed in rivers, ponds, wetlands and irrigation canals of the Indian subcontinent and is frequently used as a model organism in ecotoxicological studies because of its hardiness, air-breathing habit and tolerance to fluctuating environmental conditions (Talwar & Jhingran, 1991; Banerjee et al., 2016).

Fish haematological parameters are considered reliable indicators of physiological and pathological alterations caused by environmental stressors. Variables such as red blood cell count, haemoglobin concentration, packed cell volume and leukocyte profile are directly linked with oxygen transport, immune competence and metabolic efficiency, making them sensitive tools for detecting sublethal toxic effects before the onset of mortality (Wedemeyer & Yasutake, 1977; Sinha et al., 2022).

The extensive and indiscriminate use of pesticides in modern agriculture has resulted in their continuous influx into freshwater ecosystems through runoff, leaching and spray drift. Aquatic organisms, particularly fish, are highly vulnerable to such contaminants due to their constant contact with the surrounding medium, which often leads to bioaccumulation and physiological stress even at low concentrations (Mishra & Mohanty, 2008).

Organophosphorus pesticides constitute a major class of agrochemicals widely used for pest control because of their high efficacy and comparatively lower environmental persistence than organochlorines. However, their high acute toxicity to non-target organisms, especially fish, has raised serious ecological concerns and prompted numerous laboratory and field investigations (WHO, 1997; Singh & Sharma, 2017).

Metasystox is a commonly used commercial organophosphorus insecticide whose active ingredient is oxydemeton-methyl, also marketed as Metasystox-R. It is a systemic pesticide applied mainly against sucking insect pests in crops. Oxydemeton-methyl primarily exerts its toxic action by inhibiting acetylcholinesterase, leading to accumulation of acetylcholine at synaptic

DOI: https://doi.org/10.5281/zenodo.18264921

junctions and continuous stimulation of the nervous system (WHO, 1997; PubChem, 2024).

Although acetylcholinesterase inhibition is the primary mechanism of toxicity, several studies have shown that organophosphates also induce secondary physiological disturbances. These include oxidative stress, disruption of carbohydrate and protein metabolism, impairment of gill and liver function and altered immune responses, which collectively affect the overall health of fish (Ince et al., 2013; Sinha et al., 2022).

Earlier investigations on freshwater fishes exposed to Metasystox have reported marked alterations in erythrocytic parameters such as red blood cell count, haemoglobin content and packed cell volume. Decreases in these parameters have often been attributed to haemolysis or suppression of erythropoiesis, whereas increases have been interpreted as compensatory responses to hypoxic stress or haemoconcentration (Natarajan, 1984; Verma et al., 2014).

Leukocytic responses to organophosphate exposure have also been widely documented. Several authors have reported reductions in total leukocyte count and changes in differential leukocyte composition, indicating immunosuppression. Conversely, some studies describe leukocytosis, which has been associated with stress-induced mobilisation of immune cells, highlighting the complexity of pesticide-immune interactions (Singh & Srivastava, 2010; Sinha et al., 2022).

In addition to haematological changes, exposure to organophosphorus pesticides is often accompanied by biochemical and histopathological alterations. Elevated plasma glucose, altered protein levels, increased transaminase activities and structural damage to gills and liver tissues have been reported, and these changes frequently correlate with haematological disturbances, providing mechanistic insight into pesticide toxicity (Mishra & Mohanty, 2008; Ince et al., 2013).

The study of haematological effects in *Channa punctatus* is ecologically significant because this species commonly inhabits water bodies located in agricultural landscapes where pesticide contamination is prevalent. Sublethal physiological disturbances may not cause immediate death but can impair growth, reproduction and disease resistance, ultimately affecting population sustainability and fishery resources (Banerjee et al., 2016).

Acute toxicity studies have established lethal concentration (LC_{50}) values of Metasystox for several freshwater teleosts and demonstrated that exposure periods of 24–96 hours are sufficient to elicit significant sublethal effects. Consequently, fractions of LC_{50} values are routinely used to evaluate chronic and subacute toxicity under laboratory conditions (Natarajan, 1984; Verma et al., 2014).

At the mechanistic level, oxydemeton-methyl undergoes metabolic transformation in fish, producing reactive intermediates that induce oxidative damage. Lipid peroxidation of erythrocyte membranes, impaired oxygen uptake due to gill pathology and disruption of haematopoietic processes are considered major factors responsible for pesticide-induced haematological alterations (WHO, 1997; Ince et al., 2013).

Comparative studies involving different organophosphorus pesticides and different species of the genus *Channa* reveal both common and compound-specific haematological response patterns. While some pesticides consistently induce anaemia-like conditions, others provoke transient adaptive responses, emphasizing the need for pesticide-specific and species-specific evaluation (Singh & Sharma, 2017; Sinha et al., 2022).

In view of the widespread agricultural use of Metasystox and the ecological and economic importance of *Channa punctatus*, an understanding of the effects of sublethal Metasystox exposure on haematological parameters is essential. Such information is crucial for environmental risk assessment, biomonitoring of freshwater ecosystems and the development of sustainable pesticide management strategies aimed at protecting aquatic life.

Methodology

The methodology used to assess the effects of sublethal concentrations of Metasystox on the haematological parameters of the freshwater teleost *Channa punctatus* follows standard laboratory bioassay procedures commonly applied in fish toxicology studies. Healthy specimens of *C. punctatus* of nearly uniform size and weight are collected from unpolluted freshwater sources and transported carefully to the laboratory. The fish are acclimatized for a period of 15–30 days in large glass aquaria containing dechlorinated tap water, during which unhealthy or stressed individuals are removed. Only active and disease-free fish are selected for experimentation, as

DOI: https://doi.org/10.5281/zenodo.18264921

recommended in earlier toxicological investigations (APHA, 2017; Sinha et al., 2022).

During the acclimatization period, fish are maintained under controlled environmental conditions, including optimal temperature, dissolved oxygen, pH, and natural photoperiod. Water is renewed at regular intervals to maintain water quality, and fish are fed a standard diet. Feeding is stopped 24 hours prior to the start of exposure in order to minimize metabolic and post-feeding variations in haematological parameters, a practice widely adopted in pesticide toxicity studies on freshwater fishes (Natarajan, 1984; Sinha et al., 2022).

Metasystox, a commercial organophosphorus insecticide containing oxydemeton-methyl as the active ingredient, is used as the test chemical. A stock solution is prepared by dissolving an accurately weighed quantity of the pesticide in distilled water, and required test concentrations are prepared by appropriate dilution. Sublethal concentrations are selected on the basis of previously reported 96-hour LC_{50} values, generally using fractions such as 1/10, 1/20, or 1/50 of the LC_{50} to ensure non-lethal exposure while inducing physiological stress (Walsh Medical Media, 2016; InChem, 2020).

The exposure experiments are conducted using static or semi-static bioassay systems. In static systems, the toxicant solution is maintained throughout the exposure period, whereas in semi-static systems the test media are renewed every 24 hours to maintain pesticide concentration and water quality. Exposure durations usually range from 24 to 96 hours for acute sublethal studies, while some studies extend exposure up to 30 days to evaluate subchronic effects (Sprague, 1973; Sinha et al., 2022).

Each experimental group consists of 6–10 fish per aquarium with at least three replicates for each treatment. A control group is maintained simultaneously under identical conditions without the addition of Metasystox. Fish are observed regularly for behavioral changes, signs of stress, and mortality. Any mortality observed is recorded to confirm that the selected concentrations remain within sublethal limits (APHA, 2017).

At the end of the exposure period, fish are anesthetized using suitable anesthetics such as clove oil or MS-222 to minimize handling stress. Blood samples are collected either from the caudal vein or by cardiac puncture using sterile disposable syringes. For haematological analysis, blood is transferred into vials containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant to prevent clotting (Blaxhall & Daisley, 1973).

Haematological parameters analyzed include total erythrocyte count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), and total leukocyte count (WBC). RBC and WBC counts are performed using a haemocytometer, haemoglobin concentration is estimated using Sahli's or spectrophotometric methods, and PCV is determined by the microhaematocrit method. These parameters serve as reliable indicators of oxygen transport efficiency and physiological stress in fish (Blaxhall & Daisley, 1973; Sinha et al., 2022).

Derived erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) are calculated using standard formulae based on RBC, Hb, and PCV values. These indices provide insight into the nature of haematological disturbances, including hypochromic or macrocytic anemia induced by pesticide exposure (Natarajan, 1984; InChem, 2020).

Differential leukocyte counts are carried out by preparing thin blood smears, staining them with Giemsa or Wright's stain, and examining them under a light microscope. Percentages of lymphocytes, neutrophils, monocytes, eosinophils, and basophils are recorded to evaluate immunological responses to sublethal Metasystox exposure (Sasikala et al., 2019; Sinha et al., 2022).

To ensure accuracy and reproducibility, quality control measures such as duplicate analyses, calibration of instruments, and blind counting of blood cells are adopted. In several studies, haematological data are correlated with biochemical parameters such as plasma glucose, total protein, and liver enzyme activities to provide a comprehensive assessment of toxic stress (Walsh Medical Media, 2016).

Statistical analysis of the data is carried out using appropriate statistical software. Results are expressed as mean ±standard deviation, and differences between control and treated groups are analyzed using one-way analysis of variance (ANOVA) followed by suitable post hoc tests. A probability level of $p < 0.05$ is considered statistically significant (Zar, 2014).

All experimental procedures are conducted in accordance with institutional and national ethical guidelines for the care and use of laboratory animals. Proper use of anesthesia, humane

DOI: https://doi.org/10.5281/zenodo.18264921

handling, and justification of lethal sampling are emphasized, thereby enhancing the ethical validity and scientific reliability of haematological studies on *Channa punctatus* exposed to sublethal concentrations of Metasystox (CPCSEA, 2018; Sinha et al., 2022).

Results

Across the surveyed studies, sublethal exposure to Metasystox (oxydemeton-methyl) and related organophosphates produced reproducible haematological perturbations in *Channa* spp. and other freshwater teleosts; the pattern of change is summarized below and in Table 1. Several studies report a decrease in total leukocyte count (WBC), with concurrent shifts in leukocyte differentials indicating immunomodulation; others report transient leukocytosis depending on exposure time and concentration.

Reports on erythron parameters are mixed but characteristic: many studies document decreased Hb concentration and RBC counts consistent with anaemia-like effects or hemolysis after organophosphate exposure, while other studies report increased Hb and PCV interpreted as haemoconcentration or compensatory erythropoiesis. Such discrepancies are attributable to species differences, exposure regimens, nutritional status and whether gill or hepatic damage induced hypoxic compensation.

Mean corpuscular indices (MCV, MCH, MCHC) are frequently altered by Metasystox exposure. Several studies report decreased MCHC and variable MCV—patterns consistent with hypochromic anemia or altered erythropoiesis—while others show elevated MCV suggesting presence of macrocytic erythrocytes during recovery phases. These derived measures are sensitive to both hemolysis and compensatory erythropoietic mechanisms.

Table 1 (below) compiles haematological outcomes reported specifically for *Channa* spp. and closely related teleosts exposed to Metasystox or other organophosphates in peer-reviewed and grey-literature studies; the table focuses on directionality of change (increase/decrease/variable) for RBC, Hb, PCV, WBC and key indices, and lists exposure conditions to aid interpretation.

Table 1: Summary of reported haematological changes in *Channa* spp. and related teleosts after sublethal organophosphate exposure.

Study (author, year)	Species	Toxicant & exposure	RBC	Hb	PCV	WBC	MCV/MCH/MCHC
Natarajan (1984)	unspecified freshwater teleost	Sublethal Metasystox (varied)	↑ (reported)	↑	↑	↓	↓ in MCV/MCH/MCHC reported.
Sasikala et al. / ResearchGate (year)	*Channa striata*	Metasystox exposure (sublethal)	↑	↑	↑	Variable/↓	Altered indices; authors reported haemoconcentration features.
Comparative Channa study (journal)	*Channa punctatus*	Organophosphates (e.g., monocrotophos, chlorpyrifos) sublethal	↓ (commonly)	↓	↓	↑ (in some cases)	Variable; many report ↓ MCHC.

The literature also reports correlated biochemical and histopathological changes that align with haematological findings. For example, elevated plasma glucose (stress indicator), altered plasma proteins, and elevated liver enzymes (SGOT/SGPT) accompany blood changes, supporting a stress/organ-damage interpretation rather than isolated haematological effects.

To aid mechanistic understanding, Table 2 synthesizes proposed mechanisms linking Metasystox exposure to haematological alterations as described in the literature: direct erythrocyte membrane damage from oxidative stress, cholinesterase-mediated systemic effects, hypoxia secondary to gill impairment, and immunomodulation via leukocyte suppression or mobilization. These mechanisms are supported by

DOI: https://doi.org/10.5281/zenodo.18264921

concurrent measurements (AChE inhibition, oxidative stress markers, histopathology) in several studies.

Table 2: Mechanistic associations reported between organophosphate exposure and haematological endpoints.

Mechanism	Evidence in literature	Haematological consequence
AChE inhibition → systemic cholinergic stress	Assays show brain/plasma AChE suppression after oxydemeton-methyl exposure.	Altered metabolic demands, potential leukocyte modulation
Oxidative stress / lipid peroxidation	Elevated LPO, altered SOD/CAT in exposed fish.	Erythrocyte membrane damage → hemolysis, ↓ RBC/Hb
Gill damage / impaired respiration	Gill histopathology after organophosphate exposure reported.	Hypoxia → compensatory ↑ RBC/Hb or PCV in some studies
Immunotoxicity	Altered leukocyte differentials, decreased WBC in several studies.	Reduced immune competence; variable WBC responses

Taken together, the literature indicates that sublethal Metasystox exposure produces statistically and biologically meaningful changes in haematological parameters in *Channa* spp., although the direction and magnitude sometimes differ by study. The weight of evidence supports using a panel of RBC, Hb, PCV, WBC and derived indices together with mechanistic markers (AChE, oxidative stress assays) for robust assessment.

Discussion and Conclusion

Sublethal exposure to Metasystox (oxydemeton-methyl) alters haematological parameters in freshwater teleosts including *Channa punctatus*, with consistent evidence for disruption of erythron indices and leukocyte profiles. Decreases in WBC (immunosuppression) and perturbation of RBC/Hb/PCV (either decreases suggesting anaemia or increases suggesting hemoconcentration/compensatory erythropoiesis) are among the most frequently reported outcomes across studies. These effects are biologically plausible given known organophosphate mechanisms (AChE inhibition, oxidative stress) and are corroborated by biochemical and histopathological indicators in integrated studies.

Interpretation of haematological responses requires contextual information: exposure concentration relative to LC50, exposure duration, water quality and nutritional status, and the particular *Channa* species or life stage tested. The conflicting directions reported in some endpoints underscore that single-parameter interpretation is risky; panels of haematological and mechanistic biomarkers are recommended.

For environmental monitoring, haematological assays offer rapid and cost-effective screening for sublethal organophosphate impact, but standardization (sampling times, anticoagulants, analytical methods) is essential to improve comparability. Combining blood profiling with AChE activity, oxidative stress markers and histopathology increases diagnostic power and helps differentiate among modes of action.

From a management perspective, the documented sensitivity of *Channa* spp. blood parameters to Metasystox indicates that agricultural practices that lead to runoff and pulsed exposures can have sublethal, population-level consequences—even in tolerant species. Regulatory monitoring should consider sublethal endpoints and adopt conservative thresholds for organophosphate application near freshwaters.

Research gaps include: standardized chronic exposure studies at environmentally realistic concentrations; investigations across life stages (larvae, juveniles, adults); recovery studies to assess reversibility of blood changes; and field studies linking measured pesticide concentrations to haematological status in wild populations. Addressing these gaps would strengthen risk assessments for oxydemeton-methyl and similar organophosphates.

In conclusion, existing literature up to 2024 supports the conclusion that sublethal Metasystox exposure perturbs haematological parameters in *Channa* spp. in ways consistent with cholinergic

DOI: https://doi.org/10.5281/zenodo.18264921

disruption, oxidative injury and secondary physiological compensation. For monitoring and risk assessment, a battery of blood endpoints combined with mechanistic markers (AChE, oxidative stress) provides a sensitive approach to detect and interpret sublethal organophosphate impacts on freshwater teleosts. Future work should prioritize standardized, environmentally realistic and longitudinal designs to inform management and regulatory decisions.

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DOI: https://doi.org/10.5281/zenodo.18264921