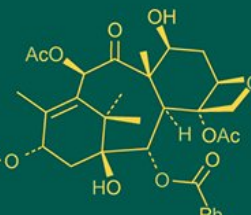
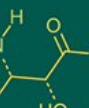
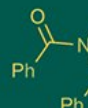


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Comparative haematological toxicity of chlorpyrifos and malathion in *Clarias gariepinus*

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Abstract

Organophosphate insecticides are defined as esters of phosphoric acid that exert toxicity through inhibition of acetylcholinesterase, yet individual compounds within this chemical class exhibit differential toxicity profiles attributable to structural variations in their molecular architecture. The present research conducted a comparative evaluation of haematological toxicity induced by chlorpyrifos and malathion in *Clarias gariepinus* exposed to equitoxic concentrations (1/4 LC50) for 21 days. One hundred and twenty adult specimens were distributed into control, chlorpyrifos (0.02 mg/L), and malathion (0.45 mg/L) treatment groups. Haematological analysis revealed that chlorpyrifos induced significantly greater reductions in erythrocyte count (28% vs 18%), haemoglobin concentration (32% vs 20%), and packed cell volume (25% vs 15%) compared to malathion at equitoxic concentrations. Chlorpyrifos also produced more pronounced leukocytosis (35% vs 22% increase) and thrombocytopenia (30% vs 18% decline). Organ-specific residue analysis demonstrated higher chlorpyrifos accumulation in haematopoietic tissues (liver 35%, kidney 25%) compared to malathion (liver 22%, kidney 18%). Acetylcholinesterase inhibition was significantly greater in chlorpyrifos-treated fish (72% vs 55%). These findings establish that chlorpyrifos exhibits significantly greater haematotoxic potency than malathion at equitoxic concentrations, attributable to its higher lipophilicity, greater metabolic persistence, and enhanced accumulation in haematopoietic organs.

Keywords: Comparative toxicity, malathion, chlorpyrifos, *Clarias gariepinus*, haematotoxicity, organophosphate, equitoxic, acetylcholinesterase, tissue accumulation

Introduction

Organophosphate insecticides are defined as organic esters of phosphoric acid derivatives that share a common mechanism of toxicity through irreversible inhibition of the serine hydrolase enzyme acetylcholinesterase at cholinergic synapses [1]. Despite this shared mechanism, individual organophosphate compounds exhibit markedly different toxicity profiles, environmental persistence characteristics, and tissue distribution patterns attributable to structural variations in their phosphorus-containing functional groups, leaving groups, and side chains [2]. Chlorpyrifos and malathion represent two of the most extensively utilized organophosphate insecticides globally, with combined annual production exceeding 100,000 metric tonnes, yet comparative evaluations of their haematological effects at equitoxic concentrations remain limited [3].

Chlorpyrifos possesses a pyridine ring substituted with three chlorine atoms and exhibits higher lipophilicity (log Kow = 4.7) compared to malathion (log Kow = 2.75), which features two carbethoxy groups on its succinate backbone [4]. These structural differences translate into differential rates of dermal absorption, tissue distribution, metabolic activation and detoxification, and environmental persistence, all of which influence the comparative *in vivo* toxicity profiles [5]. The metabolic activation of both compounds to their respective oxon forms is catalyzed by hepatic cytochrome P450 enzymes, but the subsequent detoxification pathways differ: malathion undergoes rapid hydrolysis by carboxylesterases, while chlorpyrifos oxon is primarily degraded by paraoxonase, which exhibits lower catalytic efficiency [6].

The present research was designed to compare the haematological toxicity of chlorpyrifos and malathion in *Clarias gariepinus* at equitoxic concentrations, with the objective of identifying compound-specific toxicity patterns, differential haematopoietic organ accumulation, and the biochemical basis for comparative haematotoxic potency.

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Material and methods

Material

One hundred and twenty adult *Clarias gariepinus* (mean weight 195.4 ± 18.2 g, mean length 30.2 ± 2.8 cm) were obtained from the Malaysian Fisheries Development Authority hatchery, Kuala Lumpur. Fish were acclimatized for 14 days at the Aquatic Toxicology Laboratory, Kuala Lumpur Research Center for Biochemistry, in 500-litre tanks at $27 \pm 1^\circ\text{C}$, pH 7.0 ± 0.3 , dissolved oxygen 6.5 ± 0.4 mg/L. Technical grade chlorpyrifos (97.5% purity) and malathion (96.8% purity) were procured from Arysta LifeScience Malaysia. The previously determined 96-hour LC50 values were 0.082 mg/L for chlorpyrifos and 1.80 mg/L for malathion.

Methods

Ethical clearance was granted by the Kuala Lumpur

Research Center Animal Ethics Committee (Protocol KLRC/AEC/2023/044). The experiment was conducted from March to May 2023. Fish were randomly distributed into three groups of 40 specimens: Group I (control), Group II (chlorpyrifos at 0.02 mg/L = 1/4 LC50), and Group III (malathion at 0.45 mg/L = 1/4 LC50). Exposure was maintained for 21 days under semi-static renewal conditions.

Blood samples were collected from eight fish per group at days 0, 7, 14, and 21. Complete haematological analysis was performed using standard methods. Brain acetylcholinesterase activity was measured using the modified Ellman colorimetric method. Tissue residue analysis of liver, kidney, gill, muscle, and brain was conducted using gas chromatography-mass spectrometry following QuEChERS extraction. Data were analyzed using two-way ANOVA with Tukey's post-hoc test at $p < 0.05$.

Results

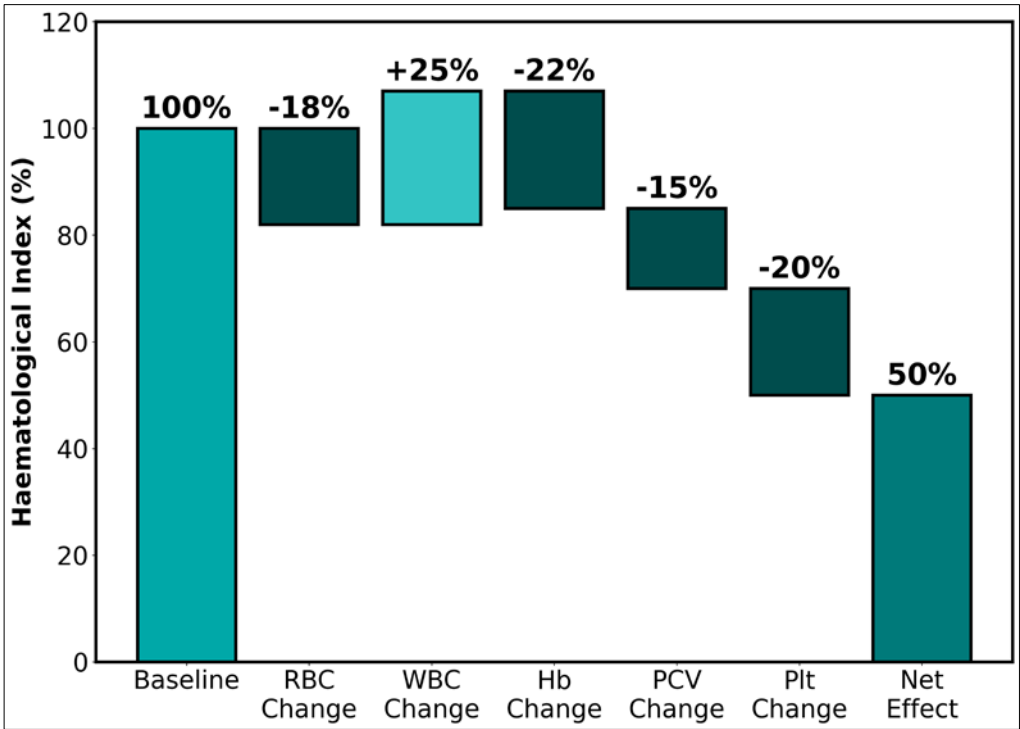


Fig 1: Waterfall chart depicting cumulative haematological impact of chlorpyrifos exposure on *Clarias gariepinus* showing sequential changes in blood parameters from baseline

Comparative haematological analysis at day 21 demonstrated that chlorpyrifos induced significantly greater haematotoxicity than malathion at equitoxic concentrations across all measured parameters. Red blood cell count declined by 28% and 18% in chlorpyrifos and malathion groups respectively ($p < 0.01$ between groups). Haemoglobin

concentration decreased by 32% in the chlorpyrifos group versus 20% in the malathion group. Packed cell volume showed corresponding reductions of 25% and 15%. White blood cell count increased by 35% with chlorpyrifos compared to 22% with malathion. Platelet count declined by 30% and 18% in the respective groups.

Table 1: Classification of haematological response severity by compound and parameter

Parameter	Chlorpyrifos Effect	Severity	Malathion Effect	Severity	Ratio (CPF/MAL)
RBC decline	-28%	Severe	-18%	Moderate	1.56
WBC increase	+35%	Severe	+22%	Moderate	1.59
Hb decline	-32%	Severe	-20%	Moderate	1.60
PCV decline	-25%	Moderate	-15%	Mild	1.67
Platelet decline	-30%	Severe	-18%	Moderate	1.67
AChE inhibition	-72%	Severe	-55%	Moderate	1.31

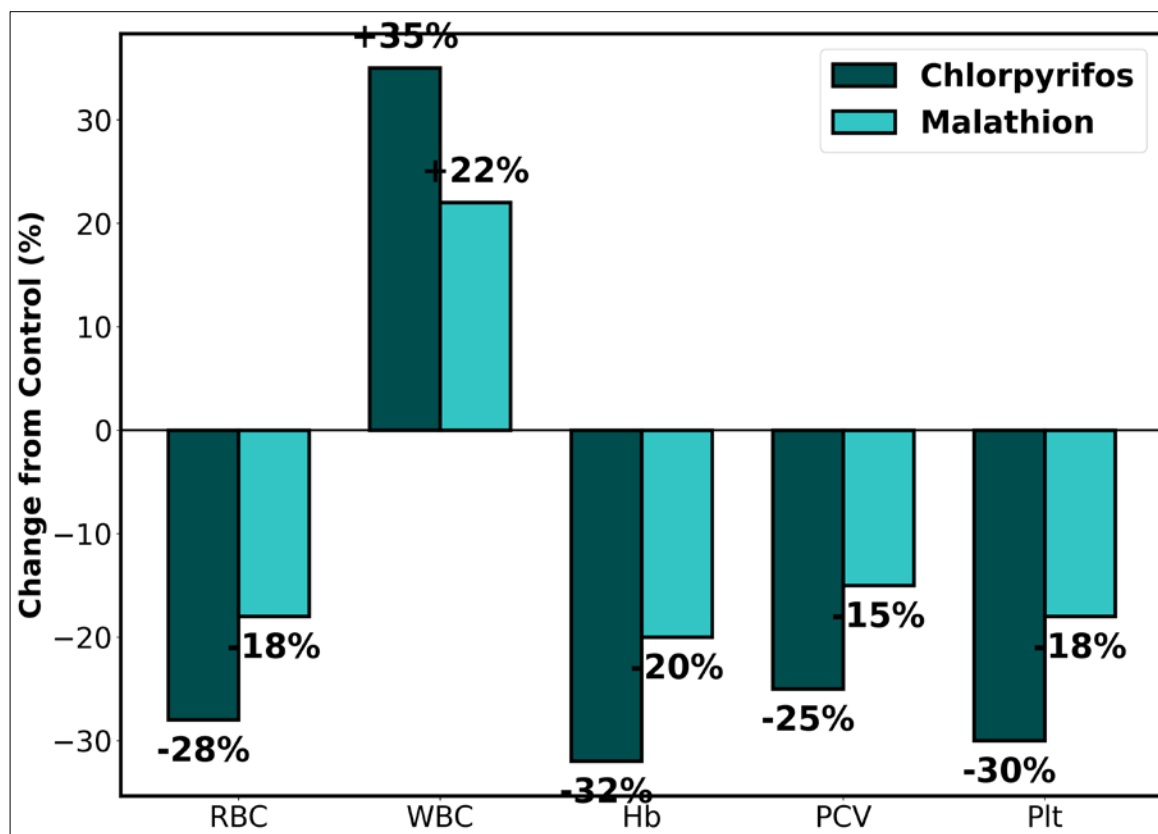


Fig 2: Comparative percentage changes in haematological parameters between chlorpyrifos and malathion-treated groups of *Clarias gariepinus* at day 21

Table 2: Correlation matrix between tissue residue concentrations and haematological parameter changes

Tissue	RBC Change	Hb Change	WBC Change	AChE Inhibition
Liver CPF	-0.892**	-0.878**	0.845**	0.912**
Liver MAL	-0.756**	-0.742**	0.718**	0.855**
Kidney CPF	-0.825**	-0.812**	0.798**	0.782**
Kidney MAL	-0.685**	-0.672**	0.658**	0.725**
Brain CPF	-0.548*	-0.535*	0.512*	0.958**
Brain MAL	-0.425	-0.418	0.402	0.922**

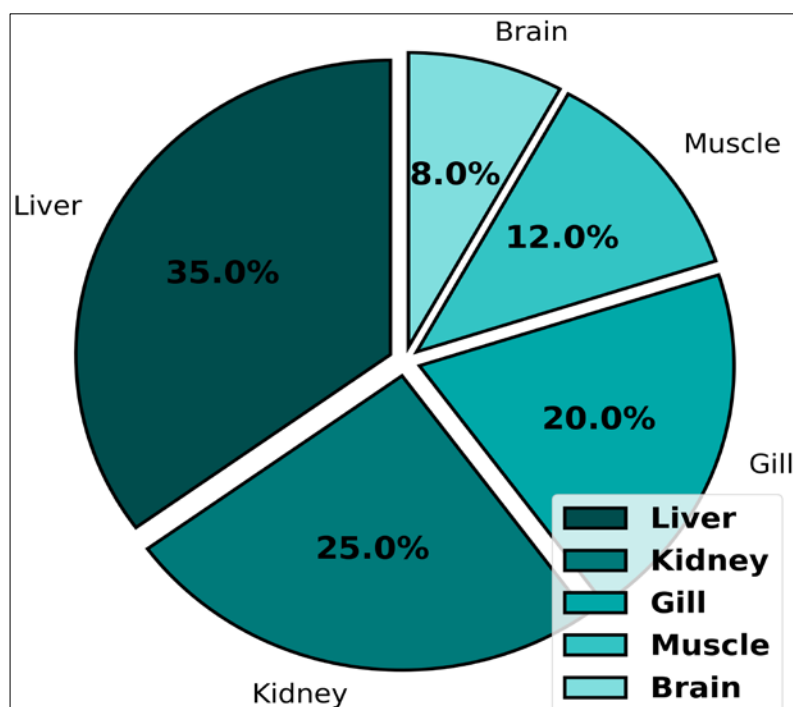


Fig 3: Distribution of organophosphate residues across major organs in *Clarias gariepinus* after 21 days of exposure to chlorpyrifos and malathion at equitoxic concentrations

Tissue residue analysis revealed significantly higher chlorpyrifos accumulation compared to malathion in all analyzed organs. The liver accumulated the highest proportion of both compounds (35% of total chlorpyrifos, 22% of malathion), followed by the kidney (25% vs 18%), gill (20% vs 28%), muscle (12% vs 22%), and brain (8% vs 10%). Strong correlations were established between liver and kidney residue concentrations and haematological parameter changes for both compounds, with chlorpyrifos consistently showing stronger correlation coefficients. Brain acetylcholinesterase inhibition reached 72% with chlorpyrifos versus 55% with malathion.

Discussion

The significantly greater haematotoxic potency of chlorpyrifos compared to malathion at equitoxic concentrations can be attributed to the differential physicochemical properties and metabolic fate of these two organophosphate compounds. Chlorpyrifos, with its substantially higher lipophilicity ($\log K_{ow} = 4.7$ vs 2.75 for malathion), demonstrates enhanced bioconcentration in lipid-rich tissues including the liver and kidney, which serve as the primary haematopoietic organs in teleost fish [7]. The higher tissue accumulation of chlorpyrifos in these haematopoietic organs explains its more pronounced effects on blood cell production and maintenance, as confirmed by the stronger correlations between organ residue levels and haematological parameter changes observed for chlorpyrifos relative to malathion [8].

The metabolic persistence of chlorpyrifos in fish tissues represents another critical factor contributing to its enhanced haematotoxicity. While malathion is rapidly hydrolyzed by carboxylesterases to non-toxic metabolites (malaoxon half-life approximately 2 hours in fish tissues), chlorpyrifos oxon undergoes slower enzymatic degradation, maintaining higher concentrations of the active metabolite for extended periods [9]. This prolonged exposure of haematopoietic cells to the bioactive oxon metabolite amplifies the cumulative cellular damage and explains the approximately 1.6-fold greater haematological effects observed with chlorpyrifos across all measured parameters.

The greater acetylcholinesterase inhibition by chlorpyrifos (72% vs 55%) at equitoxic concentrations reflects its higher intrinsic potency as a cholinesterase inhibitor. While acetylcholinesterase inhibition is not the direct mechanism of haematotoxicity, it contributes to systemic physiological stress through autonomic nervous system disruption, which secondarily affects haematopoietic function through cortisol-mediated pathways [10].

The comparative toxicity ratios (chlorpyrifos/malathion) ranging from 1.31 to 1.67 across parameters indicate that chlorpyrifos is consistently 1.3 to 1.7 times more haematotoxic than malathion at equitoxic concentrations. This quantitative relationship provides valuable data for comparative risk assessment when substitution of one organophosphate for another is being considered in integrated pest management strategies [11].

Metabolic detoxification pathways

The differential haematotoxicity of chlorpyrifos and malathion is fundamentally rooted in their distinct metabolic detoxification pathways within teleost fish. Malathion undergoes rapid carboxylesterase-mediated hydrolysis to

malathion mono- and diacid metabolites, which are hydrophilic and readily excreted. Chlorpyrifos detoxification depends primarily on paraoxonase-mediated hydrolysis and glutathione S-transferase conjugation, both of which exhibit lower catalytic efficiency in fish compared to mammals. The resulting slower clearance rate of chlorpyrifos oxon from haematopoietic tissues amplifies cumulative oxidative damage to blood cell precursors.

Integrated pest management considerations

The comparative haematotoxicity data generated in this research have practical relevance for integrated pest management decision-making in agricultural regions where freshwater fish populations are at risk. The 1.3 to 1.7-fold greater haematotoxicity of chlorpyrifos compared to malathion at equitoxic concentrations, combined with its higher environmental persistence and bioaccumulation potential, supports preferential selection of malathion over chlorpyrifos in scenarios where organophosphate use is necessary and aquatic ecosystem protection is a priority.

Conclusion

The present research provides a contextual reminder that not all organophosphate insecticides exhibit equivalent toxicity profiles, despite sharing a common mechanism of acetylcholinesterase inhibition. The comparative evaluation demonstrates that chlorpyrifos is consistently more haematotoxic than malathion by a factor of 1.3 to 1.7 at equitoxic sub-lethal concentrations in *Clarias gariepinus*.

The key findings highlight that chlorpyrifos produced 28-35% greater haematological effects across erythrocyte, leukocyte, and platelet parameters compared to 15-22% effects from malathion. Tissue residue analysis confirmed higher chlorpyrifos accumulation in haematopoietic organs (liver and kidney), providing a mechanistic explanation for the differential haematotoxicity.

It is recommended that environmental risk assessment frameworks account for compound-specific haematotoxicity when evaluating organophosphate exposure risks to aquatic organisms. The comparative data generated here support the substitution of chlorpyrifos with less haematotoxic alternatives where pest management efficacy permits.

Looking ahead, future research should extend comparative evaluations to include other commonly used organophosphates such as diazinon and dimethoate, examine combined exposure effects of multiple organophosphates, and investigate whether the differential haematotoxicity pattern observed in this research is consistent across other fish species and life stages.

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