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Physiological and Biochemical Effects of Insecticides on Catla catla from Freshwater Ecosystems: A Biomarker-Based Monitoring System for Yeldari Reservoir, Maharashtra

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Abstract: *Agricultural insecticides reach freshwater reservoirs through runoff, drainage, leaching and accidental discharge, and fish are exposed continuously through gills, skin and diet. Catla catla (Hamilton), an Indian major carp of high economic and ecological value, is a suitable sentinel species for such contamination. This paper reviews the physiological, haematological, biochemical, oxidative and histopathological responses of C. catla to organophosphate, organochlorine, pyrethroid and neonicotinoid insecticides, and identifies the gaps that limit their use in field risk assessment. Building on this evidence, we present a methodology and a proposed six-module system for Yeldari Reservoir, a hydroelectric and irrigation reservoir on the Purna River, that links catchment pesticide-use surveys, seasonal field monitoring, laboratory bioassays, a multi-biomarker panel, integrated data analysis (Integrated Biomarker Response and risk quotient) and decision support. The approach is intended to show whether agricultural insecticides cause measurable physiological stress in the reservoir's C. catla population, and to convert that evidence into management advice.*

Keywords: *Catla catla; insecticide toxicity; acetylcholinesterase; oxidative stress; biomarkers; Yeldari Reservoir; Integrated Biomarker Response; freshwater monitoring.*

I. INTRODUCTION

Freshwater reservoirs support irrigation, drinking-water supply, hydropower and capture or culture fisheries, yet they also act as sinks for agricultural chemicals from their catchments. Reservoirs already alter flow, sediment transport and habitat connectivity [13]; pesticide inputs add a chemical stressor on top of these physical changes. Insecticides are designed to act on the nervous systems of insects, but the same targets, such as cholinesterase and voltage-gated sodium channels, are conserved in fish, so non-target toxicity is expected even at low concentrations [4,5]. Catla catla is a plankton-feeding, surface-fishing carp, and it is popularly farmed and fished throughout India. It is a practical bioindicator, as it is a part of the food chain and its commercial importance and available published baseline data. There are already sub-lethal studies available for the following pesticides: deltamethrin [1], cypermethrin [8,10], endosulfan [2], methyl parathion [9], ethion [6] and acetamiprid and thiamethoxam (neonicotinoids) [3]. Most of these are single-compound testing at a single concentration, however, few relate laboratory response to a water body in real use where multiple exposure routes and seasons are involved.

The Yeldari reservoir is in an intensively cultivated catchment and our previous survey of water at the reservoir site indicated physico-chemical parameters were within WHO and BIS limits, but that we were unable to detect the agricultural run-off as a threat because it is not a routine parameter [12]. Hence, the aim of this paper is to (i) review the physiological and biochemical effects of the insecticides used on C. catla; (ii) establish a methodology appropriate to the Yeldari environment; and (iii) put forth an integrated monitoring system that correlates exposure to biological effect.

II. INSECTICIDE ENTRY INTO RESERVOIRS AND THE YELDARI SETTING

Reservoir input includes insecticides from storm-water drainage from farmland, irrigation return flow, soil erosion, deposition and drift from the air, accidental spills and re-suspension of contaminated sediment. A compound can persist, adsorb to sediments, be metabolized, or accumulate in biota once in the water, and persistence and toxicity are a function of the chemical structure, temperature, pH, organic carbon and light [4].

Hydrophobic compounds like pyrethroids and organochlorines will partition quickly to sediment and suspended particles and thus exposure can be higher than represented by water sampling because of benthic and bottom-feeding exposure, while polar neonicotinoids will remain dissolved and will readily be transported along with runoff. Chronic or pulse exposures can thus be received at adjoining reservoirs that receive multiple applications from land.

Yeldari Reservoir constructed on the Purna river is located in the Parbhani–Hingoli area of Marathwada to provide irrigation, drinking water, hydropower and fishing facilities. The water body is well oxygenated, slightly alkaline and low turbidity as reflected in our baseline measurements in 2025 (Table 1) [12]. For toxicology, these values are important because the persistence of the insecticide and the sensitivity of the fish is related to pH, temperature, and dissolved oxygen (Section 5).

Table 1. Baseline physico-chemical range of Yeldari Reservoir water, January–June 2025 [12]

Parameter	Observed range	WHO/BIS limit	Relevance to fish toxicology
Temperature	24–30 °C	Natural	Raises uptake and metabolic rate
pH	7.0–8.2	6.5–8.5	Affects hydrolysis of OP esters
Dissolved oxygen	5.5–7.5 mg/L	> 5 mg/L	Low DO worsens respiratory stress
Turbidity	2–5 NTU	< 10 NTU	Suspended solids bind hydrophobic insecticides
TDS / hardness	150–300 / 120–180 mg/L	< 500 / < 300 mg/L	Ca ²⁺ can reduce gill uptake

III. INSECTICIDE CLASSES AND REPORTED EFFECTS – CATLA CATLA

The following table (Table 2) summarises information on each of the major classes. Acute (96 h) LC₅₀ values vary over several orders of magnitude for *C. catla* ranging from approximately 1.24 µg/L for cypermethrin to 4.8 mg/L for methyl parathion [5] indicating a much greater toxicity of pyrethroids than the older organophosphates on a mass basis.

Table 2. Insecticide classes, primary targets and reported responses in *Catla catla*

Class (example)	Primary target	Reported responses in <i>C. catla</i>
Organophosphate (methyl parathion, ethion)	Acetylcholinesterase (AChE)	AChE inhibition after ethion exposure [6]; altered Hb, Hct, RBC, WBC, plasma protein and glucose after methyl parathion [9]
Organochlorine (endosulfan)	GABA-gated chloride channel	Isomer- and formulation-dependent toxicity; metabolites detected in liver, kidney and muscle [2]
Pyrethroid (cypermethrin, deltamethrin)	Voltage-gated Na ⁺ channel	60-day sub-lethal cypermethrin lowered erythrocyte and leucocyte counts, Hb and Hct, raised liver ALT and AST, and inhibited brain AChE, muscle LDH and gill ATPases [8]; hepatic and renal lesions at 0.12–0.41 µg/L [10]; deltamethrin altered blood and biochemical indices, partly reversed by vitamin C [1]; chronic low-dose cypermethrin changed brain and liver gene expression [11]
Neonicotinoid (acetamiprid, thiamethoxam)	Nicotinic ACh receptor	Single and joint exposure altered biochemical indices and antioxidant enzyme activities [3]

IV. PHYSIOLOGICAL, BIOCHEMICAL AND TISSUE-LEVEL EFFECTS

A. Behaviour, respiration and growth

The first signs of insecticide stress are often apparent; swimming may become erratic or hyperactive, may lose balance, rise up to the surface, stop eating, and may exhibit unusual movements; this is indicative of neurological stress, respiration stress, or altered energy metabolism. Since gills are the primary site of uptake, structural damage to the gills increases opercular beat frequency and decreases the amount of oxygen exchanged [5]. Sub-lethal exposure decreases growth and reproductive resources, which may result in decreased food consumption and growth [4].

B. The haematological and biochemical responses are as follows:Haematological and biochemical responses:

The following parameters are commonly employed as signs of physiological stress: haemoglobin, RBC count, haematocrit, white blood cell count and the red-cell indices (MCV, MCH, MCHC). Sub-lethal cypermethrin toxicity towards *C. catla* resulted in a decrease in the levels of erythrocyte and leucocyte, haemoglobin, haematocrit, and serum protein, albumin and globulin [8]. The same study showed increased ALT and AST levels which were a positive indicator of liver injury and reduced muscle LDH, brain AChE and gill Na⁺/K⁺-ATPase levels which was a negative indicator of disturbed ion regulation [8]. A change in plasma glucose and protein following exposure to methyl parathion suggests mobilization of carbohydrate and protein stores to satisfy the energy demands of stress [9]. Reduced haemoglobin can also be caused by the occurrence of ROS-induced damage to red cells, thereby connecting haematological and oxidative endpoints.

C. Neurotoxicity: acetylcholinesterase

AChE hydrolyses acetylcholine at cholinergic synapses. Organophosphates and carbamates inhibit it, causing acetylcholine to accumulate and producing tremor, incoordination and respiratory failure. AChE activity in brain or muscle is therefore the standard biomarker of exposure to these classes and has been measured in *C. catla* for ethion [6]. Brain AChE is also depressed by pyrethroids in this species [8], so inhibition should be interpreted alongside residue data rather than assigned to a single chemical class.

D. Oxidative stress and antioxidant defence

Reactive oxygen species (ROS) are produced during insecticide metabolism. If ROS levels are higher than antioxidant capacity, lipids in the membranes will become peroxidised (as measured by malondialdehyde, MDA), proteins will be oxidised, and DNA may be damaged. The Glutathione (GSH), Glutathione-S-Transferase (GST), Fish Counter ROS (Superoxide Dismutase (SOD): catalyzes the conversion of superoxide to hydrogen peroxide), Catalase (CAT) and Glutathione Peroxidase (GPx: removes hydrogen peroxide) are also included. This is because activities tend to increase in a moderate stress and decrease in a high stress situation, when the damage is now larger than the repair capacity, and all the way down is informative. These enzymes were found to be affected by exposure to neonicotinoids in *C. catla* [3] and oxidative and enzymatic markers were recommended for organophosphate monitoring in freshwater fish [7].

E. Histopathology, bioaccumulation and molecular responses.

The primary target organs are gills, liver, kidney and brain. Lesions reported are lifting of the epithelium, fusion of the lamellae, hyperplasia and necrosis of the gills, hepatocyte degeneration, vacuolation, and sinusoidal change in liver, tubular degeneration and glomerular change in kidney [5,10]. Residue studies indicate that the liver, kidney and muscle are target organs for the insecticide residues, with muscle being a storage tissue for endosulfan residues [2] as relevant for consumer exposure. Long-term exposure to low dose cypermethrin changed the transcriptomes of *C. catla* in the brain and liver and suggested some early-warning genes (fosab, nr4a1, tm7sf2, dhcr24) at the molecular level [11]. These genes haven't been field-tested.

V. ADAPTING THE FACTORS AND THE SELECTION OF BIOMARKERS

The toxicity is related to concentration, exposure time, water temperature, pH, dissolved oxygen, fish size, age, nutrition and chemical formulation and persistence and presence of other contaminants. Laboratory values should thus only be interpreted in the light of actual field conditions. This complexity cannot be adequately described by just one marker but can be assessed by combining neurological, oxidative, haematological, metabolic and structural markers, which provide a more reliable picture than mortality or any single parameter [5]. The panel used in this study and analytical method is shown in Table 3.

Table 3. Proposed biomarker panel, tissue and analytical method

Biomarker	Tissue / sample	Indicates	Method
AChE	Brain, muscle	Neurotoxicity	Ellman colorimetric assay [17]
SOD, CAT, GPx	Liver, gill	Antioxidant response	Standard spectrophotometric assays [19,20]
GST, GSH	Liver, gill	Detoxification	CDNB conjugation [21]; DTNB for GSH
MDA (TBARS)	Liver, gill	Lipid peroxidation	Thiobarbituric acid reaction [18]

Biomarker	Tissue / sample	Indicates	Method
Hb, RBC, WBC, Hct, MCV, MCH, MCHC	Whole blood	Haematological stress	Cyanmethaemoglobin method, haemocytometer, micro-haematocrit
AST, ALT, total protein, glucose	Serum, liver	Tissue injury, metabolism	Commercial kits; protein by Lowry [22]
Histopathology	Gill, liver, kidney, brain	Structural damage	Paraffin sections, H&E, lesion scoring
Residues	Water, sediment, muscle, liver	Exposure / accumulation	QuEChERS extraction; GC-MS/MS and LC-MS/MS

VI. METHODOLOGY

The study combines a field component (Yeldari Reservoir) and a controlled laboratory component, following the design outlined in the research synopsis [24] and extended here with seasonal sampling, residue analysis and a wider biomarker panel.

A. Study area and sampling design

Sampling will be carried out at three stations, inlet, mid-reservoir and outlet, as in our earlier physico-chemical survey [12], in three seasons (pre-monsoon, monsoon and post-monsoon) over twelve months. At each station, surface (0.5 m) and mid-depth (2 m) water will be collected in pre-cleaned amber glass bottles for residue analysis and in polyethylene bottles for physico-chemical analysis, and surface sediment will be taken with a grab sampler. Temperature, pH and dissolved oxygen will be measured in situ, and other parameters by APHA standard methods [14]. A less-impacted reference station or hatchery pond will be included for comparison. Before sampling begins, a structured questionnaire survey of farmers in the catchment will identify the insecticides actually in use, their application timing and quantities.

B. Fish collection and acclimatisation

For field assessment, 10 adult or sub-adult *C. catla* per station and season will be obtained through local fishers; length, weight, condition factor and hepatosomatic index will be recorded. For laboratory bioassays, healthy fingerlings of uniform size (about 8–12 cm, 10–15 g) will be sourced from a hatchery, transported in oxygenated bags and acclimatised for at least 15 days in dechlorinated, aerated water (12:12 h light:dark), fed a commercial pellet diet at about 2% of body weight daily. Fish will be fasted for 24 h before exposure and before sampling. Batches showing more than 5% mortality during acclimatisation will be rejected.

C. Laboratory exposure design

Candidates will be selected from the farmer survey and will be a representative from each organophosphate, pyrethroid and neonicotinoid class. (i) Acute test: 96 h static-renewal test with at least five geometrically spaced test concentrations and a control, according to OECD Test Guideline 203 [15] to determine LC50 with 95% confidence limits, using probit analysis [16]. (ii) Sub-lethal test: fish will be exposed for 21–28 days to two predetermined sub-lethal concentrations of the 96 h LC50 (e.g. 1/10 and 1/20 of the LC50, as done in previous experiments [8]), as well as to a negative control (and also to a solvent control when a carrier is used). Three replicate tanks (10 fish each) will be set up for each group. Test solutions will be replaced every 24 h and water quality (DO, pH, temperature, ammonia) will be measured daily. Sampling of fish will be conducted on days 7, 14, 21 and 28, and a recovery group of fish will be maintained in clean water for 14 days to assess reversibility. Nominal concentrations will be verified analytically. A short mixture test (binary combinations) is planned to fill the mixture gap mentioned in the literature.

D. Sample collection and analysis

Fish will be anaesthetised (clove oil or MS-222) and blood will be collected from the caudal vein and placed in a heparinised tube. Dissected gill, liver, kidney, brain and dorsal muscle will be kept on ice, one sample for enzyme assays and residue analysis stored at –80 °C and another fixed in 10% neutral buffered formalin for histology. Biochemical assays will be conducted according to the procedures in Table 3 and enzyme activity will be normalised by protein content.

A QuEChERS protocol will be used to extract residues from water, sediment and tissues, which will be quantified using both GC-MS/MS (organophosphates, pyrethroids and organochlorines) and LC-MS/MS (neonicotinoids), with procedural blanks, matrix-matched calibration and recovery checks (range 70–120% acceptable).

E. Statistical analysis and ethics

The data will be presented as the mean $\pm$ SD. One-way or two-way ANOVA (treatment $\times$ time, station $\times$ season) will be performed for the group differences, with Tukey's post-hoc test employed at $p < 0.05$, following normality and variance homogeneity testing. Principal component analysis and Pearson/correlation or Spearman correlation will be used for relating biomarker responses to the level of residue and water quality. Histological lesions will be assessed by an observer blind to treatment in a semi-quantitative manner. All animal work will be approved by the institutional animal ethics committee and follow the principle of minimising the number of animals used.

VII. PROPOSED SYSTEM: INTEGRATED BIOMARKER-BASED MONITORING FOR YELDARI RESERVOIR

Routine water-quality monitoring measures physical and chemical parameters but cannot show whether fish are biologically affected. We propose a six-module system (Figure 1) that connects pesticide sources, exposure and effect, and ends in a management decision.

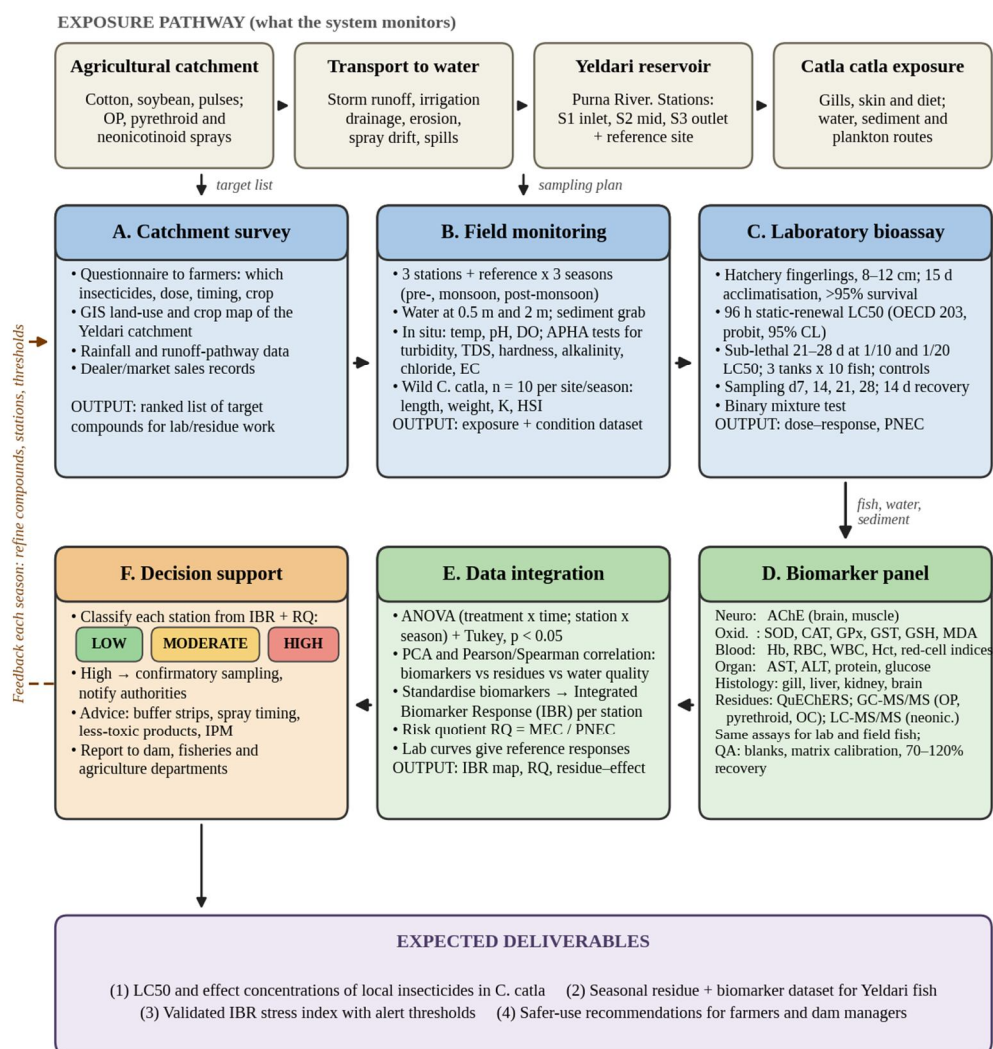


Figure 1. Proposed system: exposure pathway (top), six modules A–F with their main steps and outputs, seasonal feedback loop, and expected deliverables.

- Module A, catchment survey: identifies which insecticides are applied, when and where, and maps likely runoff pathways, so that the analytical target list reflects real use rather than assumption.
- Module B, field monitoring: seasonal sampling of water, sediment and wild *C. catla* at three stations plus a reference site, measuring physico-chemistry, residues and condition indices.
- Module C, laboratory bioassay: establishes compound-specific LC50 and sub-lethal dose–response relationships and recovery, supplying the reference responses against which field fish are interpreted.
- Module D, biomarker panel: the neurological, oxidative, haematological, metabolic, histological and residue markers listed in Table 3, measured identically in laboratory and field fish.
- Module E, data integration: biomarker values are standardised and combined into an Integrated Biomarker Response (IBR) index per station and season [23]. Exposure is characterised by a risk quotient, $RQ = \text{measured environmental concentration} / \text{predicted no-effect concentration}$ (derived from the laboratory data). Correlation and PCA test whether IBR tracks residue load.
- Module F, decision support: stations are classified into stress classes (for example low, moderate, high) based on IBR and RQ thresholds set from laboratory reference responses. A high class triggers confirmatory sampling, advice to farmers on buffer strips, application timing and integrated pest management, and reporting to reservoir and fisheries authorities.

The system is cyclic: results from each season refine the target compounds, stations and thresholds for the next. Because it uses a native, commercially important species and standard assays, it can be repeated at other Maharashtra reservoirs with modest adaptation.

VIII. EXPECTED OUTCOMES AND RESEARCH GAPS ADDRESSED

The work is expected to deliver (i) LC50 and sub-lethal effect concentrations for locally used insecticides in *C. catla*; (ii) the first seasonal dataset combining residues and biomarker responses in Yeldari fish; (iii) a validated IBR-based stress index with thresholds; and (iv) evidence-based recommendations on safer application practice. Together these address gaps we identified in the literature: lack of long-term exposure studies at environmentally relevant concentrations, limited seasonal monitoring of reservoirs, scarce data on mixtures and tissue-specific accumulation, and poor validation of laboratory findings under field conditions. Reproductive toxicity, genotoxicity and molecular biomarkers remain outside the present scope and are recommended for follow-up studies.

A. Limitations

Laboratory concentrations may not reproduce field exposure; wild fish vary in age, diet and history; and biomarker responses are influenced by temperature and season, so reference-site and seasonal controls are essential. Residue detection limits may also miss short pulses after heavy rain, which is why monsoon sampling is included.

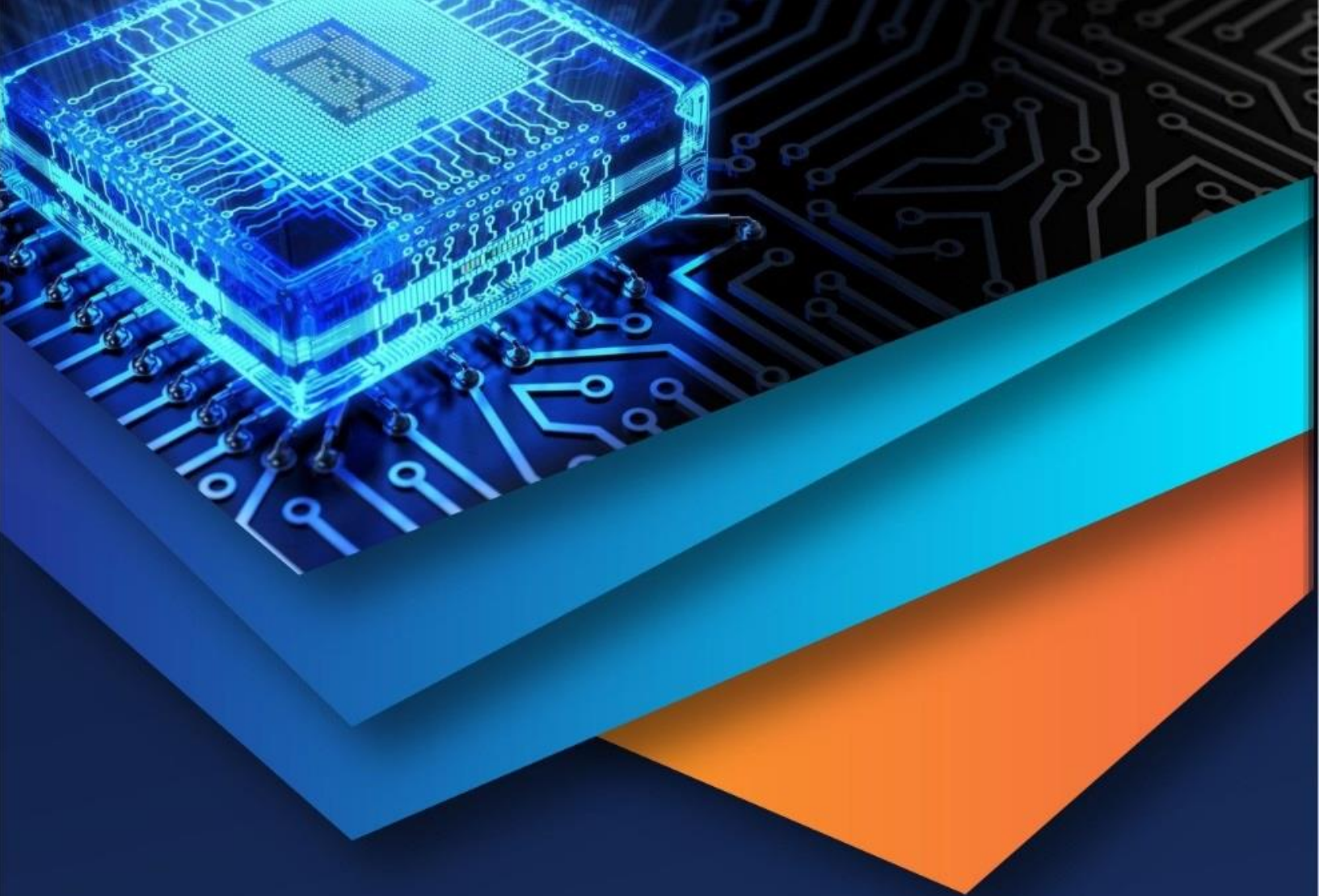
IX. CONCLUSION

Insecticides from several chemical classes produce behavioural, respiratory, haematological, metabolic, enzymatic, oxidative and histopathological changes in *Catla catla*, even at sub-lethal concentrations. Because responses are multi-level, a panel of complementary biomarkers linked to measured residues is the most defensible way to judge whether a reservoir population is under pesticide stress. The methodology and six-module system proposed here provide a practical route to do this for Yeldari Reservoir and to turn the results into management action for farmers, fisheries and reservoir authorities. Field validation is the next step.

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