



Bioaccumulation and Enzyme Activity Inhibition of Profenofos in Japanese Medaka (*Oryzias latipes* Temminck and Schlegel, 1846)

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Abstract: Effects of the organophosphate pesticide (OP) profenofos on acetylcholinesterase enzyme (AChE) activity inhibition were investigated using brain samples from Japanese medaka (*Oryzias latipes* Temminck and Schlegel, 1846). River water samples from six sampling sites along the Dalaguete River, Cebu, Philippines were tested for pesticide concentrations, the results of which were used as the basis for the Range-Finding and Exposure Tests. Japanese medaka of approximately the same weight and length were grouped into five (n=6 per group) for the RFT and were exposed to different concentrations of profenofos. For the exposure test, a total of 96 medaka were used, grouped into four groups (n=24 per group). Profenofos showed a positive, dose-dependent, and exposure-time-dependent relationship in inhibiting AChE activity. The highest concentration was already causing significant inhibition of AChE activity on Day 2. On Day 5, all concentrations were significantly different from Control. Increasing the concentration of profenofos leads to greater bioaccumulation of the pesticide in fish tissues, reaching a point of equilibrium on Day 5. The amount of pesticide accumulation declined in the fish tissues with longer exposure periods. Periodic water sampling is recommended to establish levels of profenofos and other pesticides in the river, with due consideration for the wet and dry seasons. Further studies on degradation, fate, and transport are also recommended.

Keywords: Profenofos; acetylcholinesterase inhibition; bioaccumulation

1. Introduction

The extensive and continuous global use of pesticides has resulted in their widespread occurrence, along with their transformation products, across environmental compartments such as surface water, soil, and the atmosphere. These contaminants pose emerging threats to ecosystem integrity and human health, driven by their persistence, mobility, and capacity for bioaccumulation in food webs [1]. Surface waters adjacent to intensive agricultural landscapes are particularly vulnerable to pesticide contamination, especially during rain events when surface runoff transports agrochemicals into rivers, streams, and irrigation systems [2]. This contamination is of significant concern when these water bodies are used for domestic consumption, aquaculture, or supporting biologically diverse ecosystems [3].

Aquatic organisms are among the most sensitive non-target recipients of pesticide pollution. Acute exposures to pesticides delivered to freshwater systems can cause direct mortality in fish and invertebrates, whereas chronic and sublethal exposures may trigger physiological and behavioral impairments that can reduce survival and reproductive success [4]. In particular, organophosphorus pesticides (OPPs) are well documented to disrupt nervous system function by inhibiting acetylcholinesterase (AChE), leading to neurotoxicity even at sublethal concentrations [5]. Sublethal effects of pesticide exposure in fish include reduced immune function, altered feeding and avoidance behaviors, and compromised predator escape responses, which collectively can reduce population viability [1,4]. Barangay Mantalongon and its neighboring barangays in Dalaguete, Cebu, Philippines, are recognized as major vegetable production hubs in the province and are widely referred to as the “Vegetable Basket of Cebu.” To meet market demand and optimize yields, farmers in the area typically rely on intensive pesticide applications throughout the cropping season, particularly on brassicas (cabbage and pechay), spring onions, tomatoes, and cauliflowers. Surface runoff from these cultivated fields drains into tributaries that feed the Dalaguete River, which supports aquatic life, including guppies, catfish, and freshwater shrimps, and is also used by residents for tilapia aquaculture. The increased, often unregulated use of pesticides in the area has raised environmental and public health concerns among local stakeholders, who have reported perceived increases in adverse health outcomes potentially linked to pesticide exposure; however, systematic scientific studies to examine these claims have not yet been conducted.

In this study, the freshwater fish *Oryzias latipes* (Japanese medaka) was selected as the test organism because of its small size and established ecotoxicological responses, making it suitable for laboratory toxicology assessments using limited quantities of test chemicals. Although larger aquaculture species such as tilapia are relevant to local use, their size increases the volume of pesticide required for controlled exposure studies. Japanese medaka has continued to serve as a standard model organism in aquatic toxicology, particularly for neurotoxicity and biomarker studies involving enzyme inhibition and behavioral endpoints [1, 5]. Its sensitivity to organophosphorus pesticide exposure and well-characterized AChE system have maintained its inclusion in standardized test guidelines. Profenofos, an organophosphorus insecticide widely used in agricultural systems, has raised concerns for its persistence and toxicity in aquatic environments. Recent reviews have documented that profenofos exposure negatively impacts multiple physiological systems in fish—including liver, gills, and neural function—primarily through inhibition of AChE and related cholinesterase enzymes [6, 7]. These adverse effects highlight the need for detailed assessments of profenofos bioaccumulation and neurotoxic risk to non-target aquatic organisms.

Accordingly, this study was designed to determine the bioaccumulation and enzyme-inhibition effects of profenofos in Japanese medaka (*Oryzias latipes*). The specific objectives were to (1) quantify profenofos accumulation in fish tissues and test water, (2) calculate the bioconcentration factor (BCF) of profenofos in *O. latipes*, and (3) evaluate potential ecological risk by correlating laboratory-derived AChE inhibition responses with environmentally relevant exposure levels.

2. Materials and Methods

Laboratory experiments were generally conducted at the Marine Science Education and Research Institute, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan. River water samples were analyzed for pesticide residue concentration at the Department of Agriculture Bureau of Plant Industry-Pesticide Analytical Laboratory in Diliman, Quezon City. Test organisms, chemicals, reagents, and equipment were provided by Kagoshima University.

2.1 Sampling Sites

The study area is located within the major basin of Mantalongon, Dalaguete, Cebu, Philippines, specifically in two of its major tributaries (Figure 1). Six sampling sites were identified.. Station 1 is located in the Granchina headwater area. The area, though a headwater, is still surrounded by a few vegetable farms planted with cabbage and other vegetables. Succeeding sampling sites were identified with the following criteria: pollutant source (2, 3, 4, and 5) and downstream (6).

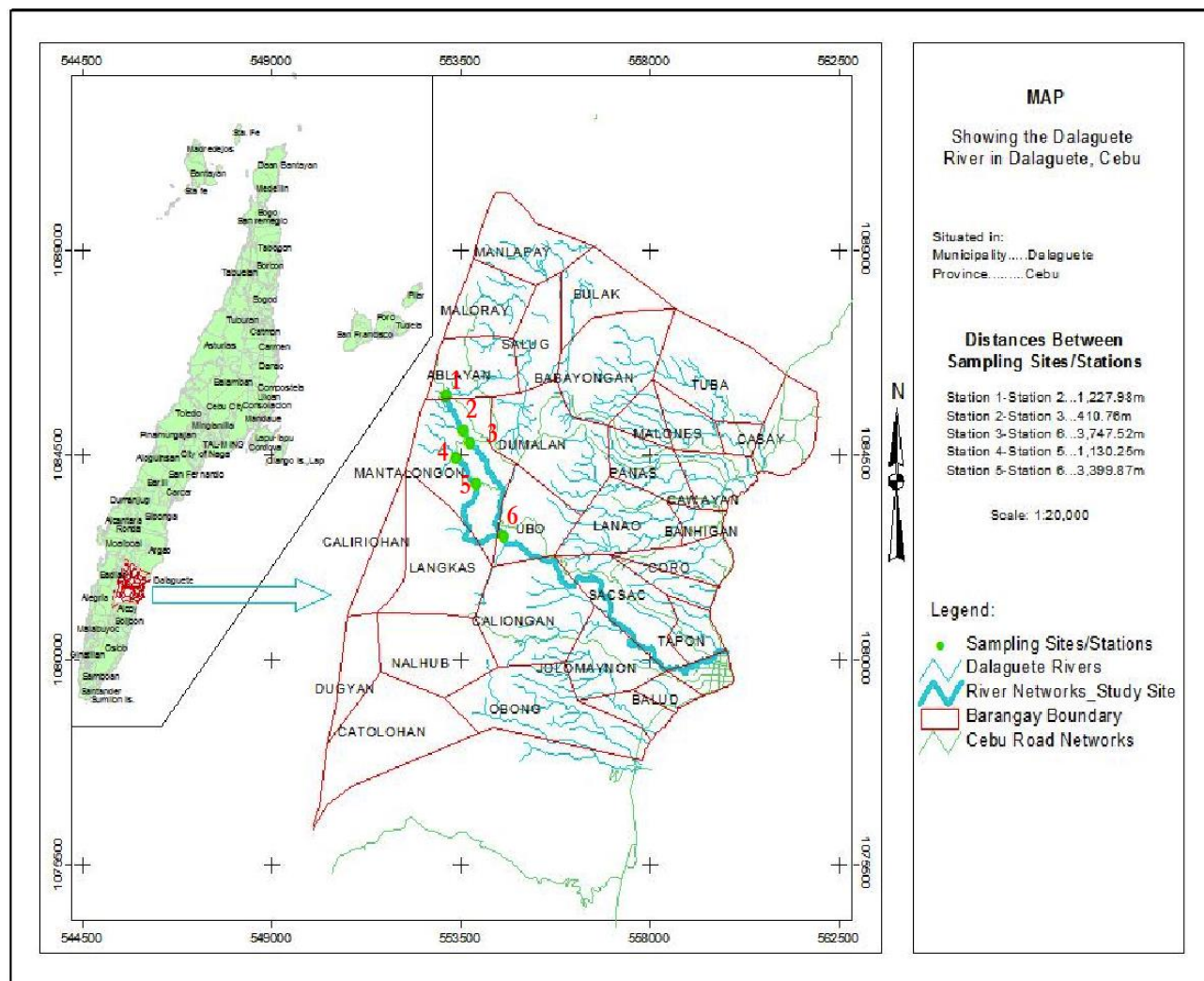


Figure 1. Map showing the location of each sampling site in the Dalaguete River.

2.2 Sample Collection

Two-liter samples were collected simultaneously from each of the identified sampling sites. The water samples were collected from the flowing parts at the sides and the middle of each sampling point and composited. The water samples were contained in acid-washed amber-colored borosilicate glass bottles, previously washed with river water, filled to the brim, tightly capped, and cooled in ice coolers, maintaining a 0–4°C temperature during transport to the laboratory. The water samples were immediately brought to the laboratory for the extraction of pesticides before analysis. Physico-chemical parameters such as temperature, pH, and dissolved oxygen (DO) were determined in situ using a digital multi-probe meter (Thermo Scientific, USA).

2.3 Chemicals

Acetylcholine iodide, 5,5'-dithiobis (2-nitrobenzoic acid), and heparin were purchased from Tokyo Chemical Industry Co., Ltd., Japan. Dichloromethane, hexane, and acetone used were all pesticide grades, while silica gel (Wakogel, C100), sodium chloride, disodium hydrogen phosphate, twelve hydrate, potassium chloride, 2-phenoxyethanol, and potassium dihydrogen phosphate were all analytical grades. These chemicals were purchased from Wako Pure Chemical Industries Ltd., Japan.

2.4 Pesticide Analysis in Water

The collected river water samples from the different sampling sites were immediately transported to the Department of Agriculture Bureau of Plant Industry-Pesticide Analytical Laboratory in Diliman, Quezon City. The pesticide residue concentrations of the samples were analyzed using Gas Chromatography (Agilent

Technologies Model HP 6890, USA). The analysis data served as the basis for identifying the target pesticide for the risk assessment.

2.5 Range Finding Test

A range-finding test was conducted prior to the exposure experiment to determine the concentration range that would give 50% mortality to the test organism, which was used as the basis for establishing the treatment concentrations. The initial concentrations used for range-finding tests were based on the reported LC50 of profenofos in zebrafish (*Danio rerio*), which is 0.2 ppm [8]. Zebrafish was used as the base organism because it closely resembled Japanese medaka in terms of biometrics such as weight, length, and size. Profenofos was used since it was the pesticide detected during the pesticide residue analysis. The setup of the range-finding test used six freshwater Japanese medaka per concentration: 3 ppm, 1 ppm, 0.2 ppm, and 0.04 ppm, and a control, with 10 liters for each concentration in a static condition. A total of 30 freshwater Japanese medaka fish with an average total body length of 38 mm and a weight of 500 mg were used. Dissolved oxygen, temperature, pH, and mortality were monitored every 2 hours for 6 hours on Day 0 and on the 24th and 48th h.

2.6 Exposure Test

There were four treatments: T₁ (control), T₂ (0.04 ppm), T₃ (0.2 ppm), and T₄ (1.0 ppm). The concentrations of the target pesticide were based on the results of the range-finding test, in which the range of concentrations that resulted in 50% mortality in the test animals was determined. Four aquariums (9.5 in x 9 in x 12 in) with 10 liters of water were set up, each containing 24 freshwater Japanese medaka, approximately 3–4 cm in length (carrying capacity of approx. 1 g/L) (Figure 2). The aerated tap water exposed to activated carbon and the Japanese medaka were provided by the Marine Science Education and Research Institute, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan. The freshwater Japanese medaka have been reared in the Institute's laboratory. Approximately 1 mL of *Artemia salina* was fed to the fish every three days. A day before pesticide exposure, eight Japanese medaka were sampled to establish baseline AChE levels. This constituted the data for Day 0. A 10-day exposure was applied. A related study found that the concentration of profenofos in zebrafish (*Brachydanio rerio*) reached equilibrium by 168 h (7 days) [8]. The flow-through exposure method was used for T₂–T₄. Static condition was followed for Control. Eight Japanese medaka fish were collected from each aquarium on the 2nd, 5th, and 10th days of the exposure period. Temperature, percent saturation, DO and pH were monitored daily for the whole duration of the study.



Figure 2. Flow-through exposure method.

Due to the small size of the fish, they were composited to represent a single replicate. A total of 96 (24×4) freshwater Japanese medaka fish were used in the risk determination. Eight fish were sampled per time point, with two fish composited, to give a total of 4 replicates per sampling time. Acetylcholinesterase enzyme inhibition tests were performed at each sampling period. Dissolved oxygen (DO), pH, and temperature of the test waters were monitored daily using a DO meter (Navi Model OM-51, Horiba, Ltd., Japan) and a pH meter (Hanna, USA).

2.6.1 Sampling of Fish for Biochemical Test

Fresh weight, body length, and total body length of the fish were determined before conducting further tests. This was to establish that the fish were approximately the same size and weight. Fishes were anesthetized with 2-phenoxyethanol (0.7 mL/L H₂O).

2.7 Acetylcholine Esterase Enzyme Inhibition Test

Brains from two Japanese medaka fish were collected and combined to compose a replicate sample. The whole brain was extracted and placed in a pre-weighed 1.5 mL microtube (Nippi, Japan). Each sample was homogenized using Power masher II (Nippi, Japan) after the addition of 0.1 M phosphate buffer (pH 8.0; 20 mg fresh tissue mL⁻¹). Homogenate was centrifuged at 10,000 g for 15 min at 4°C. Acetylcholine esterase (AChE) activity was determined using Ellman's reagent (DTNB) and acetylthiocholine [9] modified for a microplate reader (Model 550, Bio-Rad, USA) with a 96-well plate [10]. In each well, 155 µL of 0.1 M phosphate buffer (pH 8.0), 10 µL of supernatant, and 10 µL of DNTB were added. The plate was incubated at 30°C for 5 min, and then 5 µL of substrate (AChI) was added to start the reaction. Enzyme activity was measured using a Bio-Rad microplate reader every 2 min for 10 minutes at 412nm. Each enzymatic activity was carried out in triplicate. The rate of enzymatic activity was calculated according to Ellman *et al.* [9]. The protein content of the samples was quantified by the Bradford dye-binding assay using the Bio-Rad Protein Assay (Bradford, 1976) with bovine serum albumin as the standard.

2.8 Bioaccumulation of Pesticides

2.8.1 Test Water

Analysis of pesticide concentration in the test water was performed following the procedure of Añasco *et al.* [11]. Pesticide extraction was performed within 24 h of arrival in the laboratory. Solid-phase extraction was performed using C18 Sep-Pak Cartridges. The C18 Sep-Pak cartridges were conditioned by gradually adding the first 10 mL of acetone (1 drop/second) to the glass syringe fitted with the cartridge. This was followed by 10 mL of deionized distilled water (1 drop/second). Fifty mL of the water sample (1 drop/second) was then allowed to pass gradually through the glass syringe with a cartridge. The eluent was allowed to stand for 10 minutes. The cartridges were then centrifuged for 10 minutes at 3000 rpm at 4°C. The cartridges were then fitted into a new set of glass syringes and gradually eluted with 20 mL of acetone (1 drop/second). The eluents were concentrated to 1 mL by gentle nitrogen gas flow. 100 µL of 1.0 ppm anthracene-d₁₀ was added to the sample as an internal standard. Samples were finally injected into a gas chromatograph (Agilent Technologies 6890N, USA) with a mass spectrometry detector (Agilent Technologies 5973N) in splitless mode. Analytical conditions were as follows: (1) injection volume, 2 µL; (2) injector temperature, 250°C; (3) detector temperature, 280°C; (4) oven temperature, initially at 60°C (held for 1 min) then ramped to 180°C at 20°C/min (held for 5 min) then finally increased to 290°C at 3°C/min (held for 3 min); and (5) column, DB-5MS (length, 30 m; internal diameter, 0.25 mm; film thickness, 0.25 µm; J & W Scientific, USA).

2.8.2 Test Organisms

The remaining body tissues of the fish samples were freeze-dried for the determination of residual pesticide concentrations following the method of Añasco *et al.* [11] with some modifications. Dried fish tissues were cut into small pieces. The required amount for each sample was placed in 50-mL centrifuge tubes. 10 mL hexane-acetone solvent mixture (1:1) was added. The mixture was subjected to ultrasonic extraction for 15 mins, then centrifuged at 3000 rpm for 10 mins at 4°C. The supernatant was collected into another 50 mL centrifuge bottle. The precipitate was then added to 10 mL of a hexane-acetone solvent mixture (1:1), and the

process was repeated. The two batches of supernatant liquids were combined. 20 mL of deionized distilled water was then added to the supernatant. The centrifuge tube was shaken for 10 mins at 8.5 speed on a shaker, then centrifuged for 10 mins at 3000 rpm at 4°C. The supernatant liquid was then collected, to which 10 mL of hexane was added. The resulting mixture was then shaken for 10 mins at 8.5 speed, then centrifuged at 3000 rpm for 3 mins at 4°C. The supernatant liquid was collected, and the precipitate was subjected to the same process. The final supernatant was passed through a glass funnel containing quartz wool and anhydrous Na₂SO₄ to remove excess water. The filtrate was concentrated to 1 mL by a gentle stream of nitrogen gas. Clean-up was performed with Florisil containing 3% moisture. Clean-up columns were conditioned with 10 mL of hexane. The sample was passed through the column and eluted with 10 mL hexane, followed by 20 mL of 10% acetone in hexane. The eluate was concentrated to 1 mL by a gentle stream of nitrogen gas. 100 µL of 1.0 ppm anthracene-d₁₀ was added to the sample as an internal standard. Samples were finally injected into a gas chromatograph (Agilent Technologies 6890N, USA) with a mass spectrometry detector (Agilent Technologies 5973N) in splitless mode. Analytical conditions were as follows: (1) injection volume, 2 µL; (2) injector temperature, 250°C; (3) detector temperature, 280°C; (4) oven temperature, initially at 60°C (held for 1 min) then ramped to 180°C at 20°C/min (held for 5 min) then finally increased to 290°C at 3°C/min (held for 3 min); and (5) column, DB-5MS (length, 30 m; internal diameter, 0.25 mm; film thickness, 0.25 µm; J & W Scientific, USA).

2.9 Bioconcentration Factor

The bioconcentration factor of the identified pesticide in the test organisms was computed as the concentration of the pesticide in the fish tissues at steady state divided by the concentration of the chemical in water during the exposure period. The formula used was: $BCF = C_{fish} / C_{water}$, where C_{fish} is the concentration of profenofos in fish tissue (µg/g wet weight) at steady state, and C_{water} is the concentration of profenofos in the exposure water (µg/mL) at equilibrium (Day 5).

2.10 Statistical Analysis

Analysis of Variance (ANOVA) was used to test for differences between treatments and between sampling days. The Student-Newman-Keuls (SNK) Test was used to assess further whether a significant difference among treatments exists. The Spearman's rank order correlation, or Spearman's rho, was used to compute the correlation between AChE activity inhibition and bioaccumulation data.

3. Results and Discussion

3.1 Pesticide Concentration in River Water

The pesticide profenofos was the only one detected at the six identified sampling sites along the Dalaguete River, with a concentration of 481 µg/L. at Sampling Site #2. Sampling was done after a heavy rain post-application. Profenofos with an IUPAC name of 4-bromo-2-chloro-1-[ethoxy (propylsulfanyl)phosphoryl]oxybenzene, is the active ingredient of the commonly used pesticide Selectron® in Mantalongon and its neighboring barangays. Physico-chemical parameters for the different sites ranged from 7.1–7.4, 6.8–7.8 ppm, and 28.8°C–30°C for pH, DO, and temperature, respectively.

3.2 AChE Inhibition Test

Acetylcholinesterase (AChE) activity was significantly inhibited as pesticide concentration was increased. On Days 2 and 5, T34 (1.00 ppm) AChE activity was significantly lower than the other treatments; however, on Day 5, T12 (0.04 ppm) and T23 (0.20 ppm) were not significantly different from each other but were significantly lower than T₄ and Control. On the last day of sampling, T₃ and T₄ were not significantly different from each other but significantly different from T₂ and Control (Figure 3).

Table 1. Pesticide residue in the river water samples.

Sampling Site Number	Pesticide Concentration (ppm)
Station 1	<LOQ
Station 2	Profenofos = 0.481
Station 3	<LOQ
Station 4	<LOQ
Station 5	<LOQ
Station 6	<LOQ

Note: 1. Results were obtained by Gas Chromatography (Agilent Technologies HP 6890, USA).

2. The Limit of Quantification (LOQ) for organophosphates, organochlorines and pyrethroids is 10.0 µg/L.

3.2 Range Finding Tests and Setting of Pesticide Concentrations

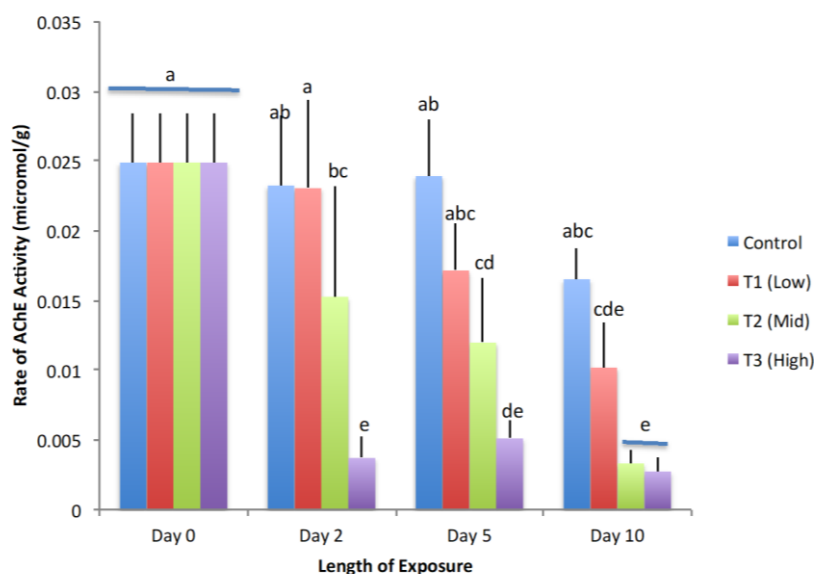
The highest concentration at 3.00 ppm produced 83.33% mortality to the test organisms after 48 hours (Table 2). The next lower concentration produced 50% mortality. However, based on actual observation the remaining *O. latipes* in T₃ were already showing behavioral alterations like continuous swimming combined with numerous jerks, partial jerks and flickering of the fins. The sole survivor in T₄ was already on its side exhibiting loss of equilibrium. Hence, approximate range causing 50% mortality was set at 0.2 ppm if the objective was to expose *O. latipes* to profenofos for 10 d. Concentrations higher and lower than 0.20 ppm were set as additional concentrations for the exposure test.

Table 2. Range Finding Test Using Profenofos (n = 6).

Treatment	Mortality (f)	% Mortality
Control	0	0
T1 (0.04ppm)	0	0
T2 (0.20ppm)	0	0
T3 (1.00ppm)	3	50
T4 (3.00ppm)	5	83.33

3.3 AChE Inhibition Test

Acetylcholinesterase (AChE) activity was significantly inhibited as pesticide concentration was increased. In Days 2 and 5, T₄ (1.00 ppm) AChE activity was significantly lower than the rest of the treatments; however, on Day 5, T₂ (0.04 ppm) and T₃ (0.20 ppm) were not significantly different from each other but significantly different from T₄ and Control. On the last day of sampling, T₃ and T₄ were not significantly different from each other but significantly different from T₂ and Control (Figure 3). F-value = 15.62, df=63.

**Figure 3.** AChE Activity on *O. latipes* exposed to different concentrations of profenofos.

The observed inhibition of acetylcholinesterase (AChE) activity in the present study is consistent with recent findings demonstrating a clear dose-dependent relationship between organophosphate (OP) exposure and AChE suppression in freshwater fish. Numerous studies over the past decade have reported significant, concentration-dependent AChE inhibition in *Oryzias latipes* and other teleost species following exposure to OP insecticides such as chlorpyrifos, diazinon, and profenofos [5, 12-14]. Similar responses have also been documented in non-target species, such as mosquito fish (*Gambusia affinis*), confirming AChE's sensitivity as a biomarker of OP neurotoxicity across taxa [15].

The inhibition of AChE is primarily due to the covalent binding of OP compounds to the serine hydroxyl group at the esteratic site of the enzyme, forming a stable phosphorylated enzyme complex that disrupts normal synaptic transmission [5,16]. Profenofos, in particular, has been shown to exert strong inhibitory effects on AChE activity in both fish and aquatic invertebrates, including freshwater shrimps and tilapia species, even at environmentally relevant concentrations [17-19]. The present results further support previous evidence on the persistence and functional irreversibility of OP-induced AChE inhibition. The absence of significant recovery in AChE activity between Days 2, 5, and 10 in treatments T3 (0.20 ppm) and T4 (1.00 ppm) suggests prolonged enzyme inhibition, consistent with reports indicating slow or incomplete enzymatic reactivation following OP exposure due to enzyme "aging" [13, 16]. Notably, significant AChE inhibition was already evident at T3, a concentration lower than measured field application rates, as early as Day 2, underscoring the potential ecological risk posed by profenofos even at low exposure levels.

Behavioral abnormalities observed in profenofos-exposed fish—including heightened reactivity to light stimuli, circular swimming, repeated collisions with aquarium walls, erratic, jerky movements, loss of equilibrium, and spiral or corkscrew swimming patterns, are characteristic manifestations of OP-induced neurotoxicity. The following behavioral abnormalities were noted as incidental observations during daily monitoring and were not subject to statistical analysis. Similar behavioral disruptions have been widely reported in fish exposed to profenofos and other OP insecticides and are closely linked to cholinergic overstimulation resulting from AChE inhibition [14, 20]. Such behavioral endpoints are now recognized as sensitive early-warning indicators of sublethal pesticide stress and are increasingly incorporated into ecological risk assessments [21].

In vitro AChE inhibition studies further corroborate the neurotoxic potential of profenofos, demonstrating concentration- and time-dependent alterations in enzyme kinetics, characterized by changes in apparent K_m and V_{max} values. Recent studies indicate that OPs, including profenofos, commonly exhibit competitive or mixed-type inhibition of AChE in fish, reflecting direct interaction with the enzyme's active site [17, 19]. These enzymatic disruptions provide a mechanistic basis for the observed behavioral impairments and reinforce the relevance of AChE inhibition as a key biomarker for assessing pesticide-induced neurotoxicity in aquatic organisms. Biochemical enzyme activity perturbations have also been reported as sensitive indicators of chemical toxicity in vertebrate models beyond aquatic systems [21].

3.4 Bioaccumulation and Bioconcentration Factor

Recent studies have demonstrated that aquatic organisms can bioaccumulate profenofos and other organophosphate insecticides in their tissues, with uptake strongly influenced by exposure concentration, duration, and species-specific metabolic capacity [6, 18]. In the present study, increasing concentrations of profenofos resulted in significantly higher levels of pesticide accumulation in Japanese medaka, indicating a clear concentration-dependent pattern of bioaccumulation. Tissue residues increased markedly as profenofos concentrations rose from 0.20 ppm (T3) to 1.00 ppm (T4), with accumulation approaching an apparent steady state by Day 5. This plateau suggests that a balance between uptake and elimination was achieved, a phenomenon commonly reported in controlled fish exposure studies involving organophosphate pesticides [17, 22].

The calculated bioconcentration factors (BCFs) further support this observation. Higher BCF values at elevated exposure concentrations indicate efficient uptake of profenofos from the surrounding water into fish tissues. However, the stabilization of BCF values over time implies that bioaccumulation did not increase indefinitely, reflecting the activation of metabolic and depuration mechanisms. According to current

ecotoxicological criteria, moderate BCF values are characteristic of compounds with intermediate lipophilicity and limited persistence in fish tissues, such as profenofos [6, 16].

Following the attainment of equilibrium, a decline in profenofos residues in fish tissues was observed with prolonged exposure. This decrease suggests that Japanese medaka can biotransform and eliminate profenofos via enzymatic detoxification pathways. Recent studies indicate that hepatic esterases and cytochrome P450-mediated metabolism play a central role in reducing internal organophosphate burdens during extended exposure periods [5, 12,13]. As depuration progressed, profenofos concentrations in the surrounding water increased, likely due to the compound's release from fish tissues back into the medium.

Table 3. Bioaccumulation and BCF of profenofos in Japanese medaka.

Days of Exposure	T ₁	T ₂	T ₃
0	5	5	5
2	441.515	455.826	1060.551
5	393.651	558.881	1496.096
10	385.522	524.057	1210.217
Average (Fish) ng/g wet weight	406.896	512.921	1255.621
Average (Water) ng/mL	13.824	61.573	281.860
BCF	29.434	8.330	4.455

The observed bioaccumulation and depuration dynamics are consistent with the physicochemical properties of profenofos, including its relatively low water solubility and moderate lipophilicity, which favor rapid initial uptake followed by gradual elimination rather than long-term retention [6, 18]. These findings suggest that although profenofos does not exhibit extreme bioaccumulative behavior, its ability to reach biologically relevant internal concentrations—especially at environmentally realistic exposure levels—poses a potential risk to aquatic organisms through repeated or chronic exposure. Similar bioaccumulation dynamics have been observed in fish from Philippine riverine systems affected by anthropogenic contamination [3].

4. Conclusions

This study demonstrates that profenofos induces concentration-dependent bioaccumulation and pronounced acetylcholinesterase (AChE) inhibition in *Oryzias latipes*, confirming its neurotoxic potential at environmentally relevant exposure levels. Although bioconcentration factors (BCFs) indicated only moderate bioaccumulation, profenofos rapidly reached biologically effective internal concentrations, with tissue residues stabilizing within 5 days. Significant and persistent AChE inhibition, accompanied by abnormal swimming behavior, was observed even at concentrations below estimated field application levels, indicating limited enzymatic recovery and substantial sublethal toxicity. These findings highlight the ecological risk posed by profenofos to freshwater organisms and underscore the need for strengthened management of organophosphate pesticide use in agricultural watersheds. Regulatory and mitigation efforts should emphasize improved pesticide application practices, runoff control measures, and adoption of integrated pest management strategies to minimize contamination of surface waters. Future research should prioritize long-term, multigenerational exposure studies, evaluate recovery dynamics following exposure cessation, and integrate laboratory biomarkers with field-monitoring data. Additionally, investigations into mixture toxicity involving profenofos and other commonly applied pesticides are essential to reflect real-world exposure scenarios better and inform more protective ecological risk assessments.

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Supplemental Table for Physico Parameters

Day 0	pH	Temp	DO	% Sat'n
Control	7.42	20.70	7.85	89.50

Day 6	pH	Temp	DO	% Sat'n
Control	7.06	24.90	6.91	89.60

T1	7.36	21.00	7.25	84.70
T2	7.20	23.60	6.37	77.50
T3	6.96	23.50	7.24	88.10
Day 1	pH	Temp	DO	% Sat'n
Control	6.92	24.50	6.57	80.10
T1	6.80	24.70	6.63	80.20
T2	6.83	24.30	6.70	81.30
T3	6.90	24.60	6.69	81.70
Day 2	pH	Temp	DO	% Sat'n
Control	6.95	23.70	6.61	80.40
T1	7.08	23.60	6.63	87.20
T2	7.00	23.80	7.04	84.50
T3	7.13	23.70	6.82	82.40
Day 3	pH	Temp	DO	% Sat'n
Control	7.24	23.70	7.24	88.00
T1	7.10	23.60	6.86	80.60
T2	7.35	23.60	7.30	88.70
T3	7.33	23.30	7.72	92.20
Day 4	pH	Temp	DO	% Sat'n
Control	7.06	22.60	7.88	92.40
T1	7.10	22.90	7.42	83.50
T2	7.04	22.50	6.94	83.60
T3	6.94	22.90	7.24	87.00
Day 5	pH	Temp	DO	% Sat'n
Control	6.76	22.70	7.13	83.40
T1	7.02	23.10	7.28	86.50
T2	7.06	24.90	6.91	89.60
T3	7.02	25.20	6.84	88.70

T1	7.02	25.20	6.84	88.70
T2	7.12	24.40	6.57	79.70
T3	7.22	24.60	6.89	86.40
Day 7	pH	Temp	DO	% Sat'n
Control	7.33	24.10	6.49	79.40
T1	7.54	24.50	6.55	81.00
T2	7.16	24.70	6.68	82.00
T3	7.34	24.70	6.60	80.30
Day 8	pH	Temp	DO	% Sat'n
Control	7.25	24.30	6.86	81.20
T1	7.60	24.10	6.63	82.00
T2	7.20	23.80	6.49	79.10
T3	7.33	24.00	6.30	79.90
Day 9	pH	Temp	DO	% Sat'n
Control	7.31	23.00	6.90	80.20
T1	7.53	23.30	6.87	80.20
T2	7.60	23.30	7.09	86.00
T3	7.54	23.60	6.91	85.50
Day 10	pH	Temp	DO	% Sat'n
Control	7.60	23.30	7.09	86.00
T1	7.54	23.60	6.91	85.50
T2	7.40	23.40	7.40	86.00
T3	7.51	23.60	7.18	88.50