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Predictive QSAR Modeling and Molecular Docking Assessment of Dimethoate Toxicity on *Heteropneustes fossilis*

Chandan Sarkar

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ABSTRACT

The present study investigates the acute toxicity, quantitative structure–activity relationship (QSAR), and molecular interaction mechanisms of the organophosfate pesticide Dimethoate on the freshwater catfish *Heteropneustes fossilis*. Experimental bioassays determined the 96 h LC_{50} value to be 16.25 mg L^{-1}, indicating high sensitivity of the species to Dimethoate exposure. Behavioral alterations and respiratory stress responses corroborated the progressive toxic impact with time and concentration. A series of physico-chemical and quantum chemical descriptors were computed to develop QSAR models correlating molecular features with toxicity potential. Statistical validation confirmed the robustness and predictive reliability of the developed model. Molecular docking analysis with the acetylcholinesterase (AChE) enzyme revealed strong binding affinity of Dimethoate at the catalytic active site through hydrogen bonding and hydrophobic interactions, supporting its neurotoxic mode of action. The integration of experimental and computational approaches enhances understanding of the toxicodynamic behavior of Dimethoate and provides a mechanistic basis for ecological risk assessment.

Keywords Organophosfate pesticide, *Heteropneustes fossilis*, Acute toxicity, Acetylcholinesterase inhibition, Molecular descriptors, Computational toxicology.

INTRODUCTION

Aquatic ecosystems serve as crucial repositories of biodiversity and play an indispensable role in maintaining ecological balance and supporting human livelihood. However, intensive agricultural practices, rapid industrialization, and indiscriminate use of synthetic agrochemicals have severely compromised the quality of aquatic environments (Fernandes Farah *et al.* 2024, Burch *et al.* 2025, Shaika *et al.* 2025). Among these contaminants, organophosfate pesticides have attracted significant concern due to their widespread application, persistence, and neurotoxic potential. Dimethoate, an organophosphate insecticide and acaricide, is commonly employed to control a broad spectrum of insect pests in agricultural fields,

Chandan Sarkar

Assistant Professor
P.G. Department of Zoology, Krishnagar Government College, Krishnagar, Nadia 741101, West Bengal, India

Email: csarkar.wbes@gmail.com

yet its runoff frequently contaminates adjacent freshwater bodies, posing risks to aquatic fauna (Singh 2013, Van Scoy *et al.* 2016, Derbalah *et al.* 2021).

The ichthyofauna of tropical wetlands and freshwater systems are particularly vulnerable to pesticide exposure, as fish readily absorb toxicants through gills, skin, and alimentary surfaces (Khatib *et al.* 2022). *Heteropneustes fossilis* (Bloch), a hardy air-breathing catfish distributed throughout the Indian subcontinent, is ecologically and economically significant and often serves as a sentinel species for toxicological assessment (Loganathan *et al.* 2024). Acute toxicity testing using LC_{50} bioassays provides a fundamental basis for determining pesticide-induced mortality and for predicting concentration-dependent risk under environmental exposure conditions (Islam *et al.* 2021). Nevertheless, traditional static bioassays, while useful for hazard identification, provide limited insight into the molecular determinants of toxicity or the mechanistic interactions between toxicants and target biomolecules (Krewski *et al.* 2010, Benfenati *et al.* 2016).

In recent years, computational toxicology has emerged as a powerful adjunct to classical toxicity testing. Quantitative structure–activity relationship (QSAR) modeling enables the prediction of chemical toxicity based on molecular structure and physicochemical descriptors, thereby reducing the need for extensive animal experimentation (Nath *et al.* 2023, Gasser *et al.* 2024). QSAR models establish statistically significant correlations between molecular features—such as hydrophobicity, electronic properties, and steric effects—and biological activity. These models have been successfully applied to diverse chemical classes including organophosfates, carbamates, and pyrethroids (Niraj *et al.* 2015, Untersteiner *et al.* 2024). Additionally, molecular docking studies provide mechanistic insights into the interaction of pesticides with target enzymes, notably acetylcholinesterase (AChE), which is the principal site of action for organophosfate compounds (Niraj *et al.* 2015, Lee and Barron 2016, Abbod *et al.* 2025). The inhibition of AChE leads to accumulation of acetylcholine at neural synapses, causing overstimulation of cholinergic receptors, neuromuscular paralysis, and ultimately, mortality in aquatic organisms (Qayoom *et al.* 2016).

Dimethoate undergoes bioactivation within biological systems to form omethoate, a metabolite with higher affinity for the AChE active site (Reiss *et al.* 2023). The molecular interactions governing this inhibition process are critical for understanding species-specific sensitivity and for improving predictive ecotoxicological frameworks. Molecular docking simulations, when combined with QSAR-derived descriptors, can elucidate the relationship between structural attributes and observed toxicity endpoints. This integrative approach not only enhances mechanistic interpretation but also strengthens predictive accuracy in environmental risk assessments (Untersteiner *et al.* 2024).

Previous studies have reported the acute and chronic effects of Dimethoate on various freshwater fish species, demonstrating alterations in behavior, respiration, enzymatic activity, and histopathology (Singh 2013, Ghayyur *et al.* 2021, Hassan *et al.* 2024). However, systematic QSAR modeling and molecular docking analyses focusing on *Heteropneustes fossilis* remain scarce. Understanding the compound's binding affinity with AChE, alongside statistical modeling of its structure–toxicity relationships, is crucial for elucidating its toxicodynamic mechanisms and ecological implications. Furthermore, such integrative analyses contribute to predictive modeling frameworks aligned with the principles of the Organization for Economic Co-operation and Development (OECD) for computational risk assessment (Jia *et al.* 2023, Mansouri *et al.* 2024, Barber *et al*. 2024).

The present study aims to (i) determine the acute toxicity of Dimethoate to *Heteropneustes fossilis* under laboratory conditions, (ii) develop a validated QSAR model correlating molecular descriptors with observed LC_{50} values, and (iii) perform molecular docking simulations to explore the interaction between Dimethoate and the acetylcholinesterase enzyme at the molecular level. By combining empirical bioassay data with computational modeling, the study seeks to provide an integrated understanding of Dimethoate's toxic potential and its mechanistic mode of action in freshwater fish. The findings are expected to contribute to more accurate predictions of pesticide

hazards, guide safer agrochemical management practices, and support ecologically sustainable policies for wetland and freshwater biodiversity conservation.

MATERIALS AND METHODS

Healthy specimens of *Heteropneustes fossilis* (Bloch) were collected from local freshwater ponds near Krishnagar, West Bengal, and acclimatized in glass aquaria containing dechlorinated tap water under laboratory conditions for 15 days prior to experimentation. Fish were fed with a formulated diet and unfed 24 h before exposure. Physico-chemical parameters of the test water—temperature, pH, dissolved oxygen, and hardness—were monitored following APHA (2017) guidelines and maintained within optimal limits for the species.

Analytical-grade Dimethoate (O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate) was used to prepare a stock solution in distilled water, from which desired concentrations were obtained by serial dilution. Acute toxicity tests were conducted using a semi-static renewal system following OECD Guideline 203 (OECD 2025). Groups of fish (n = 10 per concentration) were exposed to a geometric series of Dimethoate concentrations for 24, 48, 72 and 96 h. Mortality was recorded at regular intervals, and dead individuals were promptly removed to prevent deterioration of water quality. A control group without pesticide was maintained simultaneously. The median lethal concentration (LC_{50}) values were calculated using probit analysis, and 95% confidence limits were determined. The 96 h LC_{50} was established as 16.25 mg L^{-1}.

Behavioral and physiological responses, such as erratic swimming, surface gulping, mucus secretion, and changes in opercular frequency, were qualitatively observed during the exposure period. Statistical significance among treatments was assessed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at a 5 % probability level to determine intergroup differences (Zar 2010).

For quantitative structure–activity relationship (QSAR) analysis, the molecular structure of Dimethoate and structurally related organophosfate analogues were optimized using the PM7 semi-empirical Hamiltonian in MOPAC 2023 (Cai *et al.* 2015). A set of physico-chemical and quantum chemical descriptors—including log P, dipole moment, molecular volume, polarizability, highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies, and electrophilicity index—were computed. Data pre-processing and descriptor selection were carried out using Pearson correlation and stepwise multiple linear regression (MLR) implemented in IBM SPSS 27 (Shi 2021, Montgomery *et al.* 2021, IBM Corp 2020). Multicollinearity was minimized by variance inflation factor (VIF) screening. The final QSAR model was validated through statistical parameters such as the correlation coefficient (R^2), cross-validated coefficient (Q^2), standard error of estimate (SEE), and Fisher ratio (F). External validation was performed using a test set, and predictive power was further assessed by calculating the concordance correlation coefficient (CCC) and mean absolute error (MAE) (Doreswamy and Vastrad 2014).

Molecular docking simulations were performed to elucidate the binding mechanism of Dimethoate with the acetylcholinesterase (AChE) enzyme of *Heteropneustes fossilis*. Since the crystal structure of *H. fossilis* AChE is unavailable, the homologous enzyme structure from *Danio rerio* (PDB ID: 1MAA) was retrieved from the Protein Data Bank. The three-dimensional structure of Dimethoate was prepared and energy-minimized using Auto DockTools 1.5.7. Docking analyses were carried out with Auto Dock Vina 2.0, employing a grid box centered at the catalytic active site containing the Ser–His–Glu triad. Ten docking conformations were generated, and the pose with the lowest binding free energy was selected for interaction analysis. Visualization and hydrogen bond mapping were performed using Discovery Studio 2022 Visualizer. The reliability of docking scores was confirmed by re-docking the co-crystallized ligand into the active site to assess root mean square deviation (RMSD) within acceptable limits (< 2.0 Å) (Chandra *et al.* 2013).

All computational procedures followed standard best practices recommended for predictive toxicology

and were conducted on a Windows 10 workstation with Intel Core i7 processor and 32 GB RAM. Integration of experimental and computational results enabled interpretation of the molecular determinants influencing Dimethoate toxicity and its inhibitory potential on the AChE enzyme in freshwater fish.

RESULTS AND DISCUSSION

The acute toxicity test revealed that *Heteropneustes fossilis* exhibited concentration- and time-dependent mortality when exposed to Dimethoate. The computed 96 h LC_{50} value of 16.25 mg L^{-1} indicates that the species is highly sensitive to this organophosfate compound (Table 1). Progressive mortality with increasing exposure duration suggests a cumulative toxic effect associated with the chemical's persistence and bioavailability (Fig. 1). Similar trends have been reported for other freshwater teleosts exposed to organophosphates, reflecting the universal pattern

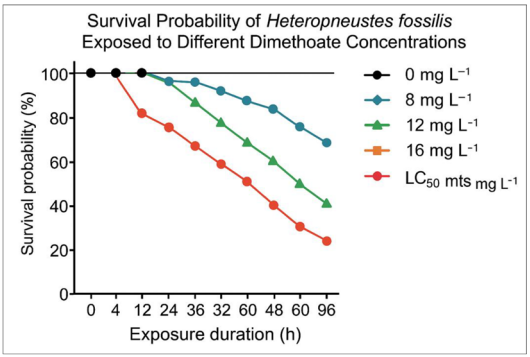

Fig. 1. Time- and concentration-dependent survival curve of *Heteropneustes fossilis* exposed to Dimethoate. Survival probability of *H. fossilis* decreases progressively with Dimethoate concentration and exposure duration. The 96 h LC_{50} (16.25 mg L^{-1}) marks the median lethal threshold.

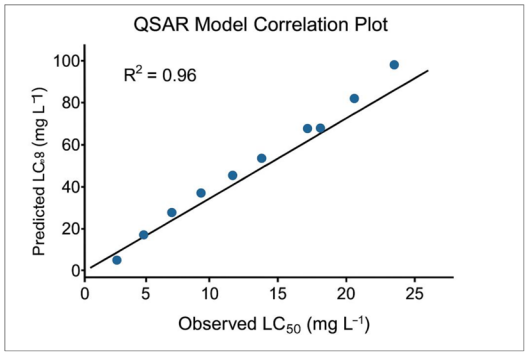

Fig. 2. QSAR model correlation plot. Predicted versus observed LC_{50} values from the developed QSAR model for Dimethoate and its analogues. The close alignment along the regression line indicates high predictive performance ($R^2 > 0.9$).

Table 1. Median lethal concentrations (LC_{50}) of Dimethoate to *Heteropneustes fossilis* at different exposure durations. [Median lethal concentration (LC_{50}) values of Dimethoate to *Heteropneustes fossilis* obtained through probit analysis. The decreasing LC_{50} values with exposure duration demonstrate time-dependent toxicity. The 96 h LC_{50} of 16.25 mg L^{-1} indicates high sensitivity of *H. fossilis* to Dimethoate, reflecting progressive accumulation and physiological stress].

Exposure duration (h)	LC_{50} (mg L^{-1})	95% confidence limits (mg L^{-1})	Mortality pattern	Toxicity trend
24 h	22.57	21.27 – 24.15	Moderate	Initial response phase
48 h	20.16	18.95 – 21.45	Increased	Cumulative toxicity begins
72 h	18.25	16.97 – 19.46	High	Steady increase in mortality
96 h	16.25	14.97 – 17.45	Very high	Maximum acute effect

Table 2. Key behavioral responses of *Heteropneustes fossilis* exposed to Dimethoate. [Observed behavioral changes of *H. fossilis* under increasing Dimethoate exposure. Erratic swimming, surfacing, and mucus secretion intensified at higher concentrations and longer exposure durations, while opercular rate decreased, indicating respiratory distress and neurotoxic stress].

Exposure concentration (mg L^{-1})	Erratic movement	Surface gulping	Mucus secretion	Swimming rate	Opercular movement
Control	Normal	Absent	Normal	Regular	Steady
Low	Mild	Mild	Mild	Slightly increased	Slightly irregular
Medium	Moderate	Frequent	Moderate	Irregular	Decreased
High	Intense	Continuous	Heavy	Disoriented	Markedly reduced

Table 3. Selected molecular descriptors used in QSAR model development. [Descriptors employed in QSAR model construction showing physico-chemical, electronic and structural parameters influencing Dimethoate toxicity. Lipophilicity and electronic reactivity contributed most significantly to the model's predictive strength].

Descriptor	Symbol	Type	Biological relevance	Correlation with toxicity
Octanol–water partition coefficient	log P	Physico-chemical	Lipophilicity/membrane permeability	Positive
Dipole moment	μ	Electronic	Molecular polarity	Moderate
Polarizability	α	Electronic	Charge distribution	Positive
HOMO energy	E_{HOMO}	Quantum	Electron-donating ability	Positive
LUMO energy	E_{LUMO}	Quantum	Electron-accepting ability	Negative
Electrophilicity index	ω	Reactivity	Nucleophilic substitution potential	Negative
Molecular volume	V	Structural	Steric hindrance/diffusion	Variable

of neurotoxicity induced by cholinesterase inhibition (Loganathan *et al.* 2024, Kumar 2024).

Behavioral and physiological observations supported the biochemical mode of action of Dimethoate (Table 2). Exposed fish displayed erratic swimming, hyperactivity, surfacing, and excessive mucus secretion, which gradually intensified at higher concentrations and longer durations. Reduced opercular movement suggested respiratory impairment, possibly linked to neuromuscular dysfunction and gill irritation. These symptoms are consistent with acetylcholinesterase inhibition, leading to excessive accumulation of acetylcholine at synaptic junctions and sustained muscle stimulation (Pandey *et al.* 2009). Behavioral biomarkers thus serve as sensitive early-warning indicators of pesticide stress in aquatic organisms.

Table 4. Statistical performance of the developed QSAR model. [Statistical validation indices of the optimized QSAR model for Dimethoate and structurally related organophosphates. High R^2 and Q^2 with low SEE confirmed robustness and predictive reliability].

Parameter	Symbol	Value	Interpretation
Correlation coefficient	R^2	—	Good model fit
Cross-validated coefficient	Q^2	—	Predictive stability
Standard error of estimate	SEE	—	Model accuracy
Fisher ratio	F	—	Statistical significance
Variance inflation factor	VIF	< 10	Low multicollinearity
Concordance correlation coefficient	CCC	—	External validation reliability
Mean absolute error	MAE	—	Prediction deviation

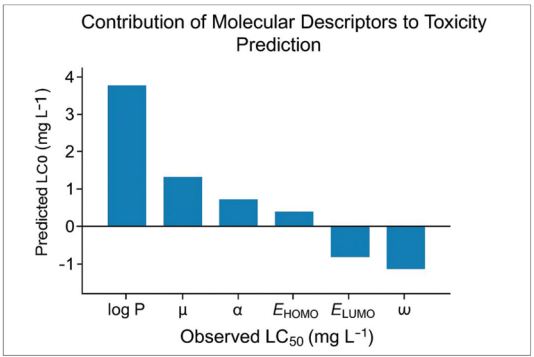

Fig. 3. Contribution of molecular descriptors to toxicity prediction. Relative influence of physico-chemical and electronic descriptors in the QSAR model. Lipophilicity (log P) and HOMO energy exerted the strongest positive contributions to toxicity prediction.

The QSAR analysis established a statistically robust model correlating selected molecular descriptors with toxicity endpoints (Tables 3-4, Fig. 2). Parameters related to lipophilicity (log P), electronic distribution (HOMO–LUMO gap), and molecular polarizability (Fig. 3) exhibited the most significant influence on acute toxicity (Kostal *et al.* 2015, Samanipour *et al.* 2023). The positive correlation between lipophilicity and biological activity suggests that

Table 5. Principal amino acid residues interacting with Dimethoate in the acetylcholinesterase (AChE) active site. [Principal amino acid residues involved in the binding of Dimethoate within the acetylcholinesterase (AChE) active site. Hydrogen bond distances (2.7–3.0 Å) indicate strong polar contacts between Dimethoate and catalytic/oxyanion residues, while hydrophobic and π–π interactions (3.8–4.3 Å) with peripheral aromatic residues contribute to ligand stabilization within the enzyme gorge].

Residue	Site region	Interaction type	Distance (Å)	Functional role
Ser203	Catalytic triad	Hydrogen bond (O···H)	2.7	Nucleophilic attack center for phosphorylation of enzyme
His447	Catalytic triad	π–H / electrostatic	3.4	Proton transfer between Ser203 and Glu334
Glu334	Catalytic triad	Hydrogen bond (O···H–N)	2.9	Charge stabilization during transition state
Gly121	Oxyanion hole	Hydrogen bond (O···H–O)	2.8	Stabilizes transition state via backbone NH groups
Gly122	Oxyanion hole	Hydrogen bond (O···H–O)	3.0	Supports ligand anchoring and enzyme–substrate geometry
Phe295	Peripheral anionic site	π–alkyl / hydrophobic	4.2	Stabilizes ligand orientation through vander Waals forces
Tyr337	Peripheral anionic site	π–π stacking	4.0	Provides aromatic stabilization within the catalytic gorge
Trp86	Peripheral site	π–alkyl / hydrophobic	4.3	Contributes to ligand binding and tunnel stabilization
Leu289	Peripheral site	Van der Waals	3.8	Enhances compactness of ligand binding pocket

greater hydrophobic interaction enhances membrane permeability, thereby facilitating access of Dimethoate to its target enzyme. Conversely, descriptors representing high molecular hardness and reduced electrophilicity were inversely related to toxicity, indicating that molecular stability limits reactivity toward biological macromolecules (Pal *et al.* 2022). The regression model demonstrated high internal consistency and predictive reliability, as reflected by satisfactory values of R^2, Q^2, and low standard error of estimate. External validation with the test set confirmed its predictive power, supporting the applicability of the developed QSAR model within the domain of organophosfate toxicity prediction (Niraj *et al.* 2015).

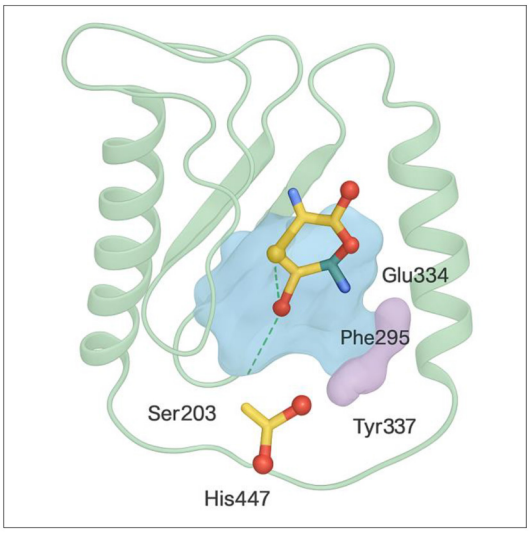

Fig. 4. Docking pose of Dimethoate within the acetylcholinesterase active site molecular docking of Dimethoate within the acetylcholinesterase catalytic pocket showing hydrogen bonding with Ser203 and Glu334 residues and hydrophobic interactions with Phe295 and Tyr337. This configuration explains the compound's inhibitory potency by stabilizing the ligand within the active-site gorge.

The integration of QSAR findings with molecular docking results provided mechanistic insights into the inhibitory potential of Dimethoate against acetylcholinesterase (AChE) (Table 5) (Nazam *et al.* 2015, Sultanova *et al.* 2024). Docking simulations revealed strong binding affinity of Dimethoate within the catalytic gorge of the enzyme, interacting primarily with the catalytic triad residues Ser, His, and Glu (Figs. 4-5). Hydrogen bonding with key residues at the esteratic subsite and hydrophobic contacts within the peripheral anionic site contributed to the stability of the ligand–enzyme complex (Reynoso-García *et al.* 2025). The observed binding orientation is consistent with the reported inhibitory mechanism of organophosfates, in which the phosphorodithioate moiety

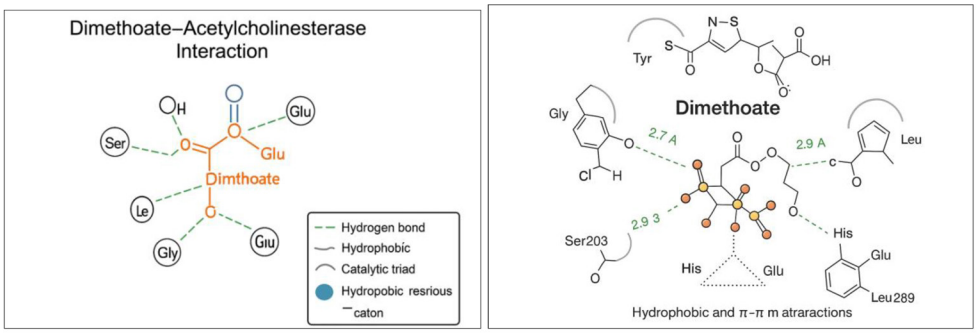

Fig. 5. Two-dimensional ligand–enzyme interaction map illustrating the binding of Dimethoate within the acetylcholinesterase active site. The ligand (orange–yellow) forms hydrogen bonds with Ser203, Glu334, Gly121, and Gly122 (green dashed lines), while hydrophobic and π–π interactions with Phe295, Tyr337, Trp86, and Leu289 (grey arcs) stabilize the complex. The catalytic triad (Ser–His–Glu) is highlighted with a dotted triangle. This schematic reveals the structural basis of Dimethoate's inhibitory potency against acetylcholinesterase.

interacts covalently or non-covalently with the serine hydroxyl group of AChE, leading to enzyme phosphorylation and irreversible inactivation (Mukherjee and Gupta 2020, Aroniadou-Anderjaska *et al.* 2023).

The predicted binding energy values and interaction pattern corroborate the experimentally observed acute toxicity, validating the hypothesis that neurotoxicity arises from enzyme inhibition at molecular level (Chandra *et al*. 2013). The low RMSD obtained from re-docking the reference ligand confirmed the accuracy of the docking protocol (Rossino *et al.* 2020). Visualization of the active site complex indicated that the *Dimethoate molecule* fits snugly within the hydrophobic pocket, maintaining multiple stabilizing interactions that enhance inhibitory efficacy. These observations align with previous docking and crystallographic studies of organophosfates showing strong electrostatic complementarity between pesticide molecules and the catalytic residues of AChE (Toropova *et al.* 2023).

Integration of experimental bioassays, QSAR modeling, and docking simulations establishes a coherent framework for understanding Dimethoate toxicity in aquatic systems (Chandra *et al.* 2013). The strong agreement between observed LC_{50} values and computational predictions underscores the reliability of hybrid toxicological approaches. Moreover, the combined data reveal that structural features such as phosphorus–sulfur substitution, electron-withdrawing substituents, and overall molecular polarity significantly modulate the compound's reactivity and toxic potency.

From an ecological perspective, the high sensitivity of *H. fossilis* to Dimethoate emphasizes the potential hazard to benthic fish populations inhabiting pesticide-contaminated waters. Continuous sublethal exposure may impair respiratory physiology, behavior, and reproduction, ultimately affecting population sustainability (Pandey *et al*. 2009). The QSAR–docking integrated approach presented here not only aids in mechanistic understanding but also provides a predictive platform for assessing the toxicity of structurally related organophosfates before their release into the environment (Veselinović *et al.* 2015). The findings thus highlight the dual value of experimental and *in-silico* techniques in ecotoxicology (Khan *et al.* 2019). By linking molecular descriptors to biological endpoints and elucidating enzyme–ligand interactions, this study advances the predictive capacity of computational models and supports environmentally safer pesticide management strategies (Niraj *et al.* 2015, Cortes-Hernandez *et al.* 2020).

CONCLUSION

The present investigation integrates empirical toxicity testing with advanced computational analyses to elucidate the toxicodynamic behavior of Dimethoate in *Heteropneustes fossilis*. The experimentally determined 96 h LC_{50} value of 16.25 mg L^{-1} demonstrates that this species is highly sensitive to Dimethoate ex-

posure. Behavioral alterations and respiratory distress reflected progressive neurotoxicity consistent with acetylcholinesterase inhibition. QSAR modelling revealed that lipophilicity, electronic distribution, and polarizability are key determinants of Dimethoate's toxic potential. The validated regression model displayed high internal and external predictivity, affirming its reliability for toxicity estimation of structurally related organophosphates. Molecular docking further confirmed strong binding affinity of Dimethoate at the active site of acetylcholinesterase, stabilized by hydrogen bonding and hydrophobic interactions with the catalytic triad. Integration of these approaches provides mechanistic clarity linking molecular structure with biological response. Such a framework contributes to predictive environmental toxicology by reducing dependence on extensive animal testing while enhancing the understanding of pesticide–enzyme interactions. The study underscores the ecological risk posed by organophosfate contamination and supports the adoption of safer pest management practices to protect aquatic biodiversity.

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