Does Size Matter? Toxicity of Polyethylene Terephthalate Nanoplastics of Different Sizes on Aquatic Organisms based on Molecular Docking and Machine Learning Methodology

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Introduction

Natural weathering processes can cause discarded plastic to break down into microplastics (MPs; 100 nm—5 mm size) and/or nanoplastics (NPs < 100 nm). NPs could be consumed by biota because of their small size. NPs are a fast-evolving topic that are important in a variety of sectors, including human toxicity, food and environmental study. Ingestion of NPs can expose aquatic biota, which can then accumulate the particles and experience negative effects. It has not been possible to directly analyse the consequences of NPs exposure in humans (Enyoh et al, 2022).

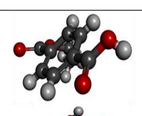
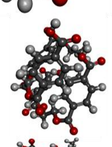
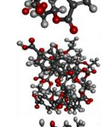
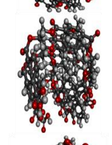
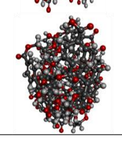
Studies have often translated toxicity on some aquatic organism to human because of similar genetic traits (Araujo et al, 2022). The aquatic organism Zebrafish, which shares genetic traits with mammals, has been utilized extensively in MPs research for assessing environmental toxicity and studying genetic developments (Bhagat et al, 2020; Araujo et al, 2022). The toxicity of chemicals to zebrafish mainly involved the cytochrome P450 (CYP) enzymes (Pallan et al, 2015). The cytochrome P450 (CYP) enzymes catalyze oxidative transformation, which results in the activation or inactivation of a wide range of endogenous and foreign substances. This has implications for both healthy physiology and disease processes.

On the other hand, an electric rays' fish (ERF) was also considered as model specie for aquatic organism in this study to evaluate toxicity of NPs to benthic organism as they often exist in the coral reefs, mud, or sandy bottoms (Last et al, 2016). ERFs are renowned for having the ability to generate an electric discharge that may range in voltage from 8 to 220 volts, depending on the species, and is used to defend themselves as well as to shock prey (Michael, 1996). The species of the genus Torpedo may be the most well-known (Last et al, 2016). To evaluate toxicity of pollutant to this organism, the Acetylcholinesterase (AChE) is the primary target. The AChE is a primary cholinesterase in organisms that catalyzes the breakdown of acetylcholine and some other choline esters that function as neurotransmitters. Compounds that inhibit AChE irreversibly may lead to convulsions, bronchial constriction, muscular paralysis, and death by asphyxiation (Colović et al., 2013).

In this study, the toxicity of Polyethylene Terepthalate (PET) NPs of different sizes to aquatic organisms were studied using a molecular docking (MD) approach coupled with machine learning (ML) methodology, using the CYP450 in zebrafish and AChE in ERF as primary targets. The properties data set collected from NPs structures designed and analyzed in silico were labelled using supervised ML and used to predict toxicity of the PET NPs to the studied aquatic organisms.

Methods

Table 1. The PET NPs prepared insilico for the study

Samples	Chain length x repeat unit (molecular size in nm)	Structure	Chemical formula	Molecular v
PET 1	1 x 1 (1 nm)		$C_{10}H_{10}O_4$	194.18
PET NPs 2	2 x 2 (4 nm)		$C_{40}H_{36}O_{16}$	772.70
PET NPs 3	3 x 3 (9 nm)		$C_{90}H_{78}O_{36}$	1747.66
PET NPs 4	4 x 4 (16 nm)		$C_{160}H_{136}O_{64}$	3082.85
PET NPs 5	5 x 5 (25 nm)		$C_{250}H_{210}O_{100}$	4814.28

- The PET NPs of different sizes (Table 1) were prepared and characterized insillico using the 3D atomistic in Biovia Material Studio 8.
- Site directed docking of the NPs was performed on the active sites of the enzymes (Fig. 1) with Autodock Vina in PyRx software version 0.8
- This study uses Multilayer Perceptrons-based (MLP) Artificial Neutral Networks (ANNs) as its machine learning strategy.
- Different statistical models, such as the average correlation factor (R^2), root mean square error (RMSE), mean absolute error (MAE), and sum of square error (SSE) were used to check and evaluate how well an ANN model predicts the output (binding affinity, kcal/mol) (Bi et al, 2020; Dubdub, 2022).

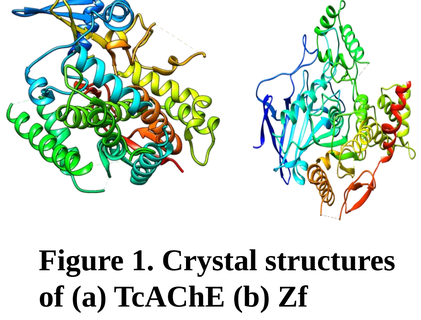


Figure 1. Crystal structures of (a) TcAChE (b) Zf CYP450

Table 2. The range of data employed in ANN training

Parameter	Type	Minimum value	Maximum value
Binding affinity for AChE (kcal/mol)	Output	-7.1	-9.9
Binding affinity for CYP450 (kcal/mol)	Output	-5.2	-8.1
Molecular size (nm)	Input	1	25
Structure Energy (kcal/mol)	Input	13.51	200.16
Occupied volume ($Å^3$)	Input	168.32	3748.59
Surface area ($Å^2$)	Input	189.78	2148.33

Results and Discussion

Toxicity study

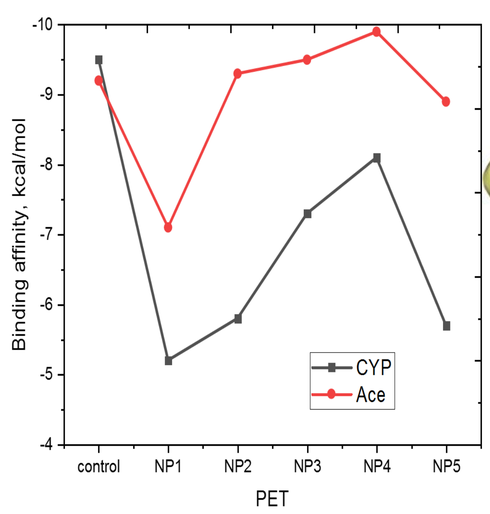


Figure 2. Binding affinities

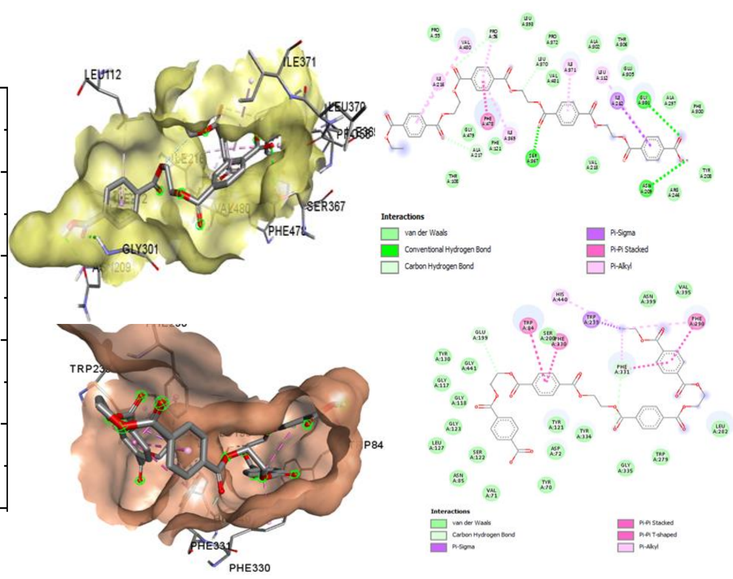


Figure 3. Protein ligand interaction

The results showed that the binding affinities of the NPs increased steadily from – 7.1 kcal/mol to – 9.9 kcal/mol for NP1 to NP4, and experienced a drop at NP5 (– 8.9 kcal/mol) for TcAChE while also Zf CYP450 had similar pattern which ranged from -5.2 kcal/mol to -8.1 kcal/mol. The binding affinity of NP2, NP3, and NP4 were higher than the control for TcAChE, indicating that they have a higher inhibitory potential at this site and therefore could impact more toxicity on the enzyme than the native inhibitor (-)-galanthamine. However, the abiraterone control for Zf CYP450 showed the highest toxicity with binding affinity of -9.5 kcal/mol compared with the PET NPs.

Machine learning

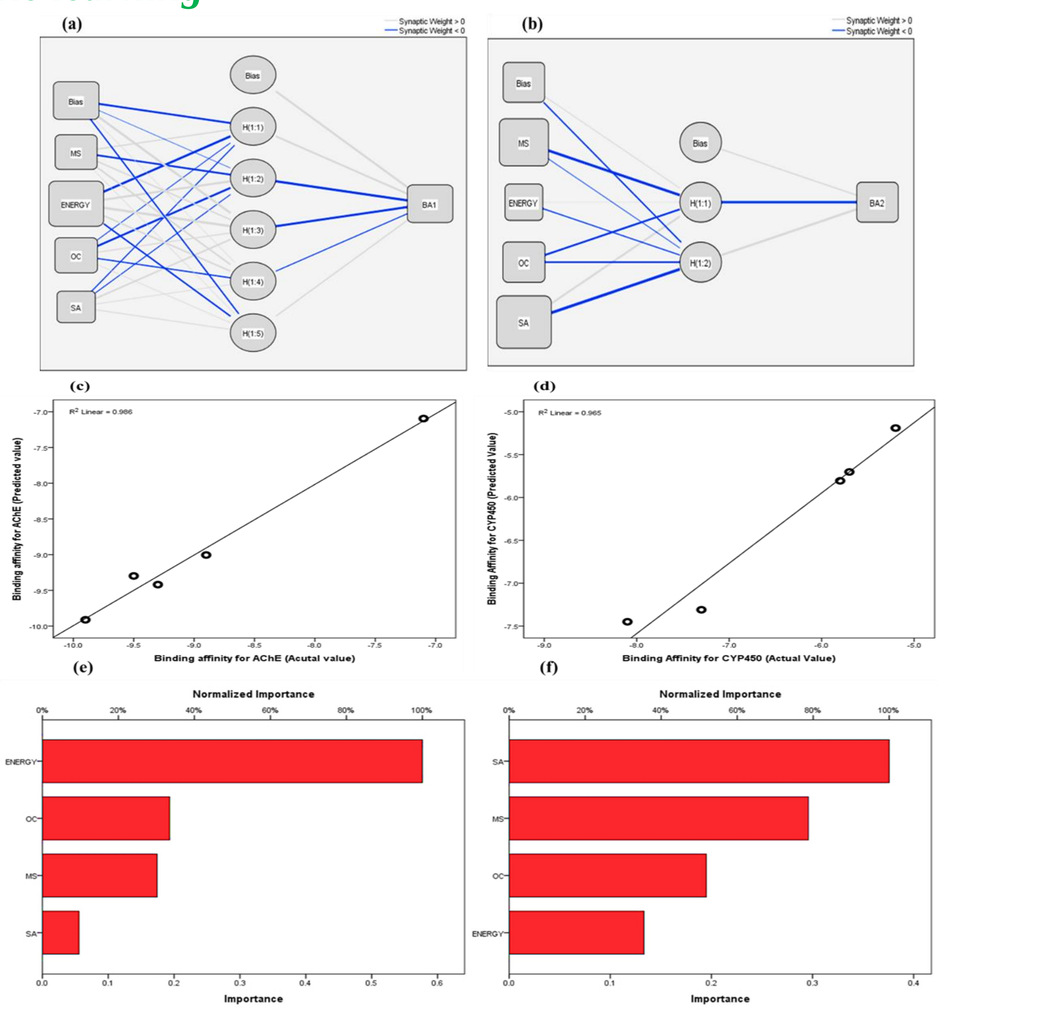


Figure 4. ANN (a,b); linear regression for predicting toxicity (c,d) and most important parameter (e,f)

The MLP ANN was able to predict the toxicity of the PET NPs based on the inherent properties with coefficients of 0.986 and 0.965 for Tc AChE and Zf CYP450 respectively. The validation of the ANN was tested by error analysis models which confirmed that the ANN was at high accuracy.

Conclusions

The toxicity of the PET NPs increased with increasing size to a certain limit (16 nm) after which there was a drop in its toxicity due to inefficient enzyme-NP binding. Using the characteristics data from the PET NPs, the factors responsible for its toxicity was evaluated by Machine Learning approach based Artificial Neural Network (ANN) model. The predicted data obtained using ANN was at high accuracy, as each of the models had, $R^2 > 0.9$ and showed that both surface area and reactivity (energy) were the most important properties for PET NPs toxicity. The study confirmed that size of PET NPs can influence its toxicity to aquatic organisms and the surface area and reactivity (energy) of the NPs are important for its toxicity.

Bibliography

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