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Article

Computational evidence for differential endocrine disruption by DEHP and PET via estrogen receptor beta binding

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SUMMARY

Nanoplastics, particularly those derived from polyethylene terephthalate (PET) and polyvinyl chloride (PVC), represent a growing environmental concern with potential impacts on human health through endocrine disruption. This study investigated the binding interactions between two nanoplastic models—di-(2-ethylhexyl) phthalate (DEHP, representing PVC) and a PET monomer—and human estrogen receptor beta (ERβ) using computational docking experiments. We hypothesized that both compounds would demonstrate strong binding affinities to ERβ, potentially disrupting normal estrogen signaling. Using DockThor for docking simulations, we found that DEHP exhibited binding affinities comparable to the native ligand estradiol (chain A: -10.02 ± 0.23 kcal/mol; chain B: -10.07 ± 0.22 kcal/mol), while PET showed significantly weaker binding (chain A: -8.25 ± 0.05 kcal/mol; chain B: -8.15 ± 0.05 kcal/mol). Toxicity predictions from ProTox and Virtual models for property Evaluation of chemicals within a Global Architecture (VEGA) platform corroborated these findings, with DEHP showing higher likelihoods of endocrine disruption and PET demonstrating minimal impact. Analysis of root mean square deviation (RMSD) values revealed that both compounds induced conformational changes in ERβ similar to estradiol, with chain-specific differences observed. These findings suggest that DEHP poses a greater risk for endocrine disruption, while PET's weaker and more variable binding indicates lower potential for direct receptor interference. This study provides computational evidence for differential endocrine disruption potential among nanoplastics and highlights the need for experimental validation through *in vitro* and *in vivo* studies.

INTRODUCTION

In the 21st century, plastic pollution has emerged as a serious environmental issue on a global scale (1). Nanoplastics, plastic particles approximately 1–1000 nanometers in diameter, are formed through the decomposition and breakdown of larger plastics, such as water bottles, food containers, or fibers from clothing (2). These nanoplastics are small enough to enter the human body through inhalation and ingestion, and then enter cells through endocytosis, a type of active transport (3). As these plastics have long half-lives, nanoplastics may not only affect broad biological processes by spreading through the bloodstream, but they may have long-term implications as they accumulate within cells and organ systems (4).

Two major classes of nanoplastics include polyethylene terephthalate (PET) and its analogs, used primarily in plastic bottles and clothing fibers, and polyvinyl chloride (PVC), used in various industrial applications (5, 6). Previous studies have shown that PET accumulation can cause digestive issues, immune dysfunction, and embryological deficiencies, as well as affect testicular function and reproductive development (7, 8). Accumulation of PVC, meanwhile, has been associated with pulmonary dysfunction and various types of cancer (9, 10).

While many studies have focused on the gross impacts of nanoplastic accumulation, their effects on specific receptor signaling pathways remain understudied. One critical signaling pathway in humans is the estrogen signaling pathway. A hormone found in both males and females, estrogen regulates the body's reproductive health while also playing a role in regulating cholesterol and blood sugar levels, circulation, and brain function (11). Estrogen binds to cytoplasmic estrogen receptors, of which there are two types. The uterus, liver, kidney, and heart primarily express estrogen receptor alpha (ERα), which promotes cellular growth (12, 14). Estrogen receptor beta (ERβ), on the other hand, is predominantly expressed in tissues of the lungs, prostate, and ovarian granulosa cells and promotes cell differentiation (13). Disruptions in estrogen signaling can have devastating health consequences, such as amenorrhea, infertility, and osteoporosis (14).

Previous studies have yielded conflicting results regarding the influence of nanoplastics on estrogen receptors in humans and other organisms. One study found that polystyrene nanospheres, a type of nanoplastic, increased expression of ERα and ERβ and estrogen-responsive activity in human cells and zebrafish. In addition, researchers found that polystyrene nanospheres exacerbate the already-known effects of homosalate, an organic compound commonly used in sunscreens, which was found to increase estrogen receptor expression (15). Another study found that higher concentrations of polystyrene nanoplastics inhibited estrogen receptor activation (16). To our knowledge, no study has investigated the impact of PET analogs and PVC-derived molecules on estrogen receptor binding using molecular docking approaches.

Our study aimed to build upon previous contradictory literature by determining whether PET or PVC might impact estrogen's binding to its cognate receptors using virtual docking

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experiments. Our computational approach provides a safe, efficient, and quantitative method of hypothesis generation and determination of interaction mechanisms. Furthermore, virtual docking experiments remedy the challenging, time-consuming, and expensive nature of traditional, wet lab binding experiments. We selected PET and PVC due to their extensive use and well-established research history. For our experiment, PET monomer was used as a model for PET plastics, and di-(2-ethylhexyl) phthalate (DEHP) was used to model PVC-derived compounds. Both molecules have been used in previous studies to model nanoplastic impact due to their ubiquitous nature and easy absorption (5,17). We focused on ERβ because of its significant role in cellular differentiation, broader tissue distribution, and specific localization in environmentally relevant tissues such as the gastrointestinal tract, where nanoplastic exposure primarily occurs (18,19).

ERα and ERβ, as nuclear receptors that bind a lipophilic ligand, are particularly vulnerable to interference from the relatively hydrophobic structures of nanoplastic particles. We therefore hypothesized that both PET and DEHP would bind to ERβ with high affinity greater than or equal to that of ERβ's native ligand estradiol (-7 kcal/mol) and potentially disrupt normal estrogen signaling. Our results showed that DEHP binds ERβ with an affinity comparable to that of estradiol, suggesting potential for competitive inhibition. In contrast, PET displayed weaker and more inconsistent binding, suggesting PET is less likely to cause direct receptor interference. Accordingly, these findings supported our hypothesis for DEHP, but did not support our hypothesis for PET. These results have important implications for understanding the health risks posed by these common plastic pollutants. By identifying DEHP as a more potent endocrine disruptor at the molecular level, our study may inform risk assessment strategies and highlight the need for prioritizing regulatory attention on PVC-derived plastics. Furthermore, our computational approach demonstrates a cost-effective method for screening nanoplastic compounds for hormonal disruption potential, which can guide future studies and public health interventions aimed at mitigating nanoplastic exposure.

RESULTS

Despite extensive research on the gross impacts of nanoplastics on human health, the specific molecular mechanisms by which nanoplastics disrupt estrogen receptor signaling remain poorly understood. We hypothesized that both DEHP and PET would bind strongly to ERβ with affinities comparable to or exceeding that of the natural ligand estradiol, thereby potentially interfering with normal estrogen signaling. To test this hypothesis, we conducted computational docking experiments and toxicity screens to evaluate the binding interactions and potential for endocrine disruption.

	Toxicity		
	LD50 (mg/kg)	*Toxicity Class*	*Predicted Estrogen Disrupting (Y/N)*
PET	3535	5	N
DEHP	1340	4	N

Table 1: Summary of the toxicity of PET and DEHP nanoplastics. The table shows data including LD50 (which represents the amount of substance lethal to half the population), toxicity class (which represents the toxicity levels of substances out of a scale of 6 based on LD50, with a score of 1 being fatal if swallowed and a score of 6 being nontoxic), and the predicted effect of whether the nanoplastic will have estrogen-disrupting effects. The summary data were collected using ProTox after digital simulations and analysis of the nanoplastics.

Toxicity Screen

To establish a preliminary understanding of the biological effects of DEHP and PET, we used modeling software to predict the toxicity parameters of our nanoplastics. ProTox is a machine-learning website equipped with 61 models for the prediction of the toxicity of chemicals, including metabolic toxicity, organ toxicity, and impact on various nuclear or molecular pathways (see Materials and Methods). ProTox analysis predicted that DEHP had a median lethal dose (LD50) of 1340 mg/kg, placing it in a toxicity class of 4 (harmful if swallowed) (**Table 1**). In contrast, PET showed a much higher LD50 at 3535 mg/kg, placing it in a toxicity class of 5 (may be harmful). Notably, ProTox predicted both compounds to be incapable of estrogen-disrupting effects.

To obtain a broader toxicity profile, we analyzed several relevant endpoints, such as biodegradability, reproductive and developmental toxicity, and estrogen receptor interactions. We used the Virtual Models for Property Evaluation of Chemicals within a Global Architecture (VEGA) quantitative structure activity relationship (QSAR), an application that provides over 100 virtual models for the property and toxicity evaluation of chemicals and outputs similarity and reliability data based on chemical similarity of the query molecule with those in the QSAR database (see Materials and Methods).

For DEHP, the Proctor and Gamble (PG) model, a very

	Model	***Prediction***	***Reliability***	***AD Index***	***Similarity Index***
PET	IRFMN-CERAPP	Possible NON-active	High (3/3)	0.952	0.906
	IRFMN	Inactive	High (3/3) Accuracy & Concordance = 1	0.966	0.933
DEHP	IRFMN-CERAPP	Possible NON-active	High (3/3)	0.952	0.906
	IRFMN	Active	High (3/3) Accuracy & Concordance = 1	1.000	1.000

Table 2: Summary of model toxicity predictions for PET and DEHP. The table shows results including predictions, reliability, applicability domain (AD) index, and similarity index. IRFMN-CERAPP models estrogen receptor-mediated effect, while IRFRM models estrogen receptor relative binding affinity. Higher AD index indicates greater confidence in model predictions; higher similarity index indicates compound similarity to training dataset molecules. Results were obtained using Virtual Models for Property Evaluation of Chemicals within a Global Architecture (VEGA).

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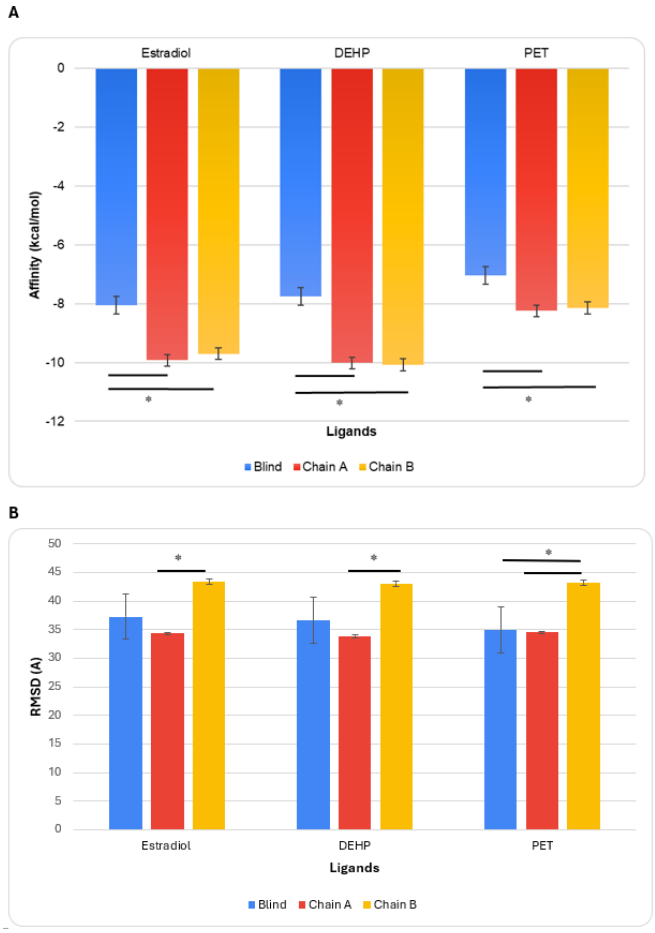

Figure 1: Results of molecular docking of estradiol, DEHP, and PET to ERβ. (A) Binding affinity (kcal/mol) for blind, chain A, and chain B docking of estradiol, DEHP, and PET. **(B)** Root mean squared deviation (RMSD) values (Å) for blind, chain A, and chain B docking of estradiol, DEHP, and PET. Values were collected using DockThor after 3D conformations of each molecule were tested against a crystallized model of ERβ and analyzed. The bar graphs show mean ± standard deviation for each value (n = 7-10). Statistical significance was determined by a two-tailed t-test, *p < 0.05.

broad reproductive toxicity algorithm, predicted that DEHP may be a reproductive and developmental toxicant that binds to estrogen receptors. The Istituto di Ricovero e Cura a Carattere Scientifico-Istituto di Ricerche Farmacologiche Mario Negri (IRCCS-IRFMN), a more specialized model, predicted that DEHP may be an endocrine disruptor, as well as a non-biodegradable substance with a reliability score of 1 out of 3 (where 1 indicates low reliability and 3 indicates high reliability based on how well the compound matches the training dataset). The estrogen receptor-mediated effect model IRFMN-Collaborative Estrogen Receptor Activity Prediction Project (IRFMN-CERAPP), an estrogen functional impact prediction model, showed high reliability that DEHP was possibly inactive (therefore, not interacting with estrogen receptors), indicated by an applicability domain (AD) index of 0.952 and a similarity index of 0.906 (**Table 2**). AD indices range from 0-1, with a higher value indicating greater confidence in model predictions based on chemical similarity to training compounds. The similarity index, also ranging from 0-1, indicates higher similarity of the query compound to molecules in the training dataset with a higher value. However, the estrogen receptor binding affinity model (IRFMN), trained on a different subset of molecules and intended for regulatory analysis, contradicted this, showing DEHP as active (activating the estrogen pathway) with high reliability (AD index: 1.0, similarity index: 1.0) (**Table 2**). Altogether, this data suggests that while there is some uncertainty regarding DEHP's estrogen receptor activity, multiple models indicate potential for reproductive toxicity and endocrine disruption.

For PET, VEGA found it to be possibly biodegradable (IRFMN model, reliability: 3/3). IRFMN-CERAPP showed high reliability (AD index: 0.952, similarity index: 0.906) that PET was possibly inactive. IRFMN confirmed similar inactivity with high reliability (AD index: 0.966, similarity index: 0.933) (**Table 2**). These results consistently indicate that PET is unlikely to directly interfere with estrogen signaling, suggesting a lower health disruption profile compared to DEHP.

Binding Affinity Analysis

Analysis of the binding affinities for estradiol, DEHP, and PET was essential for understanding how each ligand interacts with ERβ. We conducted blind docking as well as targeted docking to chains A and B of the receptor using DockThor, a protein-ligand docking program, in order to determine binding affinities. As expected for a native ligand, estradiol demonstrated strong binding across all three docking contexts (**Figure 1A, Table 3**). The blind docking showed statistically weaker affinity than chain-specific docking due to the unrestricted search space. However, there was no statistically significant difference between the binding affinity to chain A versus chain B (t=-2.344; p=0.053). DEHP exhibited strong binding affinities comparable to estradiol (**Table 3**). Similar to estradiol, blind docking showed a statistically weaker binding, and there was no significant difference between chains A and B (*t=0.59; p=0.56*). In chain A, DEHP bound with similar affinity to estradiol (*t=0.96; p=0.35*) while in chain B, DEHP showed higher binding affinity than estradiol (*t=3.74; p<0.01*).

PET demonstrated weaker binding affinity compared to both estradiol and DEHP across all contexts (**Table 3**).

	Affinity (kcal/mol)								
	Blind Docking			*Chain A Docking*			*Chain B Docking*		
Binding Poses	**Estradiol**	**DEHP**	**PET**	**Estradiol**	**DEHP**	**PET**	**Estradiol**	**DEHP**	**PET**
1	-9.918	-7.827	-7.365	-10.169	-10.023	-8.243	-9.976	-10.014	-8.252
2	-9.854	-7.611	-7.163	-10.139	-9.954	-8.310	-9.959	-10.127	-8.160
3	-8.125	-7.773	-6.997	-10.010	-9.976	-8.295	-9.882	-10.437	-8.109
4	-7.503	-7.714	-7.293	-9.902	-10.394	-8.277	-9.853	-9.853	-8.139
5	-6.474	-7.429	-6.740	-9.829	-10.281	-8.204	-9.673	-10.192	-8.166
6	-7.850	-7.350	-6.776	-9.816	-9.708	-8.208	-9.580	-10.313	-8.061
7	-7.722	-7.922	-7.227	-9.786	-9.903	-8.180	-9.487	-10.167	-8.124
8	-7.659	-8.190	-6.841	-9.741	-9.802		-9.444	-9.952	-8.162
9	-7.749	-7.529	-6.883		-9.851		-9.473	-9.721	-8.140
10	-7.711	-8.225			-10.27			-9.969	
Average	-8.057	-7.757	-7.032	-9.924	-10.016	-8.245	-9.703	-10.075	-8.146
Standard Deviation	1.056	0.296	0.236	0.164	0.227	0.050	0.217	0.2153	0.052

Table 3: Binding affinity values for all conformational docking poses. Binding affinities are reported in kcal/mol. Multiple docking poses were examined to ensure the data were accurate and consistent. Only 10 maximum possible binding poses were available, and DockThor was only able to provide 10 binding poses for some binding contexts.

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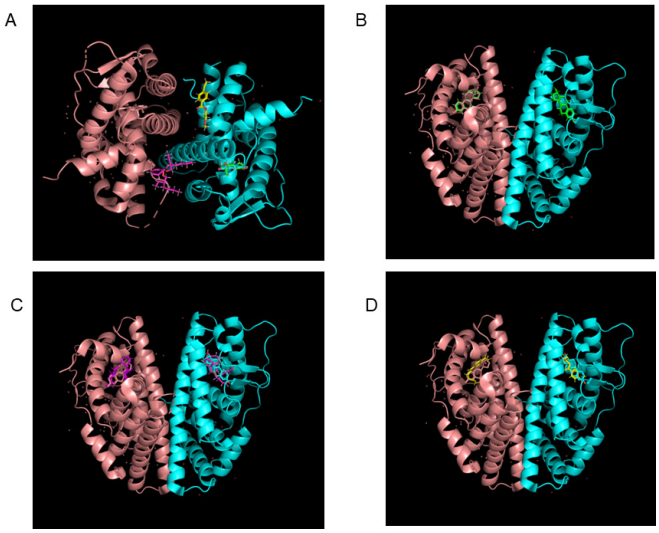

Figure 2: Representation of best-ranked binding poses. (A) Blind docking of estradiol (green), DEHP (magenta), and PET (yellow) to ERβ in its native estradiol-bound conformation. The native ligand of estrogen is already present in both chains (shown in pink on chain A (pink) and blue on chain B(blue)). **(B)** Binding to chain A (pink) and chain B (blue) for estradiol (green) compared to its native conformation. **(C)** Binding to chain A (pink) and chain B (blue) for DEHP (magenta) compared to native estradiol. **(D)** Binding to chain A (pink) and chain B (blue) for PET (yellow) compared to native estradiol. Best-ranked poses were labelled by DockThor after docking experiments, and results were visualized using PyMol.

Furthermore, PET showed differential binding with statistically different results across all three contexts *(t=13.29* and *p<.00001* for blind versus chain A; *t=13.85* and *p<.00001* for blind versus chain B; *t=-3.87* and *p<.001* for chain A versus chain B). Therefore, PET binding to ERβ may potentially be context-dependent, as PET demonstrated the greatest variability in binding affinity across the three contexts.

RMSD Analysis

Analysis of the root mean square deviation (RMSD) values was necessary to determine the conformational changes in ERβ upon ligand binding, with a higher RMSD indicating a greater conformational change. All three compounds demonstrated similar RMSD values in blind docking and chain A contexts. In chain B, all compounds demonstrated a higher RMSD value than in blind docking and chain A, with a marginal difference between estradiol and DEHP (*t=2.13, p=0.04*) (**Figure 1B**). Estradiol binding induced greater conformational changes in chain B compared to chain A (*t=-51.64, p< 0.00001*). DEHP similarly induced greater conformational changes in chain B compared to chain A (*t= -91.10, p< 0.00001*), as did PET (*t=-34.93, p< 0.00001*). In chain A, DEHP had statistically lower RMSD values than both estradiol and PET, suggesting less conformational impact in this chain.

Analysis of the Best Ranked Docking Poses

To understand binding patterns, we analyzed the best-ranked poses for each ligand across three binding contexts as determined by DockThor (**Figure 2**). In blind docking, estradiol showed the highest affinity in its best ranked pose compared to DEHP and PET. This high affinity was driven by favorable van der Waals and electrostatic interactions. However, estradiol showed slightly higher total energy when compared to DEHP. PET, meanwhile, demonstrated the weakest binding and the only positive total energy value with lower electrostatic and van der Waals energy (**Table 4**).

For chain A binding, estradiol also showed the best binding profile, once again emphasizing strong van der Waals and electrostatic interactions. DEHP showed similar affinity, but with significant structural strain indicated by high total energy, positive van der Waals, and weak electrostatic interactions. PET showed weaker affinity with stronger van der Waals interactions but lower electrostatic interactions. In chain B docking, estradiol and DEHP demonstrated similar binding affinities. Estradiol showed higher van der Waals and electrostatic interactions compared to DEHP. PET showed weaker affinity but with a similar van der Waals contribution to estradiol. However, it had an almost negligible electrostatic contribution (**Table 4**).

RMSD values in best-ranked poses demonstrated higher variability in chain B compared to chain A. DEHP showed the lowest RMSD in chain-specific docks compared to estradiol, with PET showing intermediate values (**Figure 1B, Table 5**).

DISCUSSION

This study sought to address a gap in understanding the molecular mechanisms by which nanoplastics may disrupt endocrine signaling. Specifically, we investigated whether PET and PVC-derived nanoplastics bind to ERβ with affinity comparable to the natural ligand estradiol, thereby potentially interfering with normal hormonal function. Using computational docking simulations and toxicity prediction models, we evaluated the binding interactions of DEHP (as a representative of PVC) and PET monomer with ERβ. Our principal finding demonstrated a higher potential for endocrine disruption from DEHP compared to PET, thereby suggesting differential health risks among different nanoplastic types.

Before we analyzed our experimental conditions, we first validated different binding metrics with our control, the estradiol model. Our estradiol model successfully replicated native ligand behavior, binding strongly and consistently across all three contexts (blind docking to ERβ, chain A docking, and chain B docking). The best docking pose reflected this behavior, showing high affinity in optimal binding configurations with favorable van der Waals forces and electrostatic interactions. RMSD values were generally low and stable for estradiol, indicating consistent binding.

Interestingly, our study revealed differential binding

	Ligand	Total Energy (kcal/mol)	vdW Energy (kcal/mol)	Electrical Energy (kcal/mol)
Blind Dock	*Estradiol*	-0.576	-23.506	-17.043
	DEHP	-0.602	-17.425	-20.434
	PET	12.723	-12.433	-15.970
Chain A Dock	*Estradiol*	-3.235	-25.399	-17.315
	DEHP	70.855	14.518	-1.503
	PET	16.969	-23.614	-2.203
Chain B Dock	*Estradiol*	-3.202	-23.909	-18.865
	DEHP	57.487	-1.850	-0.888
	PET	16.320	-23.399	-0.303

Table 4: Summary of energetic data from the best-ranked poses for each binding context. The table shows data including total energy, van der Waals (vdW) energy, and electrical energy. Summary data was provided by DockThor after docking experimentation.

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Root Mean Squared Deviation (RMSD) (Å)									
	Blind Dock RMSD			*Chain A RMSD*			*Chain B RMSD*		
Binding Poses	**Estradiol**	**DEHP**	**PET**	**Estradiol**	**DEHP**	**PET**	**Estradiol**	**DEHP**	**PET**
1	43.148	34.092	40.543	34.474	33.977	34.497	43.150	42.922	42.320
2	43.090	33.695	29.628	34.147	34.155	34.407	43.010	43.250	43.254
3	41.368	42.517	41.248	34.181	33.664	34.287	43.078	42.930	43.169
4	31.356	33.769	41.619	34.416	33.739	34.287	43.354	42.929	44.105
5	45.291	41.757	30.321	34.021	34.108	34.638	43.057	42.808	42.370
6	31.529	41.842	30.179	34.546	34.124	34.419	43.184	43.125	43.176
7	32.229	42.158	41.400	34.449	33.778	34.981	43.768	43.155	44.020
8	30.596	30.771	29.700	34.221	33.982		43.182	42.601	42.907
9	43.065	34.568	30.088		33.500		44.426	43.356	43.190
10	30.926	30.872			33.686			43.011	
Average	37.260	36.604	34.970	34.308	33.871	34.502	43.357	43.009	43.168
Standard Deviation	6.336	4.872	5.924	0.188	0.227	0.244	0.461	0.221	0.616

Table 5: RMSD values for all conformational docking poses. RMSD is reported in Å for all DockThor conformational docking poses compared to the reference 3D crystallized structure of ERβ. Only 10 maximum possible binding poses were available, and DockThor was unable to provide 10 for all binding conformations.

patterns between chain A and B of ERβ, particularly regarding RMSD values. While this mechanism has not been studied in depth, one study showed that some metabolites, such as estriol, exhibit preferential binding affinity for one chain over another, suggesting that metabolite structure may influence chain selectivity (20). Another study examining estrogen receptor ligand selectivity found that selectivity is determined by the overall 3D structure of the molecule, as well as specific substituents and functional groups present (21).

We hypothesized that DEHP would bind with strong affinity to ERβ, stronger than estradiol, potentially interrupting its function. Our results indeed indicated that DEHP bound strongly to the receptor with affinities below -8 kcal/mol, comparable to estradiol in both blind and chain A docking. In chain B, DEHP demonstrated even higher affinity than estradiol, which supported our hypothesis and indicates possible strong competitive binding between estradiol and DEHP at this site. RMSD values for DEHP were similar to those of estradiol in blind and chain A docking as well, but showed differences in chain B. These results raise concerns for estrogen signaling disruption by DEHP, consistent with predictions from the IRFMN model in VEGA.

Previous studies corroborate these findings. In a study evaluating DEHP, maternal exposure was shown to reduce estrogen and progesterone levels of fetal mice (22). Another study analyzing the role of DEHP in cancer initiation found that its correlative carcinogenic properties were mediated through estrogen receptor activation (23), demonstrating a connection between DEHP, toxicity, and estrogen receptors. Therefore, our results are consistent with previous studies showing DEHP as a toxic substance affecting the endocrine system.

We also hypothesized that PET would bind ERβ with high affinity, stronger than estrogen. While PET demonstrated relatively high binding affinities in our study, it exhibited weaker binding across all three contexts compared to estradiol and DEHP, suggesting that PET is a weaker competitor for ERβ binding. Our hypothesis was that PET nanoplastics would have a higher binding affinity than estrogen, but this claim was not supported by our findings. RMSD values for PET showed higher variability compared to both estradiol and DEHP, though similar chain-specific differences were observed. These results suggest that PET binds less tightly and more inconsistently than estradiol and DEHP, indicating limited true engagement with ERβ. This aligns with findings from all toxicity reports, as both VEGA and ProTox predicted minimal estrogen receptor engagement for PET.

Although research on PET's impact on estrogen signaling is limited, previous studies have found that PET may facilitate the migration of endocrine-disrupting chemicals such as antimony and acetaldehyde derived from plastics into bottled water (24, 25). These studies concluded that PET may be indirectly related to endocrine disruption through facilitation of other endocrine-disrupting substances. Other studies examining PET and endocrine effects have found varying results. One study found widespread xenoestrogen contamination from plastic materials in bottled water (26). Another study on PET nanoplastic exposure in fish found correlations with increased estrogen levels (27). Our results highlight the need for continued research, as PET's impact on estrogen signaling remains unclear.

Our hypothesis was that both DEHP and PET would bind strongly to ERβ, stronger than estrogen. We found that DEHP bound more strongly than PET, altered receptor conformation more than PET, and was predicted to have lower biodegradability and a more consequential effect by toxicity models. Because DEHP behaves similarly to estradiol, it raises concerns for disrupting estrogen receptor functionality. These concerns, however, are somewhat limited by the overall high total energy, positive van der Waals, and weak electrostatic interactions demonstrated by DEHP in its best ranked pose and highlight the need for further research to elucidate the impact of DEHP binding to ERβ.

As previously highlighted, prior studies have emphasized the impact of DEHP and PET on endocrine disruption. However, specific molecular mechanisms by which these nanoplastics may disrupt estrogen signaling remain understudied. To our knowledge, our study is the first to examine the binding of DEHP and PET to ERβ using a molecular docking approach and provides a novel insight into the binding affinities and interactions of these nanoplastics with ERβ.

Several limitations could have influenced our results. We set the number of binding modes to 10 to balance computational efficiency with result quality; more binding modes could provide more accurate results. We used small monomers to model nanoplastics instead of more complex polymers for simplicity, which could have influenced binding interactions. All experiments were conducted computationally without experimental validation. Additionally, this study modeled protein-ligand interactions using static snapshots rather than dynamic simulations, helpful for short-term interactions but unable to capture long-term effects. Finally, we did not account for solvents and cofactors that may influence binding under physiological conditions. Future studies should address these limitations by conducting experimental validation, using molecular dynamics simulations, testing with larger polymer structures, and including solvents and cofactors to mimic physiological conditions. Future studies can also validate these results by conducting cell-based reporter assays using estrogen-responsive cell lines or competitive binding assays using fluorescently labelled estradiol to directly measure

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nanoplastic interference with natural hormone binding. Isothermal titration calorimetry experiments could provide thermodynamic parameters for nanoplastic-receptor binding to complement the computational energetic calculations presented here. Additionally, other nanoplastic types should be investigated to better understand the broader impact of nanoplastics on the endocrine system and human health.

In conclusion, our computational analysis demonstrates differential endocrine potential between DEHP and PET. In particular, DEHP exhibits strong binding to ERβ, comparable to natural estradiol, supported by toxicity models predicting significant endocrine disruption potential. PET, on the other hand, shows consistently weaker binding and minimal predicted hormonal disruption. These findings have important implications for risk assessment and regulation of plastic pollutants. Our results suggest that PVC-derived compounds, such as DEHP, warrant greater concern and stricter regulatory oversight in terms of direct endocrine disruption. Furthermore, this study demonstrates the utility of computational molecular docking approaches as a cost-effective screening tool for nanoplastic toxicity, enabling rapid assessment of multiple compounds before committing resources to experimental validation. As nanoplastic pollution continues to increase globally, such molecular insights are crucial for informing evidence-based policies and public health interventions, with goals of protecting human health from environmental contaminants.

MATERIALS AND METHODS

Ligand Preparation

One compound was selected to represent each nanoplastic category: polyethylene terephthalate (PET) monomer (PubChem CID: 18721140) to represent PET (28) and di(2-ethylhexyl) phthalate (DEHP) (PubChem CID: 8343) to represent PVC (29). Estradiol was used as the control (PubChem CID: 5757) (30). 3D conformer structures were downloaded from PubChem (31). For compounds lacking 3D structures, 2D ligand files were converted to 3D via OpenBabel 3.1.1 (32, 33). OpenBabel was also used for any formatting conversions as needed.

ProTox Toxicity Screen

ProTox 3.0 (34, 35) was used as a web-based toxicity prediction platform with built in machine learning and similarity-based models. Simplified Molecular Input Line Entry System (SMILES) representations of DEHP and PET, acquired from PubChem, were entered into ProTox to obtain the following: median lethal dose (LD50) based on rat oral exposure, toxicity class based on the globally harmonised system (GHS), and Tox21 nuclear receptor signaling pathway toxicity endpoints (including estrogen receptor ligand binding domain). Pathways labeled as active by ProTox with a probability of more than 0.5 were considered active toxicity endpoints in our study.

VEGA Toxicity Screen

Virtual Models for Property Evaluation of Chemicals within a Global Architecture (VEGA) quantitative structure-activity relationship (QSAR) platform was used to perform *in silico* toxicity screening (version 1.2.4) (36, 37). Models used within VEGA included: developmental toxicity, reproductive toxicity, estrogen receptor-mediated effect (IRFMN-CERAPP), estrogen receptor relative binding affinity model (IRFMN), endocrine disruptor screening model, and biodegradability. Each compound was analyzed independently. Predictions included qualitative classifications as well as reliability scores based on the applicability domain index (ADI) and similarity index. The similarity index determines the similarity of the query ligands with others in the VEGA database (with an index of 1 meaning that it is the most similar to another molecule). The reliability of VEGA QSAR predictions, split into three grades (with 1 being least reliable, and 3 most reliable) is based on the ADI. The ADI is a quantitative score that evaluates prediction confidence based on chemical similarity to training compounds, descriptor range compatibility, and concordance between predicted and experimental values of structurally similar molecules. The results from VEGA and ProTox were compared to assess congruency between the two platforms, with a special focus on estrogen receptor binding and endocrine disruption capacities.

Protein Preparation

Human ERβ was used as the target protein. The 3D crystal structure was obtained from the Protein Data Bank (PDB) (38) using the structure with the highest validation ranking (PDB ID: 5TOA) (39, 40).

Docking Simulation

Docking experiments were conducted using DockThor 2.0, a protein-ligand docking program that optimizes for the best possible binding of a ligand to a defined region and provides affinity and energetic values associated with the binding (41–43). Each ligand was prepared individually using DockThor's internal ligand preparation pipeline in order to reduce steric clashes. ERβ was likewise prepared by DockThor by removing nonessential molecules. Each ligand was then docked individually onto ERβ. Blind docking experiments were conducted using DockThor's built in blind docking algorithm. Docking experiments to chain A and B were conducted by setting a binding site based on the original ligand binding pocket, with each dimension of the grid set to 20 Å (coordinates for chain A: X=19.723, Y=43.462, Z=15.481; coordinates for chain B: X=18.145, Y=33.322, Z=42.824). Chain A and B were separately docked due to DockThor's limitations in defining one set binding site. All other default parameters were used, with the number of runs set to 10 to explore multiple binding conformations. Multiple docking poses were examined to capture the inherent flexibility of protein-ligand interactions, and to identify the most energetically favorable binding configurations. DockThor provided binding affinity scores and RMSD values via comparison to the original ERβ molecule file. The top ranked binding pose for each ligand was visualized via PyMol (44).

Statistical Analysis

Statistical analyses were performed using Microsoft Excel. All data are presented as mean ± standard deviation (SD). For comparisons between two groups, unpaired two-tailed Student's t-tests were conducted to determine statistical significance. Comparisons were made between different ligands within the same docking context (e.g., DEHP vs. estradiol in chain A) and between different docking contexts for the same ligand (e.g., chain A vs. chain B for DEHP). A p-value < 0.01 was considered statistically significant. For

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binding affinity analyses, independent docking runs were performed for each condition, as indicated in the figure legends (n=10 for DEHP, n=10 for blind estradiol docking, n=8 for chain A estradiol docking, n=9 for chain B estradiol docking, n=9 for blind and chain B PET docking, and n=7 for chain A PET docking). All statistical tests assumed normal distribution of data and equal variances between groups.

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