

ORIGINAL RESEARCH ARTICLE

Evaluating thyroid transport protein binding activity and structure–activity relationship of hydroxylated polybrominated diphenyl ethers using three-dimensional quantitative structure–activity relationship models

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Abstract

Polybrominated diphenyl ethers (PBDEs) have become globally significant environmental pollutants because of their persistence, bioaccumulative nature, and potential toxicity. Hydroxylated PBDEs (OH-PBDEs) are environmental thyroid-disrupting chemicals. Their toxicity primarily manifests through competitive binding with thyroid hormone transport proteins, including transthyretin (TTR) and thyroxine-binding globulin (TBG), disrupting the endocrine system. In this study, a three-dimensional quantitative structure–activity relationship (3D-QSAR) model was developed to predict the binding activity of OH-PBDEs. Binding activity data for 14 representative OH-PBDEs (containing 1–6 bromine atoms) against TTR and TBG were selected. 3D-QSAR models were constructed using comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA). For TTR, the CoMFA model exhibited the best predictive capability and robustness, with a cross-validated coefficient of $Q^2 = 0.611$, a fitted correlation coefficient of $R^2 = 0.999$, and a standard error of estimate (SEE) of 0.014. The CoMSIA model for TTR yielded $Q^2 = 0.502$, $R^2 = 0.999$, and $SEE = 0.017$. For TBG, all models yielded $Q^2 < 0.5$. This indicates insufficient predictive ability and was therefore not used for subsequent prediction. Field contribution analysis identified electrostatic field as the predominant factor governing binding activity (contribution rate: 65.4% in CoMFA). Using the optimal TTR-CoMFA model, the activities of 40 OH-PBDE isomers were predicted. T3 and T4 were used separately as endogenous reference ligands (predicted $pIC_{50} = 7.573$ and 7.921, respectively) and were not included in the OH-PBDE ranking. Among the predicted OH-PBDEs, OH-BDE-085 and OH-BDE-154 showed higher activity than T4, whereas OH-BDE-003 and OH-BDE-007 showed lower activity than T3. The remaining OH-PBDEs fell between T3 and T4. These results indicate that, within the range of 1–6 bromine atoms and under specific substitution patterns, increasing bromination generally enhances TTR binding activity. This study provides an efficient computational tool for the rapid screening of high-risk OH-PBDE congeners, establishing a scientific basis for environmental health risk assessment.

Keywords: Hydroxylated polybrominated diphenyl ethers; Three dimensional quantitative structure–activity relationship; Comparative molecular field analysis; Thyroid transport protein; Transthyretin

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants¹ extensively utilized in textiles, chemical products, electronic products, furniture, and building materials.² Based on the number of substituted bromine atoms, they range from monobrominated to decabrominated diphenyl ethers, with 209 congeners.³ Because brominated flame retardants are highly effective and suitable alternatives are difficult to secure in the short term, they continue to be used in large quantities worldwide.⁴ PBDEs are now widely detected in environmental matrices worldwide, including soil, air, water bodies, sediments, and a wide range of organisms such as fish, birds, and mammals.^{5–8} In regions with intensive industrial activity, such as electronic waste dismantling sites and flame-retardant manufacturing plants,⁹ environmental media show markedly elevated PBDE concentrations. PBDEs have also been detected in remote polar regions, confirming their long-range transport potential.¹⁰ Athanasiadou *et al.*¹¹ reported elevated PBDE levels in children from certain African cities. This confirmed that their metabolite, hydroxylated PBDEs (OH-PBDEs), can undergo bioaccumulation in human blood. A big-data analysis of recent literature on polybrominated diphenyl ethers indexed in Web of Science was conducted using CiteSpace software (Figure 1). The results show that research focus in this field has gradually shifted from early fundamental pollution characterization and identification studies to risk assessment and human health exposure studies.

Research findings revealed that PBDEs affect hormone secretion, transport, and metabolism through competitive binding to hormone receptors or by interfering with the function of hormone transport proteins. This disrupts the endocrine system and can lead to various diseases.^{12,13} Among these, the thyroid hormone system is the primary target of PBDEs. Specifically, by mimicking the structural characteristics of endogenous thyroid hormones, PBDEs disrupt the blood circulatory transport of T₄, peripheral deiodination activation, and nuclear receptor-mediated gene transcription. This consequently results in thyroid dysfunction.¹⁴

Thyroid hormones (T₄ and T₃) are essential for metabolism, growth, and development. Their synthesis, transport, and receptor signaling are tightly regulated.¹⁵

Pollutants that disrupt these processes are defined as thyroid-disrupting chemicals.¹⁶ OH-PBDEs are representative thyroid-disrupting chemicals. Notably, OH-PBDEs exhibit a higher binding affinity for transthyretin (TTR), thereby forming a competitive relationship with T₄ and suppressing thyroid hormone secretion.^{17,18} In addition, because OH-PBDEs are structurally similar to T₄, OH-PBDEs can displace T₄ from TTR binding. Consequently, normal thyroid hormone transport and function are impaired. Endocrine disruption is induced, and a potential threat to human health is posed.¹⁹

Quantitative structure–activity relationship (QSAR) has been established as an effective means of correlating the molecular structures of compounds with their biological or environmental activities through mathematical models.²⁰ Among these, three-dimensional QSAR (3D-QSAR) permits a more intuitive reflection of the interactions between molecular fields in 3D space and biological activity.^{21,22} Constructing a QSAR model primarily comprises four steps: dataset collection, determination of structural descriptors, QSAR model construction, and model evaluation and validation. In the present study, based on the binding activity data between OH-PBDEs and TTR as well as thyroxine-binding globulin (TBG), two classical 3D-QSAR methodologies—namely, comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA)²³—were applied to construct predictive models.

In CoMFA, molecular-level interactions governing biological activity generally correspond to non-covalent forces such as van der Waals and electrostatic forces. After placing the molecules on a 3D grid, probe atoms on the grid are used to analyze steric and electrostatic fields, and the sum of van der Waals and electrostatic interactions between the compounds is calculated. The methodological procedure is as follows: (i) the optimal conformations of the compounds are sought and optimized; (ii) molecular alignment is carried out, and all compounds are superimposed based on a common scaffold; (iii) the CoMFA fields are computed (typically, an sp³ orbital is selected as the probe to calculate the interaction energy); (iv) model construction: the molecular potential energies are employed as independent variables, and the compound activities as dependent variables; a model is established by applying partial least squares (PLS), and the model results

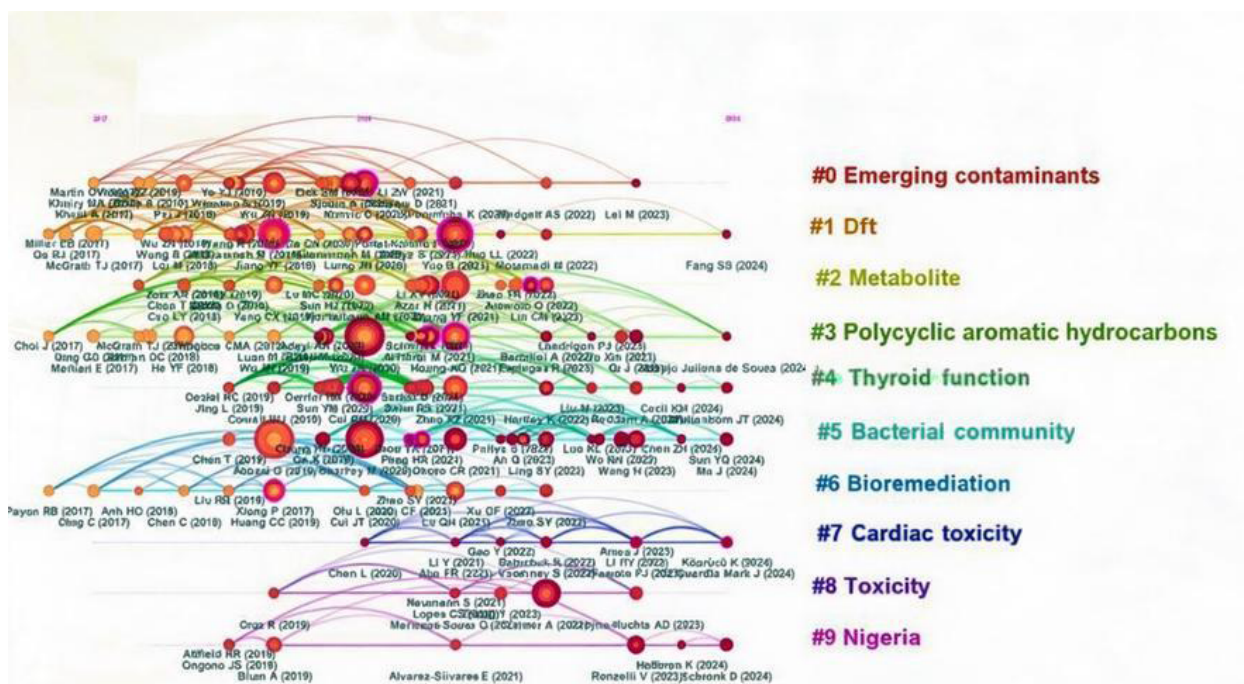


Figure 1. Temporal distribution of research hotspots on polybrominated diphenyl ethers

are presented in the form of equations and 3D contour maps.

Compared with CoMFA, CoMSIA not only requires establishing model elements, such as selecting bioactive molecular conformations, aligning molecules, and defining the spatial field extent, but also uses a distance-dependent Gaussian functional form to calculate interactions between the probe atom and the molecules. Moreover, the influences of five fields—steric, electrostatic, hydrophobic, hydrogen-bond acceptor, and hydrogen-bond donor—on the model are primarily investigated.

The objective of the present study was to establish a reliable and robust activity prediction model, whereby the key molecular fields and structural factors governing binding activity were identified; the binding activities of unknown OH-PBDE congeners were predicted using the established model; and a theoretical basis for their environmental and health risk assessment was consequently provided.

2. Materials and methods

2.1. Data collection and processing

Fourteen OH-PBDE congeners (containing 1–6 bromine atoms), together with T3 and T4, were selected for investigation, and binding activity data for OH-PBDEs toward TTR and TBG were used to construct the QSAR

model.²⁴ T3 and T4 represent the core endogenous ligands of the thyroid hormone system; the structural difference between the two molecules is confined to the presence of an iodine substitution at the 5'-position of the 3',5'-diiodotyrosine ring of T4, whereas T3 lacks an iodine atom at the corresponding site. From a functional perspective, T4 is the predominant secretory product of the thyroid gland, accounting for more than 90% of total plasma thyroid hormones, and serves as the principal binding substrate for the transport proteins TTR and TBG, exhibiting significantly higher affinity than T3. T3 is largely generated through type II deiodinase-catalyzed deiodination of T4 in peripheral tissues;²⁵ although its biological activity is approximately 3–5 times that of T4, its binding capacity toward the two transport proteins amounts to only 10–50% of that of T4, and consequently, it is not considered the key competitive ligand at the transport stage. The structural characteristics of T4, as a representative endogenous thyroid hormone, constitute the basis for understanding the mechanism of competitive disruption by pollutants. Accordingly, the molecular structure of T4 was constructed using SYBYL-X 2.0 software, and an initial 3D structure was generated (Figure 2).

The sample size of 14 OH-PBDE congeners was relatively small for 3D-QSAR modeling. This is because experimentally determined binding affinities of OH-PBDEs

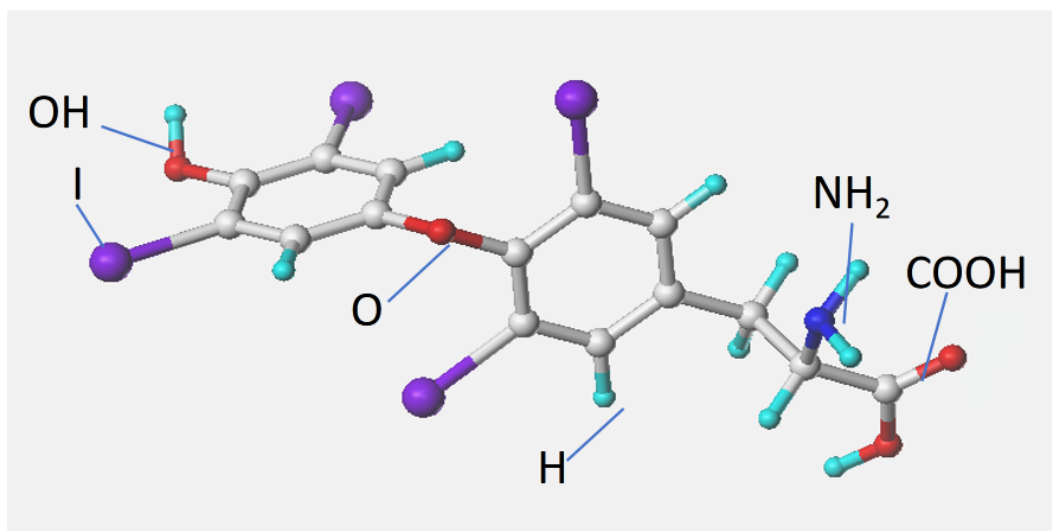


Figure 2. Molecular structure of T4

toward both TTR and TBG under consistent conditions are scarce. The dataset used in this study was based on Cao *et al.*,²⁴ one of the few available sources that provides consistent experimental data for OH-PBDEs containing 1–6 bromine atoms. Despite the limited number, these 14 congeners cover the mono- to hexa-brominated range and represent the major substitution patterns of environmental OH-PBDEs. Therefore, this dataset was selected as the most suitable basis for the present 3D-QSAR analysis.

2.2. Molecular structure optimization and alignment

In this study, the Tripos force field was used to geometrically optimize the initial molecular structures, yielding energetically minimized stable conformations. The optimization parameters were set as follows: Gasteiger–Hückel charges, a convergence criterion of 0.01 kcal·mol⁻¹, and a maximum of 1,000 iterations. Among the optimized sample molecules, 2'-OH-BDE-003 was selected as the template molecule for molecular alignment. The selection was based on two considerations. First, as a monobrominated congener, 2'-OH-BDE-003 possesses the simplest structure and lacks complex steric hindrance, thereby providing a unified core alignment reference for all molecules. Second, its rigid diphenyl ether backbone ensures conformational stability and alignment consistency. The template molecule was chosen for alignment purposes rather than for its binding activity. This is consistent with Cramer and Wendt,²⁶ who noted that template selection can be based on structural simplicity and alignment consistency rather than on highest activity. Template activity does not directly determine the predictive performance of the PLS model; the model is established by

correlating the field descriptors of all training compounds with their experimental activities. The alignment was further validated by optimizing the cross-validated coefficient Q^2 .²⁷ In the optimal alignment (Figure 3), the molecular skeletons were aligned along the central ether-bond axis, and the spatial distribution of most substituents showed pronounced regularity. This provided a reasonable spatial basis for the subsequent extraction of the steric and electrostatic fields.²⁸

2.3. Three-dimensional quantitative structure–activity model construction and validation

Based on the aligned molecular set, CoMFA and CoMSIA methodologies were respectively applied to construct QSAR models. For model construction, the CoMFA model primarily examined the contributions of the steric and electrostatic fields to binding activity, whereas the CoMSIA model also included the hydrophobic and hydrogenbond donor/acceptor fields, thereby more comprehensively characterizing the interaction features between the molecules and the protein. For model validation, a strategy combining internal and external validation was adopted to ensure the reliability of the models. Internal validation (leave-one-out cross-validation²⁹) was primarily employed to assess model stability and goodnessofit, while external validation was performed by partitioning the dataset into training and test sets to verify the predictive capability of the model for unknown samples.³⁰ The main statistical indices included the cross-validated correlation coefficient, the fitted correlation coefficient, the standard error of estimate (SEE), and the *F*value from the validation. External validation was conducted by dividing the 14

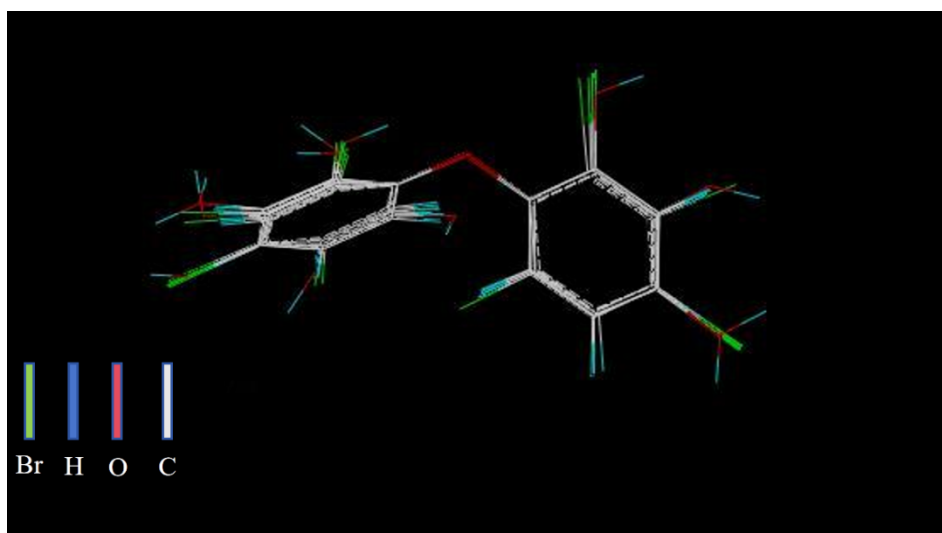


Figure 3. Molecular alignment of the compounds

OH-PBDE congeners into a training set and a test set. The compounds were first ranked by experimental pIC_{50} values, and then stratified sampling was performed: every third compound was assigned to the test set, and the remaining compounds were assigned to the training set. This yielded a training set of 10 compounds and a test set of 4 compounds, following the dataset division strategy of Cao *et al.*²⁴ The CoMFA and CoMSIA models were rebuilt using only the training set, and the test set compounds were used for blind prediction.³¹

The calculation formula for the cross-validated coefficient Q^2 is given in Equation 1:

$$Q^2 = 1 - \frac{\sum_{i=1}^n (\bar{y}_i - y_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \quad (1)$$

where y_i denotes the experimental value of each sample, $\bar{y}_i$ denotes the predicted value of each sample, and $\bar{y}$ denotes the mean of all experimental values.

2.3.1. Comparative molecular field analysis model

Model construction was conducted based on the aligned molecular set. The compound names, binding activity data, and aligned molecular set were imported into the QSAR module of SYBYLX 2.0. CoMFA was applied to compute the interaction energies of the steric and electrostatic fields, and a QSAR model was established using PLS regression. Leaveoneout crossvalidation was employed as an internal validation, from which the cross-validated coefficient Q^2 and the optimal number of components were derived. Subsequently, non-cross-validation was performed, yielding the fitted correlation coefficient R^2 , the SEE, and

the F -test value; these indices were employed to evaluate the robustness and predictive capability of the model. The detailed CoMFA model construction data for TTR and TBG are provided in Tables S1 and S2, respectively. Furthermore, to additionally confirm the generalization ability of the model, the sample set was partitioned into a training set and a test set by stratified sampling according to activity range, with reference to the dataset division strategy of Cao *et al.*²⁴ The final predictive model was built using the training set, and blind prediction for the unknown samples in the test set was carried out.

2.3.2. Comparative molecular similarity indices analysis model

In this study, CoMSIA was applied to preliminarily investigate the influence of the steric field, electrostatic field, hydrophobic field, and hydrogen-bond donor/acceptor fields on binding activity. Through model screening, the fields exhibiting significant contributions to activity without collinearity were ultimately selected and employed for modeling analysis. PLS regression was applied to establish the structure–activity relationship model. The cross-validated coefficient Q^2 was obtained via leave-one-out cross-validation to evaluate the robustness of the model, and the fitted correlation coefficient R^2 , SEE, and F -test value were calculated through non-cross-validation to assess the predictive capability and goodness-of-fit of the model. The detailed CoMSIA model construction data for TBG and TTR are provided in Tables S3 and S4, respectively. The key statistical parameters of the TTR-CoMFA and TTR-CoMSIA models are summarized in Section 3.1. For the TBG system, all models yielded $Q^2 <$

0.5, indicating insufficient predictive ability; therefore, they were not used for subsequent activity prediction. CoMSIA model evaluation parameters are given in Table S5.

2.4. Activity prediction

The model demonstrating the best performance through internal validation was selected, and virtual screening of potential OH-PBDE environmental pollutants was conducted. Based on the diphenyl ether core, all possible substitution positions accommodating 1 to 6 bromine atoms were enumerated. After excluding the 14 compounds included in the training set and configurations rendered invalid by excessive steric hindrance, 40 OH-PBDE isomers were ultimately obtained. Standardized alignment of the aforementioned molecules was performed, and the aligned set was input into the model. Their binding activities toward TTR were subsequently predicted and comparatively analyzed against the activities of the endogenous ligands T3 and T4. The prediction process was carried out within the SYBYL software environment, and the preprocessing, alignment procedure, and parameter settings for all molecules in the prediction set were applied in complete accordance with those employed for the training set. First, the 40 target OH-PBDEs and the reference ligands T3 and T4 were subjected to geometric optimization under the same optimization parameters as the training set (Tripos force field, Gasteiger–Huckel charges, convergence criterion of 0.01 kcal·mol⁻¹). Subsequently, the training set template molecule, 2'-OH-BDE-003, and the diphenyl ether backbone were designated as the rigid alignment scaffold, and all molecules were aligned. The pre-constructed TTR-CoMFA model, with fixed PLS regression coefficients and principal component number, was loaded, and the steric and electrostatic field potential energies at the 3D grid nodes for the aligned molecules were substituted into the established quantitative structure–activity relationship equation. The predicted pIC₅₀ value for each compound was thereby calculated, yielding the final predicted activity data.

3. Results and discussion

3.1. Three-dimensional quantitative structure–activity relationship model evaluation and field analysis

The key parameters for model evaluation are presented in Table 1. For the binding system of OH-PBDEs with TTR, the CoMFA model was found to exhibit superior predictive capability and robustness ($Q^2 = 0.611$, $R^2 = 0.999$, SEE = 0.014). In contrast, the performance of the CoMSIA model was assessed to be somewhat inferior ($Q^2 = 0.502$, $R^2 = 0.999$). The experimental and CoMFA-predicted pIC₅₀ values, residuals, number of bromine atoms, and CoMFA

field energies for OH-PBDEs binding to TTR and TBG are shown in Figures 4 and 5. Analysis of the field contributions revealed that, in the CoMFA model, the electrostatic field accounted for 65.4% of the contribution, substantially exceeding that of the steric field (34.6%), indicating that electrostatic interactions constitute the dominant physical force governing the binding between OHPBDEs and TTR. In the CoMSIA model, the electrostatic field contribution was predominant at 74.3%, followed by the hydrophobic field at 24.6%. Liang *et al.*³² previously constructed 3DQSAR models for OHPBDEs with TBG and TTR based on molecular docking-derived bioactive conformations and reported that CoMSIA outperformed CoMFA in terms of overall performance (TTRCoMSIA $Q^2 = 0.525$; TTRCoMFA $Q^2 = 0.317$; TBGCoMFA $Q^2 = 0.270$). However, a different conclusion was drawn in the present study under a rigid alignment strategy: the Q^2 of the TTRCoMFA model reached 0.611, surpassing that of the CoMSIA model ($Q^2 = 0.502$), and for the TBG system, neither predictive model yielded satisfactory results ($Q^2 < 0.5$). This is likely attributable to TBG's deeper, more specific binding pocket than TTR's, and the binding mechanism may impose stringent requirements not only on electrostatic and steric effects but also on the hydrophobic microenvironment and hydrogenbond geometry. Because the currently applied CoMFA and CoMSIA methods are primarily 2D or 3D empirical descriptors based on field potential energy distributions on molecular surfaces, they have inherent limitations in capturing the complex electron-cloud distribution characteristics and entropic effects at the TBG binding site. Consequently, future research should introduce additional structural descriptors, particularly quantum chemical descriptors or molecular topological indices, to characterize microscopic interactions between the molecules and the TBG protein more precisely and potentially overcome the predictive limitations of the present models.

3.2. External validation of model predictive capability and three-dimensional contour map analysis

To intuitively verify the reliability of the model, a fitted plot of experimental versus predicted values for the binding activity of OHPBDEs to TTR was generated to further provide external validation of the model's reliability, as shown in Figure 6.

The linear regression equations were derived as $y = 0.9997x + 0.0021$ ($R^2 = 0.9996$) and $y = 0.9995x + 0.0035$ ($R^2 = 0.9994$), respectively. The highly correlated linear relationships confirm the excellent external predictive capability of the CoMFA model.

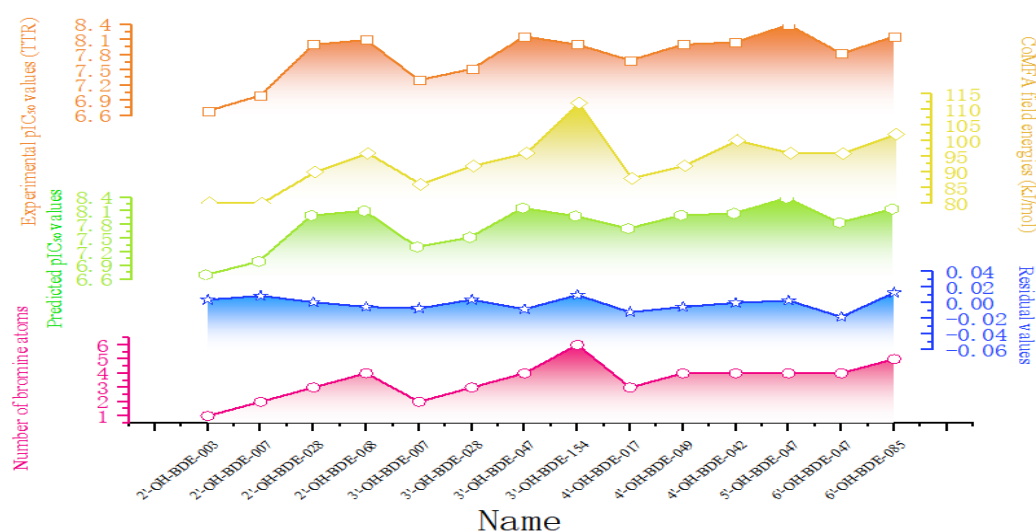


Figure 4. Experimental and comparative molecular field analysis (CoMFA)-predicted pIC_{50} values, residuals, number of bromine atoms, and CoMFA field energies for hydroxylated polybrominated diphenyl ethers binding to transthyretin (TTR)

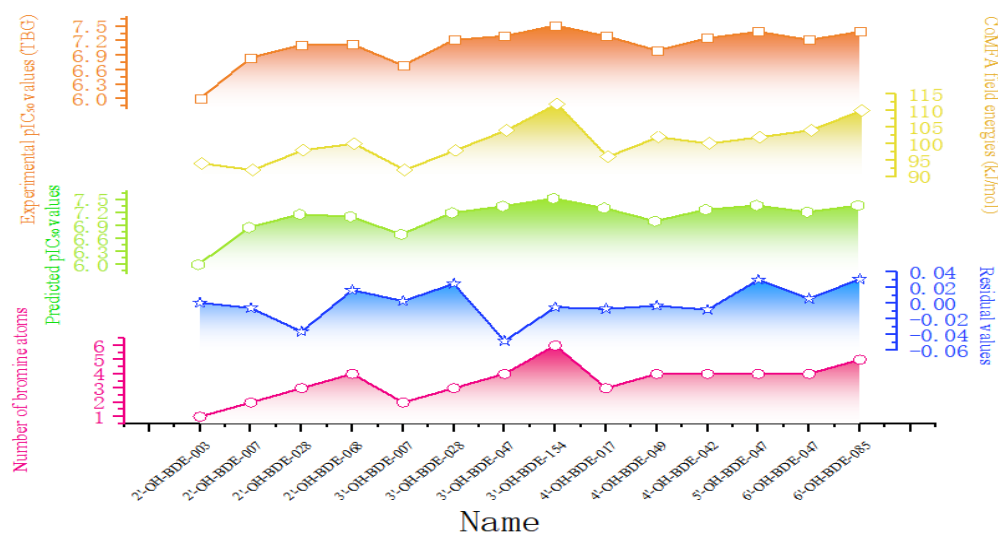


Figure 5. Experimental and comparative molecular field analysis (CoMFA)-predicted pIC_{50} values, residuals, number of bromine atoms, and CoMFA field energies for hydroxylated polybrominated diphenyl ethers binding to thyroxine-binding globulin (TBG)

Table 1. Comparative molecular field analysis model evaluation parameters

Protein	Methods	R^2	Q^2	SEE	Steric field (%)	Electrostatic field (%)	Hydrophobic field (%)
TTR	CoMFA	0.999	0.611	0.014	34.6	65.4	—
	CoMSIA	0.999	0.502	0.017	1.2	74.3	24.6

Abbreviations: CoMFA: Comparative molecular field analysis; CoMSIA: Comparative molecular similarity indices analysis; SEE: Standard error of estimate; TTR: Transthyretin.

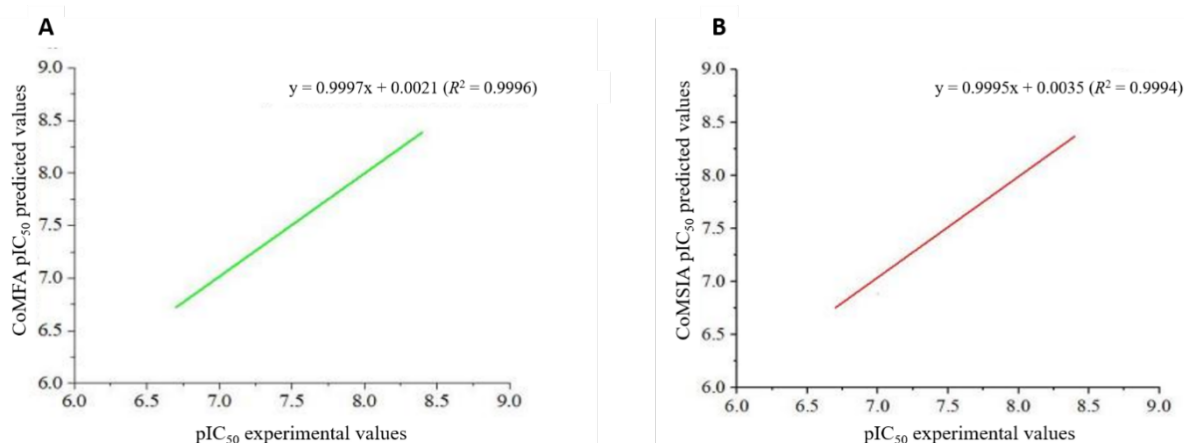


Figure 6. Comparison between experimental binding constants and (A) comparative molecular field analysis (CoMFA)-predicted constants and (B) comparative molecular similarity indices analysis (CoMSIA)-predicted constants

The characteristics of the TTR binding site elucidated by the CoMFA model are presented in Figure 7. The grey wireframe structure represents the template molecule 2'-OHBDE003, and the surrounding colored contours reflect the field distribution characteristics of the TTR binding pocket. In Figure 7A (steric field), a prominent yellow isopotential surface was observed around a specific phenyl ring substitution position of the template molecule 2'-OHBDE003, corresponding to a PLS positive coefficient region. This indicates that spatial filling (e.g., the addition of bromine atoms) at this site constitutes the primary driving force for activity enhancement. No green region (negative coefficient-restricted zone) reaching the display threshold was observed. In Figure 7B (electrostatic field), the blue region encompasses the hydroxyl group and electron-rich aromatic rings, suggesting that a positive electrostatic potential is favorable for binding. Conversely, the red region indicates an electron-deficient environment, and the introduction of positively charged groups at this location would result in an increase in binding energy (i.e., a decrease in activity), thereby verifying the dominant role of the electrostatic field contribution.

The 3D contour maps of TTR generated from the CoMSIA model are presented in Figure 8. Although the CoMSIA methodology can theoretically encompass steric, electrostatic, hydrophobic, and hydrogen-bond donor/acceptor fields, modeling results based on the present dataset showed that introducing the hydrogen-bond donor and acceptor fields markedly worsened the model's fitting and cross-validated coefficients. Accordingly, only the steric, electrostatic, and hydrophobic fields were retained in the final CoMSIA model constructed. In Figure 8A (steric and electrostatic fields), the yellow regions indicate that

steric bulk at these positions favors activity enhancement, meaning that introducing substituents such as bromine atoms can further strengthen complementarity between the molecule and the TTR binding pocket. The blue regions suggest that introducing positively charged groups enhances activity, whereas the red regions suggest that introducing negatively charged groups enhances activity. In Figure 8B (hydrophobic field), the grey regions indicate that introducing hydrophobic groups favors improved compound activity.

3.3. Prediction of hydroxylated polybrominated diphenyl ether isomer activity and structure-activity relationship

We selected the CoMFA model based on binding data with TTR, which showed the best predictive capability and robustness, and used it to predict activity for 40 OH-PBDE isomers. T3 and T4 were not included in the OH-PBDE ranking; they were used as endogenous reference ligands to define the activity thresholds. The predicted results are presented in Table 2.

The results indicated that OH-BDE-85, which contains five bromine atoms, showed the highest activity; its activity exceeded that of T3 and T4, demonstrating a stronger competitive relationship. OH-BDE-154, bearing six bromine atoms, had the next-highest activity and also surpassed that of T3 and T4. The activities of OHBDE003, containing one bromine atom, and OHBDE007, containing two bromine atoms, were both low and lower than the binding activities of T3 and T4; these two compounds were thus evaluated as having relatively weaker competitive ability, i.e., lower toxicity. The other compounds showed intermediate activity between T3

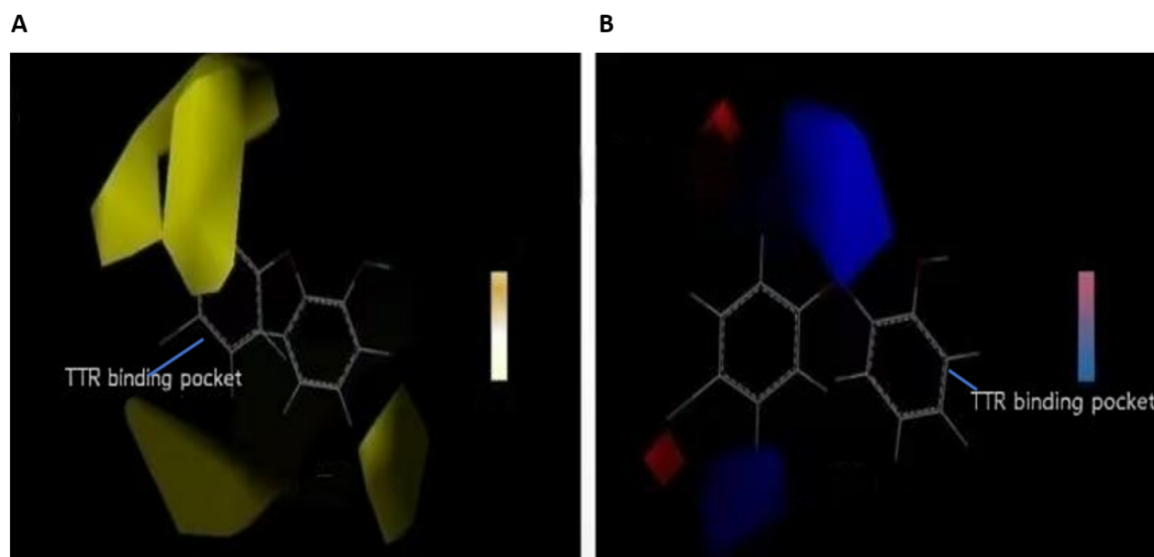


Figure 7. Three-dimensional contour maps of the transthyretin (TTR) comparative molecular field analysis model based on the template molecule 2'-OHBDE003: (A) steric field (kcal/mol) and (B) electrostatic field (kcal/mol)

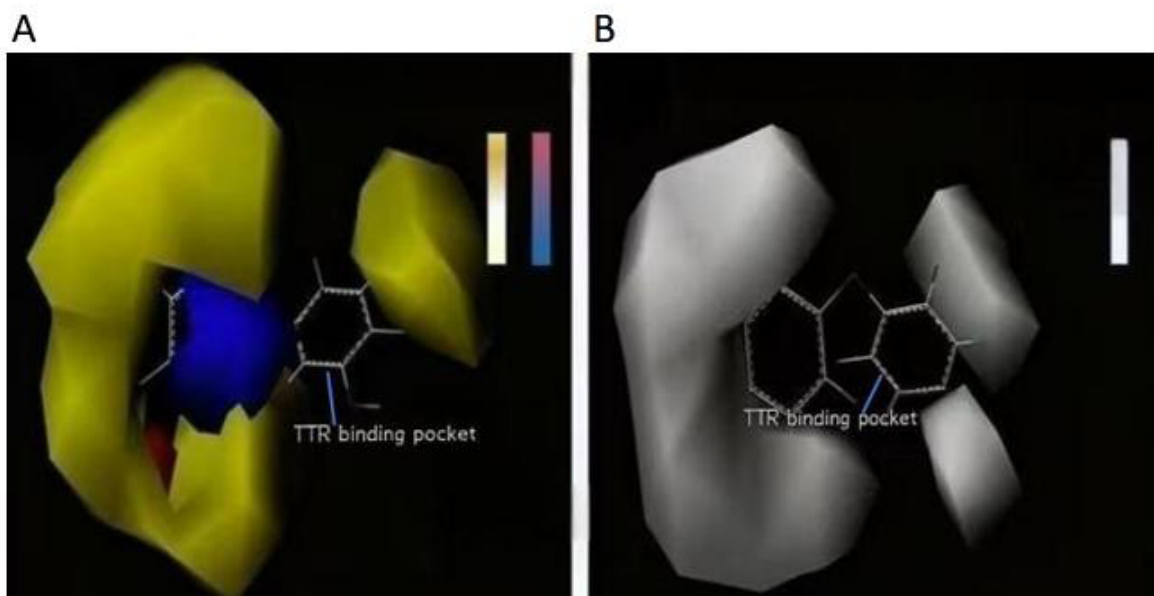


Figure 8. Three-dimensional contour maps of the transthyretin (TTR) comparative molecular similarity indices analysis model based on the template molecule 2'-OHBDE003: (A) steric and electrostatic fields; (B) hydrophobic field

and T4. The activity order of the 14 selected OH-PBDEs was: OHBDE003 < OHBDE007 < OHBDE028 < T3 < OHBDE017 < OHBDE047 < OHBDE049 < OHBDE042 < T4 < OHBDE154 < OHBDE085. As the number of bromine atoms increased, the binding activity of OH-PBDEs increased; however, beyond a certain point, the change was no longer pronounced. It is therefore inferred that, within the lower bromine atom range, increasing the number

of bromine atoms favors activity. The corresponding results were largely consistent with the conclusions of Yi *et al.*⁴ Moreover, CoMFA contour maps elucidated the physicochemical origin of the influence of the degree of bromination on activity at the microscopic level of steric and electrostatic fields, revealing a positive correlation between the degree of halogenation and toxicity.

Table 2. Predicted transthyretin binding activities of 40 hydroxylated polybrominated diphenyl ether isomers and the endogenous reference ligands T3 and T4

Sequence	Name	Number of bromine atoms	Predicted pIC ₅₀
1	2'-OH-BDE-003	1	6.695
2	2-OH-BDE-003	1	7.389
3	3'-OH-BDE-003	1	6.368
4	3'-OH-BDE-007	2	7.312
5	3'-OH-BDE-017	3	7.773
6	3'-OH-BDE-042	4	8.022
7	3'-OH-BDE-047	4	7.427
8	3'-OH-BDE-049	4	7.785
9	3'-OH-BDE-085	5	8.377
10	3-OH-BDE-003	1	7.127
11	3-OH-BDE-007	2	7.180
12	3-OH-BDE-017	3	7.829
13	3-OH-BDE-028	3	7.397
14	3-OH-BDE-049	4	7.484
15	3-OH-BDE-154	6	8.304
16	4'-OH-BDE-003	1	7.366
17	4'-OH-BDE-007	2	7.449
18	4'-OH-BDE-017	3	7.715
19	5'-OH-BDE-017	3	7.831
20	5'-OH-BDE-042	4	7.909
21	5'-OH-BDE-085	5	8.412
22	5'-OH-BDE-154	6	8.238
23	5-OH-BDE-007	2	7.423
24	5-OH-BDE-017	3	7.937
25	5-OH-BDE-028	3	7.639
26	5-OH-BDE-042	4	7.557
27	5-OH-BDE-047	4	7.719
28	5-OH-BDE-049	4	7.739
29	5-OH-BDE-085	5	8.300
30	6'-OH-BDE-017	3	7.742
31	6'-OH-BDE-042	4	7.738
32	6'-OH-BDE-047	4	7.557
33	6'-OH-BDE-049	4	7.673
34	6'-OH-BDE-085	5	8.030
35	6-OH-BDE-007	2	7.318
36	6-OH-BDE-017	3	7.530
37	6-OH-BDE-028	3	7.525
38	6-OH-BDE-042	4	7.930

(Cont'd...)

Table 2. (Continued)

Sequence	Name	Number of bromine atoms	Predicted pIC ₅₀
39	6-OH-BDE-049	4	7.633
40	6-OH-BDE-154	6	8.134
-	T3	-	7.573
-	T4	-	7.921

Note: T3 and T4 are endogenous thyroid hormones and were used as reference ligands. They were not included in the ranking of OH-PBDE isomers.

Abbreviations: OH-BDE; Hydroxylated polybrominated diphenyl ether; T3: Triiodothyronine; T4: Thyroxine; TTR: Transthyretin.

Liang *et al.*³² also reported that OH-PBDEs disrupt normal physiological functions of the human thyroid hormone system through competitive binding with the thyroxine transport proteins TBG and TTR; however, that study was largely limited to qualitative descriptions of the toxic phenomena of known compounds and lacked the capability to quantitatively predict the vast number of unknown congeners. The 3DQSAR model constructed in the present study has compensated for these deficiencies. Specifically, unlike previous studies that could only compare relative potency among a small number of compounds, the present model established a quantitative mathematical equation for the “structure–activity” relationship, enabling direct calculation of pIC₅₀ values for any OHPBDE isomer. Thus, a transition from “qualitative ranking” to “quantitative prediction” was achieved. Based on the model prediction results, it is proposed that OHBDE85 and OHBDE154 be designated as priority substances for environmental risk management: the binding activities of both compounds toward TTR surpass that of the endogenous thyroid hormone T4. Consequently, a clear priority control list can be provided for the targeted monitoring of OHPBDEs in environmental media and for ecological health risk assessment.

3.4. Structural and mechanistic basis of competitive binding between hydroxylated polybrominated diphenyl etherS and thyroid hormones

The chemical structures of T4, T3, and OH-PBDEs are shown in Figure 9. All three compounds share a hydroxylated diphenyl ether core. This structural similarity underlies their competitive binding.

Thyroid hormones comprise T4 and T3. Their physiological circulation is governed by precise molecular regulation. After secretion by the thyroid gland, T4 travels to peripheral tissues in the bloodstream, mainly bound to TTR and TBG. It is then converted to highly active T3 by

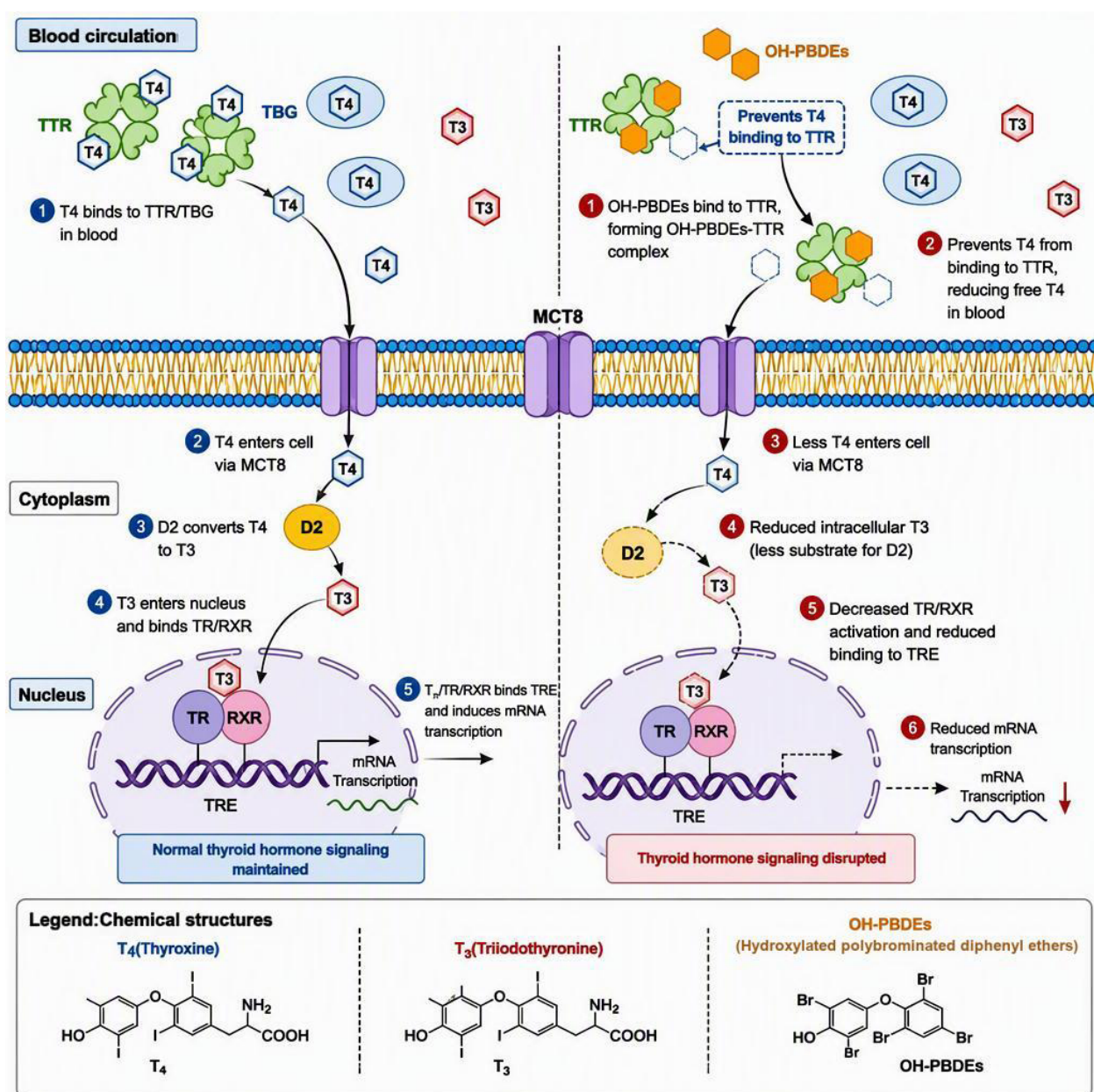


Figure 9. Structural basis and mechanism of competitive binding between hydroxylated polybrominated diphenyl ethers and thyroid hormones. The figure was created using ChemDraw Professional (PerkinElmer/Revvity Signals Software) for chemical structures (T₄, T₃, OH-PBDEs) and WPS Office for schematic assembly and layout.

Abbreviations: D2: Deiodinase 2; MCT8: Monocarboxylate transporter 8; OH-PBDEs: Hydroxylated polybrominated diphenyl ethers; RXR: Retinoid X receptor; T₃: Triiodothyronine; T₄: Thyroxine; TBG: Thyroxine-binding globulin; TR: Thyroid hormone receptor; TRE: Thyroid response element; TTR: Transthyretin.

type II iodothyronine deiodinase (D2). T₃ enters the cell nucleus and binds to the thyroid hormone receptor, thereby regulating target gene transcription and maintaining homeostasis of metabolism, growth, and development.

Hydroxylated polybrominated diphenyl ethers disrupt

this process mainly through three mechanisms. First, they inhibit iodine uptake by the thyroid gland, reducing thyroid hormone synthesis. Second, they compete with T₄ for binding to TTR and TBG. OH-PBDEs show higher binding affinity for TTR, forming an OH-PBDEs-TTR

complex. This reduces free T4 in the blood and decreases T4 transport into cells via monocarboxylate transporter 8. As a result, less T4 is available as substrate for D2, leading to lower intracellular T3. Third, OH-PBDEs directly bind to the thyroid hormone receptor and compete with T3, suppressing thyroid hormone production.³³

Reduced intracellular T3 decreases thyroid hormone receptor/retinoid X receptor activation and binding to thyroid hormone response elements in DNA. Consequently, mRNA transcription is downregulated, disrupting thyroid hormone signaling. This competitive binding is the mechanistic basis for the thyroid-disrupting effects predicted by the 3D-QSAR model.³⁴

4. Conclusion

In the present study, CoMFA and CoMSIA models were constructed using the QSAR module within SYBYLX 2.0, and the respective structural parameters and field contributions of the two models were comparatively analyzed. Ultimately, CoMFA and CoMSIA models were established based on binding data with TTR, and comparative analysis showed that the CoMFA model was more suitable for the present dataset. Furthermore, we used the constructed CoMFA model to predict the activity of a series of OH-PBDE compounds and generated corresponding 3D contour maps, which can be used to reduce compound activity through structural modifications. The principal conclusions are as follows:

- (i) The cross-validated coefficients Q^2 of the 3DQSAR models (CoMFA and CoMSIA) constructed in this study were determined to be 0.611 and 0.502 (>0.5), respectively; the fitting coefficients R^2 were calculated to be 0.999 and 0.999 (>0.6), respectively; and the SEES were identified as 0.014 and 0.017, respectively. It is thereby indicated that both models possess satisfactory predictive capability and robustness, and that the predicted results are relatively reliable.
- (ii) With respect to field contributions, the contribution rates of the steric and electrostatic fields in the CoMFA model were determined to be 0.346 and 0.654, respectively, while those of the steric, electrostatic, and hydrophobic fields in the CoMSIA model were identified as 0.012, 0.743, and 0.246, respectively; the influence of the hydrogenbond donor/acceptor fields on the binding activity of the compounds was not considered. This indicates that the electrostatic field constitutes the principal field governing the binding activity of OH-PBDEs, and that the compositional ratios of different fields exert a substantial influence on the predictive capability and robustness of the models.

- (iii) A comparative activity analysis conducted on the predicted compounds permitted the preliminary inference that the number of bromine atoms influences the competitive binding activity between OH-PBDEs and T3 as well as T4, and that, under specific substitution patterns and within a certain range, a greater number of bromine atoms tends to be associated with stronger binding activity—that is, elevated toxicity and greater potential hazard to human health.
- (iv) It is recommended that OHBDE85 and OHBDE154 be designated as priority substances for environmental risk management: the binding activities of both compounds toward TTR surpass that of the endogenous thyroid hormone T4. Consequently, a clear priority control list can be provided for the targeted monitoring of OHPBDEs in environmental media and for ecological health risk assessment.

It is anticipated that in future research, molecular dynamics simulation techniques may be employed to dynamically characterize the binding stability between this class of compounds and biological macromolecules, and that computational toxicology models may be further extended to multiple toxicity endpoints, such as neurodevelopmental toxicity and reproductive toxicity, thereby enabling the construction of a more systematic, integrated health risk assessment framework.

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Conflict of interest

The authors declare they have no competing interests.

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Availability of data

Data used in this study are available from the corresponding author upon reasonable request.

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