International Journal of Bioorganic Chemistry
2017; 2(3): 107-117
http://www.sciencepublishinggroup.com/j/ijbc
doi: 10.11648/j.ijbc.20170203.15

Quantitative Structure Toxicity Relationship (QSTR) Models for Predicting Toxicity of Polychlorinated Biphenyls (PCBs) Using Quantum Chemical Descriptors

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To cite this article:

Sabitu Babatunde Olasupo, Adamu Uzairu, Balarabe Sarki Sagagi. Quantitative Structure Toxicity Relationship (QSTR) Models for Predicting Toxicity of Polychlorinated Biphenyls (PCBs) Using Quantum Chemical Descriptors. *International Journal of Bioorganic Chemistry.* Vol. 2, No. 3, 2017, pp. 107-117. doi: 10.11648/j.ijbc.20170203.15

Received: February 25, 2017; **Accepted**: March 22, 2017; **Published**: April 7, 2017

Abstract: The Density functional theory (DFT) at B_3LYP of 6-31G* basis set was employed to optimize 30 polychlorinated Biphenyls (PCBs) involved in this study by using Genetic function appropriation algorithm (GFA) approach to develop regression models in order to predict the toxicity of the compounds. The optimum model which has squared correlation coefficient (R^2) = 0.9382, cross validated correlation coefficient (R^2cv) = 0.9056, adjusted squared correlation coefficient (R^2_{Adj}) = 0.9228 and external prediction (R^2_{pred}) =0.7238 was selected. The robustness of the model was confirmed by method of Y- randomization and the accuracy of the proposed model was also illustrated by using cross-Validation, validation through an external test set and applicability domain techniques. This QSTR model proved to be a useful tool in the prediction of toxicity of the congeneric compounds and a guide in the identification of structural features that could be responsible for toxicity of other polychlorinated aromatic compounds.

Keywords: QSAR, Dioxins, PCBs, QSTR, Polychlorinated Biphenyls

1. Introduction

Polychlorinated biphenyls (PCBs) are among the most environmentally dangerous chemicals belonging to the class of polychlorinated aromatic compounds [1], ubiquitously present in every compartment of the environment including soils, sediments, plants, animals, and human beings [2]. These compounds are of environmental and human health concern, because of their wide range of acute and chronic health effects on humans such as cancer, endocrine disruptors, neurological damage, reproductive disorders and immune suppression [3-4]. The physico-chemical properties exhibit by these chemicals such as hydrophobicity, low water solubility and lipophilicity, make them to accumulate in soil, sediments, biota and in humans and food webs and other indirect exposure [5-6] posing significant health threats to well-being of humans and animals [6].

Polychlorinated biphenyls are dioxin-like compounds (DLCs) formed and get released to the environment as by – products of various industrial processes which includes incomplete combustion of organic matter in industrial operations, medical waste incinerators, power plants, vehicle engines, household wood fires and forest fires [7], and are commonly regarded as highly toxic chemicals that are environmental contaminants and persistent organic pollutants (POP) [8].

Therefore, investigations on toxicity of PCBs are of great importance to understand their risk to human health and to the environment at large by making use of their toxicity data of the compounds to evaluate their risk to organisms and further adopt effective measures to reduce the adverse effects of this toxic chemical or pollutant in our environment.

However, because of high cost, time-consuming process, limits of detection and lack of adequate standard materials, toxicity data are rather scarce for non-genotoxic adverse effects of compounds. In order to conquer these problems and quickly estimate the environmental behaviors of

compounds, quantitative structure–activity/toxicity relationship (QSAR/QSTR) models, which correlate and predict toxicity data of the compounds (PCBs) from their molecular structural descriptors were developed, provide valuable approach in research into the toxicity of compounds without any experiments widely applied to evaluate and predict toxicity of chemicals efficiently [9] was employed in this study. Studies have shown that reliable QSAR/QSTR models are not only applied to predict toxicity and provide basic data to risk assessment, but also used to explain the toxicity mechanisms [10].

QSAR/QSTR have been widely used in research to explain acute toxicity [11], endocrine disrupting activities [12] and photo induced toxicity of organic compounds. This approach can fill the data gap of organic pollutants, decreases experimental expenses and reduces animal testing [13] and it can predict the bioactivity such as toxicity, mutagenicity and carcinogenicity based on structural parameters of compounds and appropriate mathematical models.

The alternative hypothesis to this study includes:

The magnitude of the observed toxicity log ($1/EC_{50}$) of Polychlorinated biphenyls (PCBs) are direct function of the empirical property (ies) or the theoretical parameter(s) which makes the descriptor of the total chemical structure of the compounds under investigation.

The null hypothesis to this research includes;

The observed toxicity log ($1/EC_{50}$) of Polychlorinated biphenyls (PCBs) is independent of the descriptors of their total chemical structures.

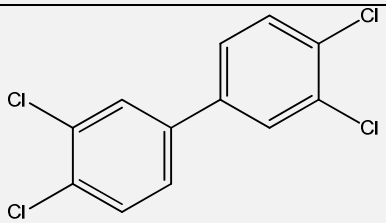
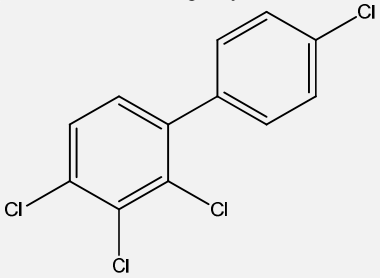
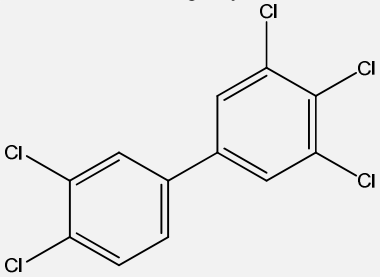
This present study is aimed to build robust and rational Genetic function approximation (GFA) based QSTR models for the predicting the toxicity of Polychlorinated biphenyls (PCBs) by exploring the correlations between the experimental log ($1/EC_{50}$) of the compounds and their calculated molecular descriptors. It is envisaged that the wealth of information in this study would provide a fast, economical, more environmentally friendly and less time consuming techniques of accessing the toxicity of Polychlorinated biphenyls (PCBs) and other related toxic Polychlorinated aromatic chemicals/ pollutants that could endanger our environment.

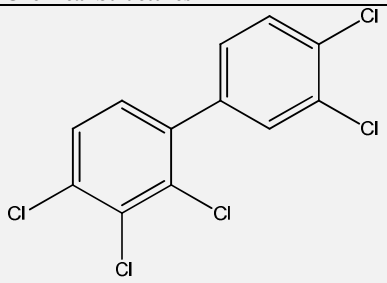
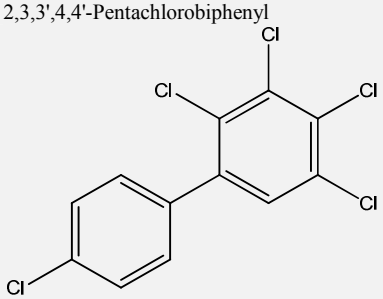
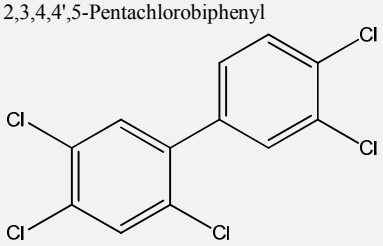
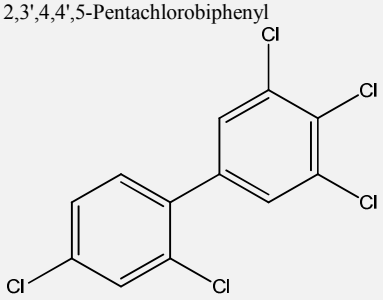
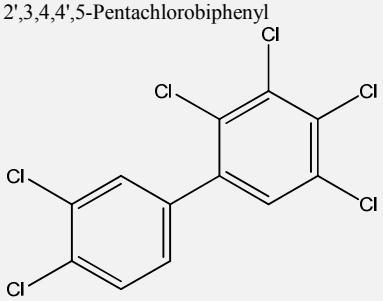
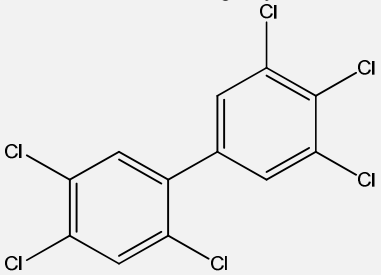
2. Materials and Methods

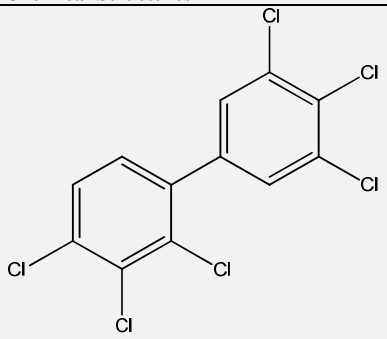
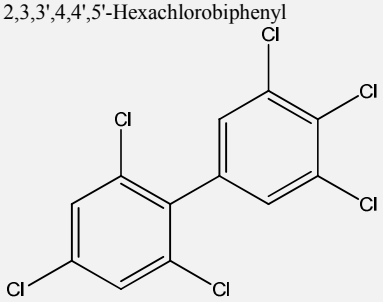
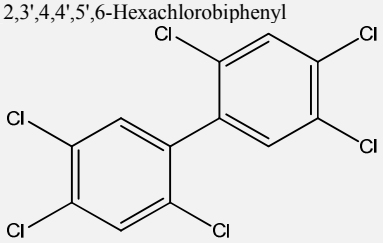
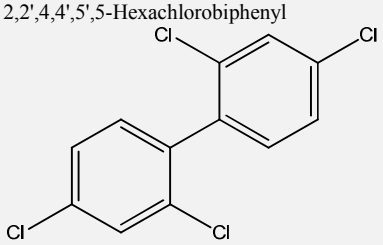
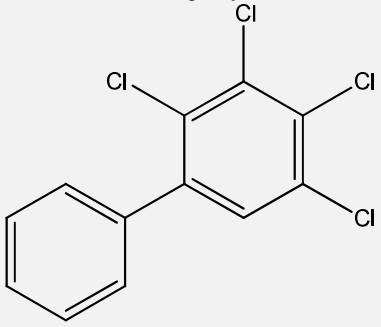
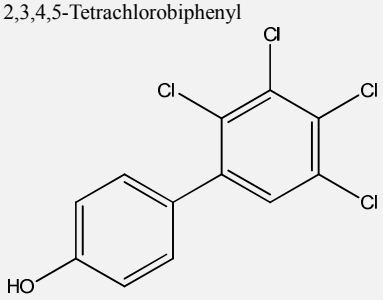
2.1. Data Collection

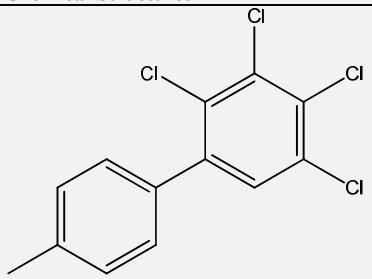
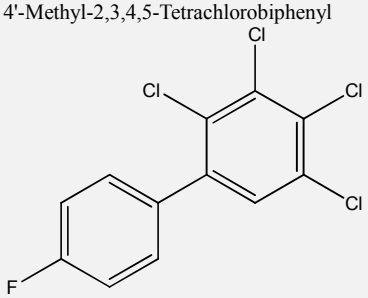
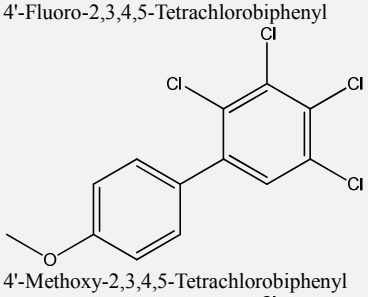
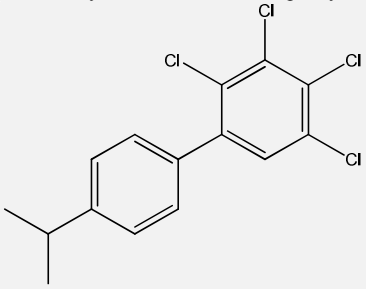
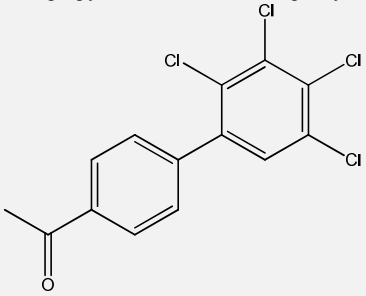
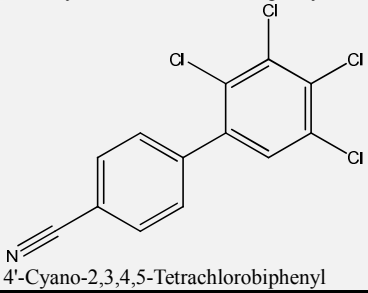
A data set of Polychlorinated biphenyls (30 PCBs) used for the QSTR analysis was selected from the literature [14]. The Chemical structures and corresponding log ($1/EC_{50}$) values for studied compounds are represented in Table 1.

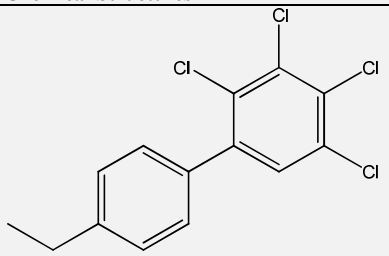
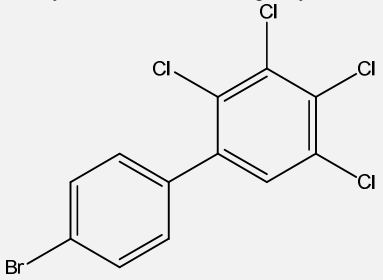
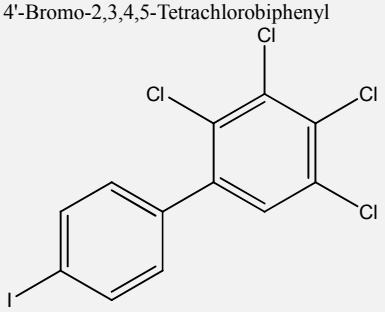
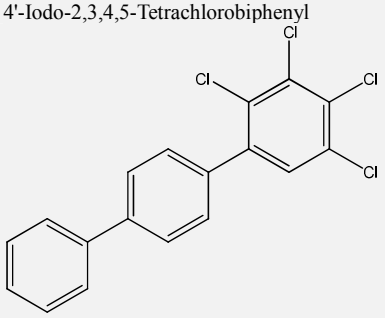
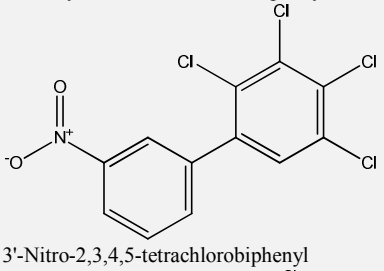
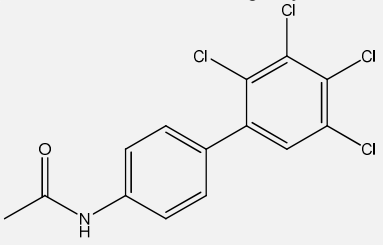
Table 1. *Chemical structures and experimental log ($1/EC_{50}$) values for studied compounds (30 PCBs).*

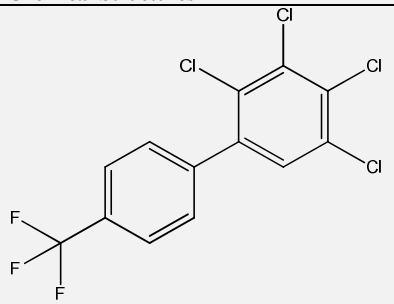
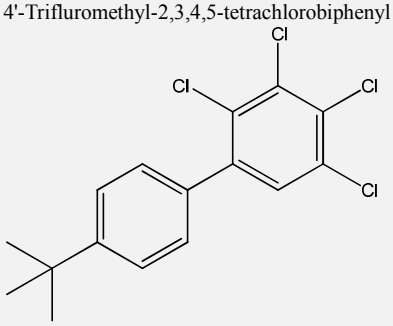
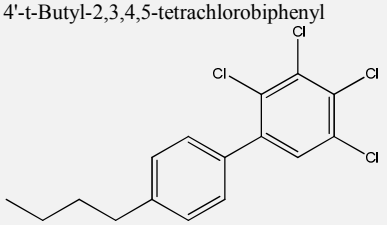
S/N	Chemical Structures	Log ($1/EC_{50}$)
1	 3,3',4,4'-Tetrachlorobiphenyl	6.15
2	 2,3',4,4'-Tetrachlorobiphenyl	4.55
3	 3,3',4,4',5-Pentachlorobiphenyl	6.89

S/N	Chemical Structures	Log (1/EC_{50})
4	 2,3,3',4,4'-Pentachlorobiphenyl	5.37
5	 2,3,4,4',5-Pentachlorobiphenyl	5.39
6	 2,3',4,4',5-Pentachlorobiphenyl	5.04
7	 2',3,4,4',5-Pentachlorobiphenyl	4.85
8	 2,3,3',4,4',5-Hexachlorobiphenyl	5.15
9	 2,3',4,4',5,5'-Hexachlorobiphenyl	4.80

S/N	Chemical Structures	Log ($1/EC_{50}$)
10	 2,3,3',4,4',5'-Hexachlorobiphenyl	5.33
11	 2,3',4,4',5',6-Hexachlorobiphenyl	4.00
12	 2,2',4,4',5',5-Hexachlorobiphenyl	4.10
13	 2,2',4,4'-Tetrachlorobiphenyl	3.89
14	 2,3,4,5-Tetrachlorobiphenyl	3.85
15	 4'-Hydroxy-2,3,4,5-Tetrachlorobiphenyl	4.05

S/N	Chemical Structures	Log (1/EC_{50})
16	 4'-Methyl-2,3,4,5-Tetrachlorobiphenyl	4.51
17	 4'-Fluoro-2,3,4,5-Tetrachlorobiphenyl	4.60
18	 4'-Methoxy-2,3,4,5-Tetrachlorobiphenyl	4.80
19	 4'-Isopropyl-2,3,4,5-Tetrachlorobiphenyl	5.89
20	 4'-Acetyl-2,3,4,5-Tetrachlorobiphenyl	5.17
21	 4'-Cyano-2,3,4,5-Tetrachlorobiphenyl	5.27

S/N	Chemical Structures	Log (1/EC_{50})
22	 4'-Ethyl-2,3,4,5-Tetrachlorobiphenyl	5.46
23	 4'-Bromo-2,3,4,5-Tetrachlorobiphenyl	5.60
24	 4'-Iodo-2,3,4,5-Tetrachlorobiphenyl	5.82
25	 4'-Phenyl-2,3,4,5-Tetrachlorobiphenyl	5.18
26	 3'-Nitro-2,3,4,5-tetrachlorobiphenyl	4.85
27	 4'-N-Acetylamino-2,3,4,5-tetrachlorobiphenyl	5.09

S/N	Chemical Structures	Log (1/EC_{50})
28	 4'-Triflurомethyl-2,3,4,5-tetrachlorobiphenyl	6.43
29	 4'-t-Butyl-2,3,4,5-tetrachlorobiphenyl	5.17
30	 4'-n-Butyl-2,3,4,5-tetrachlorobiphenyl	5.13

2.2. Molecular Optimization and Descriptors Calculation

Optimization is the process of finding the equilibrium or lowest energy geometry of molecules. The chemical structure of each compound was drawn with ChemDraw ultra [15] version 12.02 module of the program and subsequently imported into Wave function program Spartan '14' [16] version 1.2.2 for structural minimization. The geometries of all the compounds (30 PCBs) were optimized by means of Density functional theory (DFT) using B_3LYP level of theory and 6-31G* as the basis set. The molecular descriptors were calculated by using paDel descriptor tool kit and Spartan "14" software. The most significant descriptors were identified using the Genetic Function Approximation (GFA) algorithm.

2.3. Genetic Function Algorithm and Model Building

In this study, a statistical technique of analysis by Genetic function approximation algorithm was employed to build the models. Genetic function approximation (GFA) algorithm is a search method to find exact or approximation solution to optimization and search problems which is based on the principles of Darwinian evolution [17].

A peculiar features of Genetic function approximation (GFA) algorithm is that it generate a population of equations rather than a single equation as do most other statistical methods. The range of variations in this population gives added information on the quality of fit and importance of the descriptors [18]. The fitness function or Lack of Fit (LOF) used to estimate the quality of the model here was the leave one out gross validated correlation coefficient (Q^2_{LOO}) and is calculated by equation 1.

$$LOF = \frac{\text{LSE}}{\{[1-[c+d*p]\}2}_{m} \quad (1)$$

Where c= number of basic function

d = smoothing parameter

m = number of samples in the training set

LSE = least square error

P = total number of features contained in all basics functions [19].

2.4. Validation of Developed Model

The predictive ability of the developed QSTR model were evaluated using both internal and external statistical validation parameters. The validation parameters were compare with the minimum recommended value for a generally acceptable QSTR model proposed by Revinchandran et al. [20] shown in Table 2.

Table 2. *Validation parameters for a generally acceptable QSAR/QSTR model.*

S/N	Symbol	Name	Range
1	R^2	Coefficient of determination	≥ 0.6
2	Q^2	Gross validation coefficient	> 0.5

S/N	Symbol	Name	Range
3	R^2 pred.	Coefficient of determination for external test set	≥ 0.6
4	R^2 adj	Adjusted square correlation coefficient	> 0.5
5	p (95%)	Confidence interval at 95%	≤ 0.05
6	Next test set	Minimum number of extend test set	≥ 5
7	$R^2 - Q^2$	Difference between R^2 and Q^2	≤ 0.3

3. QSTR Results

3.1. QSTR Study Using DFT

A QSTR study was carried out for a set of 30 PCBs compounds in order to build a robust and rational Genetic function approximation (GFA) based QSTR models for predicting the toxicity of Polychlorinated biphenyls (PCBs) by exploring the correlations between the experimental p1/EC_{50} of the compounds and their calculated molecular descriptors that are responsible for the toxicity of the compound. Dataset Division GUI v 1.2 software was employed to divide the data set of studied compounds into a training set of 21 PCBs (70%) which was used to build the model and a prediction set (test set) of 9 PCBs (30%), which was applied to test the built model. The training set for the studied compounds with corresponding experimental, predicted and residual values in log (1/EC_{50}) was presented in Table 3.

Table 3. *IUPAC Name of the Training set with corresponding Experimental, Predicted and Residual Toxicity Values.*

S/N	IUPAC NAME	Experimental log (1/EC_{50})	Predicted log (1/EC_{50})	Residual log (1/EC_{50})
1	2,3',4,4'-Tetrachlorobiphenyl	4.550	4.964	-0.414
2	3,3',4,4',5-Pentachlorobiphenyl	6.890	6.808	0.082
3	2,3,3',4,4'-Pentachlorobiphenyl	5.370	5.482	-0.112
4	2,3,4,4',5-Pentachlorobiphenyl	5.390	4.954	0.436
5	2,3',4,4',5-Pentachlorobiphenyl	5.040	5.176	-0.136
6	2',3,4,4',5-Pentachlorobiphenyl	4.850	4.759	0.091
7	2,3,3',4,4',5-Hexachlorobiphenyl	5.150	5.312	-0.162
8	2,3',4,4',5,5'-Hexachlorobiphenyl	4.800	4.939	-0.139
9	2,3,3',4,4',5'-Hexachlorobiphenyl	5.330	5.114	0.216
10	2,3',4,4',5',6-Hexachlorobiphenyl	4.000	3.961	0.039
11	2,2',4,4'-Tetrachlorobiphenyl	3.890	3.873	0.017
12	4'-Hydroxy-2,3,4,5-Tetrachlorobiphenyl	4.050	4.098	-0.048
13	4'-Fluoro-2,3,4,5-Tetrachlorobiphenyl	4.600	4.671	-0.071
14	4'-Isopropyl-2,3,4,5-Tetrachlorobiphenyl	5.890	5.642	0.248
15	4'-Bromo-2,3,4,5-Tetrachlorobiphenyl	5.600	5.562	0.038
16	4'-Phenyl-2,3,4,5-Tetrachlorobiphenyl	5.180	5.185	-0.005
17	3'-Nitro-2,3,4,5-tetrachlorobiphenyl	4.850	4.735	0.115
18	4'-N-Acetylamino-2,3,4,5-tetrachlorobiphenyl	5.090	4.944	0.146
19	4'-Trifluromethyl-2,3,4,5-tetrachlorobiphenyl	6.430	6.485	-0.055
20	4'-t-Butyl-2,3,4,5-tetrachlorobiphenyl	5.170	5.367	-0.197
21	4'-n-Butyl-2,3,4,5-tetrachlorobiphenyl	5.130	5.218	-0.088

From the Table 3, the compound numbered 11 in the dataset, (*2,2',4,4'-Tetrachlorobiphenyl*) shown to has its predicted value as the most closest to experimental value compared to all other compounds in the dataset with the lowest positive residual value, this indicates that it is the best predicted compound of the dataset.

The GFA analysis generated five models out of which the most statistically significant model (model-1) was selected as presented in Table 4. The statistical parameters of the best model was presented in Table 5. A brief description of the descriptors of the model was shown in Table 6.

Table 4. *Best selected model; Model-1.*

S/N	Equation	Definition
1.	Y = - 1.443 * X490 + 2.841* X556 + 1.765 * X1332+ 4.895 * X1438 + 3.434	X490: SpMin8_Bhm X556: SpMax2_Bhs X1332: RDF65i X1438: E1p

Table 5. *Statistical parameters of the best model.*

Model	R^2	R^2adj	*Friedman LOF*	R^2cv	*F-Value*
1	0.938	0.923	0.185	0.906	60.731

Table 6. *A brief description of the selected descriptors of the best model.*

Descriptor	Regression coefficient	Description	Descriptor Class
SpMin8_Bhm	-1.443	Smallest absolute eigenvalue of Burden modified matrix - n 8 / weighted by relative mass	Burden Modified Eigenvalues Descriptor
SpMax2_Bhs	2.841	Largest absolute eigenvalue of Burden modified matrix - n 2 / weighted by relative I-state	Burden Modified Eigenvalues Descriptor
RDF65i	1.765	Radial distribution function - 065 / weighted by relative first ionization potential	RDF Descriptor
E1p	4.895	1st component accessibility directional WHIM index / weighted by relative polarizabilities	PaDEL WHIM Descriptor

The QSTR results in Table 5 were in good agreement with optimum acceptable parameters of a QSAR/QSTR model reported in Table 2.

The scattered plot between the experimental and predicted in log ($^1/EC_{50}$) of test set (External set validation) is presented in Figure 1. The Figure 2 shows a plot of experimental and predicted in log ($^1/EC_{50}$) of training set. The scattered plot of predicted versus experimental in log ($^1/EC_{50}$) for all of the 30 PCBs compounds studied (training and test set) is presented Figure.

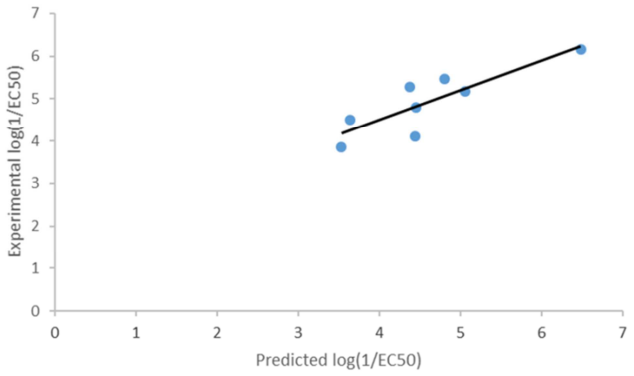


Figure 1. *A graphical representation of the model-1 Validation (Test Set).*

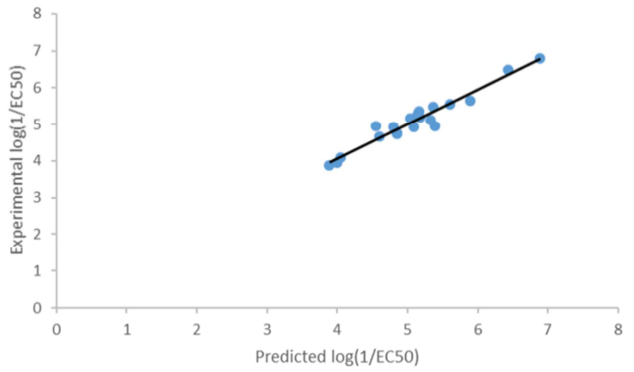


Figure 2. *A graphical representation of the model-1 (Training Set).*

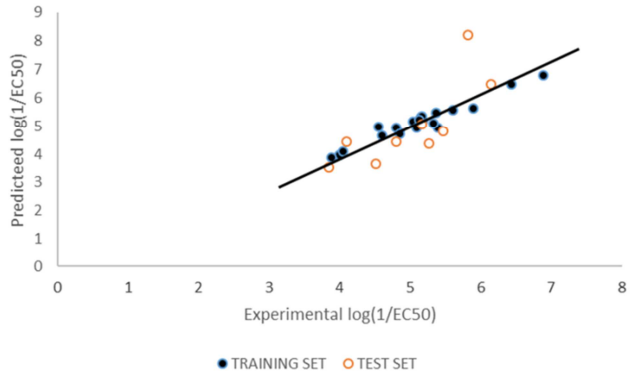


Figure 3. *A graphical representation of predicted log ($1/EC_{50}$) (training & test set) versus Experimental log (I/EC_{50}) values by GFA modeling.*

3.2. Evaluation of the GFA Model

The robustness GFA model was tested by applying Y-randomization. The low R^2 and Q^2 values that were obtained showing that the good results in the original model is not due to a chance correlation or structural depending of the training set. The results of Y-randomization test are presented in Table 7. The Random models parameters are shown in Table 8.

Table 7. *The results of Y-randomization of the Training set.*

Model	R	R^2	Q^2
Original	0.905	0.818	0.713
Random 1	0.426	0.181	-0.197
Random 2	0.452	0.204	-0.486
Random 3	0.347	0.121	-0.392
Random 4	0.262	0.069	-0.363
Random 5	0.221	0.049	-0.801

Table 8. *Random models parameters.*

Average R:	0.394
Average r^2:	0.179
Average Q^2:	-0.333
cRp^2:	0.737

3.3. Applicability Domain (AD)

Since the model 1 cannot predict the toxicity of all compounds in the universe, its applicability domain was determined using William's plot; *the plot of the standardized residuals versus the leverage as shown in* Figure *4*. This was exploited to visualize the applicability domain (AD) [21]. Leverage indicates a compound's distance from the centroid of X. The leverage of a compound in the original space is defined as;

$$hi = \boldsymbol{xi}\ (\boldsymbol{X}\ \boldsymbol{X})^{-1}\boldsymbol{xi}$$

Where xi the descriptor vector of the considered compound and X is the descriptor matrix derived from the training set descriptor values.

The warning leverage (h*) is defined as:

$$h = 3\frac{(P+1)}{n}$$

Where n = number of training compounds
p = number of predictor variables

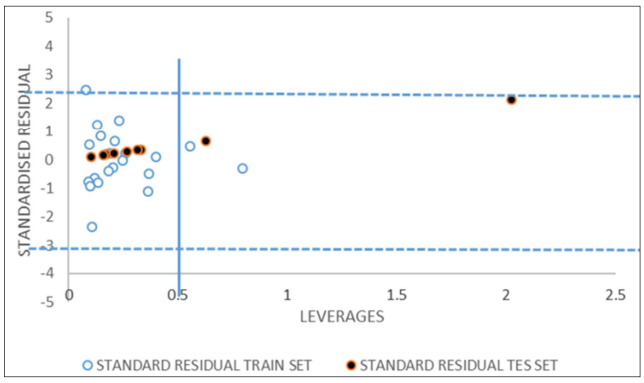


Figure 4. *Williams plot of the model.*

From the Williams plot (Figure 4) above, it is observed that compound in the test set fall inside the domain of the GFA model (the warning leverage h* =0.50) except only four compounds (*two in the training set and two in the test set)* which have the leverage higher than the warning h* value, thus they can be regarded as structural outliers.

3.4. Discussions

The result of the GFA QSTR model reported in Table 4 is in agreement with the standard shown in Table 2 as R^2 = 0.938, R^2_{adj} = 0.923, R^2cv = 0.906, F-value = 60.731, $P_{95\%}$ < 0.05, $R^2_{pred.}$ = 0.7238. This confirms the goodness and reliability of the model. Figure 1 and Figure 2 also reveal the agreement between the experimental and the predicted log ($1/EC_{50}$) values of the molecules in the training and the test set respectively. The high Linearity of these plots indicate a sound agreement between the experimental and predicted values indicative of the high internal and external predictive power of the model.

Likewise, Figure 3 gives a combine plot of the experimental and the predicted values of log ($1/EC_{50}$) training and test set molecules. The high linearity of the plot is indicative of an excellent external predictive power of the model. The comparison of observed and predicted log ($1/EC_{50}$) of the compounds is presented in Table 3. The predictability of model 1 is evidenced by the low residual values observed in the Table.

The P-value of the optimization model at 95% confidence level shown has α value < 0.05. This reveals that the alternative hypothesis that the magnitude of the observed toxicity of PCBs is a direct function of the descriptors of their total chemical structures takes preference over the null hypothesis which states otherwise.

The statistical significance of the relationship between the toxicity of PCBs and their molecular descriptors was further demonstrated by Y-randomization procedure. The results of Y-randomization test as well as the random models parameters are shown in Tables 7 and Table 8 respectively. The low R^2 and Q^2 values obtained show that the optimization model is robust and reliable and was not obtained due to a chance correlation.

Since the model 1 cannot predict the toxicity of all compounds in the universe, its applicability domain was determined using William's plot shown in Figure 4. All the compounds in the test set fall inside the domain of the GFA model (the warning leverage h* =0.50) except for four compounds (two in the training set and two in the test set) which have the leverage higher than the warning h* value as shown in the plot. This implies that the models can be successfully applied to this series of Polychlorinated biphenyls (PCBs). The few compounds with higher leverage than h* that are outside the domain are most likely to be structural outliers.

3.5. Significance of the Descriptors in the Model 1

The positive coefficient of the descriptors; SpMax2_Bhs, RDF65i and E1p reveal that the toxicity of PCBs increases with increase in the values of these descriptors. Thus, the higher the values of these descriptors in a PCB, the more the toxicity of the molecule and vice versa. Also, the negative coefficient of SpMin8_Bhm descriptor is an indication that the value of this descriptor in a PCB varies inversely with its toxicity. The percentage contribution of each descriptor in the model include; 45% (E1p), 16% (RDF65i), 26% (SpMax2_Bhs), 13% (SpMin8_Bhm). This reveals that 1st component accessibility directional WHIM index / weighted by relative polarizabilities descriptor (E1p) plays the most dominant role in influencing the observed toxicity of PCBs.

This can be rationalized thus:

E1p is a descriptor of molecular polarity. The result of the QSTR model reveals that the toxicity of PCBs increases with increase in the value of this descriptor in them. This is in consonance with similar theoretical study on toxicity of dibenzo-p-dioxins by Ya-Ying *et al*. (2008) [22] in which a descriptor of molecular polarity, α_{xx} was found to overwhelmingly influence the toxicity of the dioxins. The result of Quantitative structure-toxicity relationship study of some dioxins using molecular descriptors by Hassan *et al*. (2015) [23] also reveals the strong influence of molecular polarity on toxicity of this class of compounds. In their work, the observed toxicity of studied dioxins was found to be positively influenced by a descriptor of molecular polarity.

Though polarity of molecule enhances its water solubility and promotes its metabolism. The increase in toxicity with increase in polarity as depicted by the model may be as a result of the production of toxic intermediates or final products due to enhanced metabolism of PCBs owing to their enhanced water solubility orchestrated by their increasing polarity.

4. Conclusion

In this study, QSTR modelling for the toxicity of Polychlorinated biphenyls (30 PCBs) to explore the structural features that are responsible for its toxicity was successfully performed using Genetic Function Approximation (GFA) approach at B_3LYP level of theory and 6-31G* as the basis set. The observed log ($1/EC_{50}$) of the Polychlorinated biphenyls (PCBs) was found to be predominantly influenced by SpMin8_Bhm, SpMax2_Bhs, RDF65i and E1p descriptors. The robustness, reliability, stability and applicability of the QSTR models was established by internal and external validation techniques (R^2 = 0.938, R^2_{adj} = 0.923, R^2cv = 0.906, F-value = 60.731, $P_{95\%}$ < 0.05, $R^2_{pred.}$ = 0.7238). It is envisaged that the wealth of information in this model will provide a fast, economical and more environmental friendly techniques of accessing the toxicity of Polychlorinated biphenyls and other related toxic Polychlorinated aromatic chemicals.

Acknowledgement

The author appreciate the support from his dear wife Mrs

Olasupo Rasheedat Titilayo at all times. We also acknowledged the tremendous support of Mr David Ebuka Arthur, Philip Ameji, and Samuel Hassan in the course of this study.

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