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Ecotoxicological prediction of organic chemicals toward *Pseudokirchneriella subcapitata* by Monte Carlo approach†

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In the ecotoxicological risk assessment, acute toxicity is one of the most significant criteria. Green alga *Pseudokirchneriella subcapitata* has been used for ecotoxicological studies to assess the toxicity of different toxic chemicals in freshwater. Quantitative Structure Activity Relationships (QSAR) are mathematical models to relate chemical structure and activity/physicochemical properties of chemicals quantitatively. Herein, Quantitative Structure Toxicity Relationship (QSTR) modeling is applied to assess the toxicity of a data set of 334 different chemicals on *Pseudokirchneriella subcapitata*, in terms of EC₁₀ and EC₅₀ values. The QSTR models are established using CORAL software by utilizing the target function (TF₂) with the index of ideality of correlation (IIC). A hybrid optimal descriptor computed from SMILES and molecular hydrogen-suppressed graphs (HSG) is employed to construct QSTR models. The results of various statistical parameters of the QSTR model developed for pEC₁₀ and pEC₅₀ range from excellent to good and are in line with the standard parameters. The models prepared with IIC for Split 3 are chosen as the best model for both endpoints (pEC₁₀ and pEC₅₀). The numerical value of the determination coefficient of the validation set of split 3 for the endpoint pEC₁₀ is 0.7849 and for the endpoint pEC₅₀, it is 0.8150. The structural fractions accountable for the toxicity of chemicals are also extracted. The hydrophilic attributes like 1...n...(...) and S...(...=... exert positive contributions to controlling the aquatic toxicity and reducing algal toxicity, whereas attributes such as c...c...c..., C...C...C... enhance lipophilicity of the molecules and consequently enhance algal toxicity.

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1. Introduction

The organic chemicals released into the environment by factories can be potentially toxic pollutants of the environment. Contamination of aquatic ecosystems with organic chemicals is a serious concern because these can affect multiple levels of biological organization, from the molecular to the ecosystem level. The goal of all global communities is to achieve the management of chemicals and hazardous wastes that minimizes notable harmful effects on human health and the environment. The eco-toxicities of chemicals at different endpoints are measured according to the test guidelines of the OECD (Organization for Economic Co-operation and Development) and are utilized for regulative purposes.¹ *Pseudokirchneriella subcapitata* (*P. subcapitata*) is a microalga and it is frequently

employed as a bioindicator species in freshwater habitats to measure nutrient or hazardous chemical levels. The OECD and US-EPA (United States Environmental Protection Agency) recommend *P. subcapitata* for ecotoxicological bioassays since this microalga exhibit faster growth rates and better susceptibility to diverse toxins than other algae.^{1–4} In the ecological risk assessment, the most commonly used measurement to summarize ecotoxicological effects is the EC_x (effective concentration) where *x* can be 5–100.^{5,6}

However, the toxicological *in vivo* studies of all potential chemicals are practically impossible because these bioassays are expensive and time-consuming. Therefore, replacement approaches based on computational techniques are needed to mitigate these difficulties. In this regard, the Quantitative structure–activity/toxicity relationship (QSAR/QSTR), a significant computational technique, has been suggested to estimate the statistical relationship between the toxicity of a group of compounds with their molecular structure.^{7–10} A set of mathematical equations that equate the chemical structure to biological activity are designated as QSTR/QSAR models.

CORAL (CORrelation And Logic) software has been recommended for the construction of QSAR/QSTR models for various endpoints employing the inbuilt Monte Carlo algorithm.^{11–18} In the CORAL software, SMILES (Simplified Molecular Input Line

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Entry System) notations of the compound structures are employed as input to determine the descriptor of correlation weight (DCW). The DCW depends on the compound structure and the property under analysis but does not depend on the 3D geometry of the molecule. However, the QSAR/QSTR models of the CORAL software can be developed from three types of descriptors: SMILES-based, graph-based, and hybrid descriptors (a combination of SMILES and graphs). The models constructed based on the hybrid descriptors are statistically better than the models constructed by individually SMILES or graph descriptors.^{19–21}

The index of ideality of correlation (IIC) has been implemented by the theoretical chemist to validate and improve the predictive potential constructed QSAR/QSTR models.^{14,22–25} The IIC is a parameter for assessing the predictive capability of QSPR/QSAR models that takes into account not only the coefficient of correlation, but also the organization of the group of dots images relative to the diagonal, in “observed-calculated” endpoint coordinates.^{26–28}

The aim of the present work is to develop reliable QSTR models with the use of IIC to compute pEC₁₀ and pEC₅₀ of organic pollutants against *P. subcapitata*. A hybrid optimal descriptor is employed to design QSTR models for 334 diverse organic chemicals including pharmaceuticals, agrochemicals and personal care products using the Monte Carlo approach. Four random splits are implemented to assess the reliability and accuracy of the designed QSTR models.

2. Method

2.1. Data

In the present QSTR model development study, the prediction of effective concentration for 10% inhibition (EC₁₀) and half-maximal effective concentration (EC₅₀) was described for 334 diverse organic chemicals. Only those numerical values of EC₁₀ and EC₅₀ were taken into account for model development, which was calculated at the uniform exposure interval of 24 hours (EC₁₀-24 h and EC₅₀-24 h). The experimental acute toxicity of organic pollutants against *P. subcapitata* was taken from the article published by K. Khan & K. Roy.²⁹ The functional group amines, esters, halohydrocarbons, aldehydes, isothiocyanates, organic acids, alcohols (or phenols), ketones, etc. were present in the dataset. The pEC₁₀ (mol L⁻¹) and pEC₅₀ (mol L⁻¹) of organic compounds against *P. subcapitata* were utilized as two separate endpoints. The range of pEC₁₀ was from 0.54 to 9.3 mol L⁻¹ whereas the range of pEC₅₀ was from 0.3 to 9.1 mol L⁻¹.³⁰

The BIOVIA Draw 2019 tool was used to sketch the molecular structures of all 334 organic chemicals and the SMILES notations. Three splits were made for the above-mentioned organic chemicals ($n = 334$) and each split was unselectively split into the training ($\approx 35\%$), invisible training ($\approx 25\%$), calibration ($\approx 16\%$), and validation ($\approx 25\%$) set.

The responsibility of each set was fixed and these were: (i) the active training set provided the information on which the model was built (correlation weights are calculated); (ii) the passive training set gave the information to check how well the correlation weights fitted for the molecular structures of the active

set which were suitable for the structures not present in the active training set (“model quality inspector”); (iii) the calibration set should catch the moment when, despite the increase in the statistical quality of the model for the active and passive sets, the statistical quality for the calibration set begins to fall. In fact, this is the moment of the start of overtraining. (iv) The validation set was applied for the final assessment of the statistical quality of the model.

Table S1† represents the list of compounds IDs and SMILES code, as well as the corresponding experimental and estimated pEC₅₀ and pEC₁₀ values of chemicals.

2.2. Hybrid optimal descriptor

Herein, for designing the QSTR model of pEC₁₀ and pEC₅₀, the hybrid optimal descriptor was implemented and it was computed by the combination of molecular features extracted from SMILES and hydrogen suppressed molecular graph (HSG). In terms of statistical quality, the literature survey revealed that better models were generated by hybrid descriptors than the descriptors based only on SMILES or molecular graphs.³¹ The inbuilt Monte Carlo algorithm CORAL tool was employed to calculate the hybrid descriptors of correlation weights (DCW).

According to the OECD second principle, the algorithm used at each start performs the same sequence of actions. However, since the algorithm performs a stochastic process of processing the presented (input) information, the numerical values of the model quality criteria used are not identical. But, in the case of comparing the results of several such processes (for example, with different partitions into training and testing sets), reproducible means and variances will be obtained for the statistical characteristics (correlation coefficient and RMSE).

The QSTR model to predict pEC₁₀ and pEC₅₀ of organic chemicals is computed using the following mathematical relationship:

$$\text{pEC}_{10} \text{ or } \text{pEC}_{50} = C_0 + C_1 \times \text{HybridDCW}(T^*, N^*) \quad (1)$$

C_0 , C_1 , and DCW (descriptor of correlation weights) are the regression coefficients, the slope, and the optimal hybrid descriptor, respectively. The T^* denotes the threshold value and N^* denotes the number of epochs for the Monte Carlo optimization.

$$\text{HybridDCW}(T^*, N^*) = \text{SMILESDCW}(T, N^*) + \text{GraphDCW}(T^*, N^*) \quad (2)$$

$$\text{SMILESDCW}(T^*, N^*) = \sum \text{CW}(\text{SSS}_k) + \text{CW}(\text{BOND}) + \text{CW}(\text{NOSP}) + \text{CW}(\text{PAIR}) + \text{CW}(\text{HARD}) \quad (3)$$

$$\text{GraphDCW}(T^*, N^*) = \sum \text{CW}(\text{e1}_k) + \sum \text{CW}(\text{pt4}_k) + \sum \text{CW}(\text{nn}_k) + \sum \text{W}(\text{C5}) + \sum (\text{C6}) \quad (4)$$

In eqn (3) the code SSS_k is the local SMILES attributes described as a combination of three SMILES-atoms; NOSP is global SMILES features and it denotes the absence or presence of N (nitrogen), O (oxygen), S (sulfur), and P (phosphorus); BOND displays the presence or absence of double (=), triple



(#) and stereochemical ('@' or '@@') bonds; PAIR illustrates the combination of BOND and NOSP; HARD imply the existence or absence of NOSP, HALO (halogens), and BOND in the molecular structure.

In eqn (4), $e1_k$, $pt4_k$, and nn_k are local graph attributes. The $e1_k$ is Morgan extended connectivity of first order; $pt4_k$ is the number of paths of length 4 beginning from a given vertex in HSG; nn_k is the nearest neighbours code; C5 and C6 display the role of five- and six-member rings, respectively.

In CORAL software, two kinds of target functions (TF₁ and TF₂) can be applied to build the QSTR model with the Monte Carlo optimization. The balance of the correlation method was used to compute TF₁ whereas the IIC was added to the TF₁ to attain the modified target function TF₂.³²

$$TF_1 = R_{TRN} + R_{ITRN} - |R_{TRN} - R_{ITRN}| \times 0.1 \quad (5)$$

$$TF_2 = TF_1 + IIC_{CAL} \times C \quad (6)$$

Here, R_{TRN} and R_{ITRN} are the correlation coefficients for the training and invisible training sets, respectively. The C is an empirical coefficient or weight of IIC, here $C = 0.2$.

The IIC_{CAL} for the calibration (CAL) set is computed utilizing the following equation:

$$IIC = R_{CAL} \times \frac{\min(-MAE_{CAL}, +MAE_{CAL})}{\max(-MAE_{CAL}, +MAE_{CAL})} \quad (7)$$

R_{CAL} is the correlation coefficient between observed values and calculated values of pEC₁₀ or pEC₅₀ for the calibration set. The negative and positive mean absolute errors are indicated with $-MAE$ and $+MAE$, which are computed by the subsequent equations:

$$-MAE_{CAL} = -\frac{1}{N} \sum_{j=1}^{N^-} |\Delta_k| \quad (8)$$

$$\Delta_k < 0, \quad N^- \text{ is the number of } \Delta_k < 0$$

$$+MAE_{CAL} = +\frac{1}{N} \sum_{j=1}^{N^+} |\Delta_k| \quad (9)$$

$$\Delta_k < 0, \quad N^+ \text{ is the number of } \Delta_k \geq 0$$

$$\Delta_k = \text{Observed}_k - \text{Calculated}_k \quad (10)$$

The ' k ' is the index (1, 2, ... N) and the observed _{k} and calculated _{k} are related to the endpoint.

2.3. Applicability domain

According to the third principle of the OECD,^{33,34} a QSAR model should have a well-defined applicability domain (AD).

The domain of applicability for the model obtained as a result of stochastic Monte Carlo optimization varies depending on the split into training and validation sets. The applicability domain is determined according to the prevalence of molecular features extracted from SMILES (*e.g.* nitrogen 'N', oxygen 'O', double bonds '=', *etc.*) in the active training set.

Thus, for several splits into training and validation sets, the domain of applicability may change, but not significantly. The corresponding calculations give only a qualitative picture in terms such as "this compound is suspicious, because its constituent molecular features are poorly represented in the active training set." In CORAL software, AD is defined using the following relationship³⁰

$$\text{Defect}_{A_k} = \frac{|P_{TRN}(A_k) - P_{CAL}(A_k)|}{N_{TRN}(A_k) + N_{CAL}(A_k)} \quad \text{If } A_k > 0 \quad (11)$$

$$\text{Defect}_{A_k} = 1 \quad \text{If } A_k = 0$$

$P_{TRN}(A_k)$ and $P_{TCAL}(A_k)$ are the probability of an attribute ' A_k ' in the training and the calibration sets; and are the number of times or frequency of ' A_k ' in the training and calibration sets, respectively.

The statistical defect can be defined as the sum of statistical defects of all attributes present in the SMILES notation.

$$\text{Defect}_{\text{Molecule}} = \sum_{k=1}^{NA} \text{Defect}_{A_k} \quad (12)$$

NA is the number of active SMILES attributes for the given compounds.

In CORAL, a substance is an outlier if inequality 13 is fulfilled:

$$\text{Defect}_{\text{molecule}} > 2 \times \overline{\text{Defect}}_{TRN} \quad (13)$$

$\overline{\text{Defect}}_{TRN}$ is an average of statistical defects for the dataset of the training set.

3. Results and discussion

3.1. QSAR modeling for pEC₁₀ and pEC₅₀

In order to build up the trustworthy QSAR model(s), the T^* (optimal threshold) and the N^* (number of epochs) for the calibration set was calculated by analyzing the best statistical characteristics. The optimum value of T^* for models of pEC₁₀ and pEC₅₀ was 1 and N^* was 10 for all splits. All QSTR models for pEC₁₀ and pEC₅₀ of organic compounds against *P. subcapitata* were constructed using the target function TF₂ ($W_{IIC} = 0.2$).

The QSTR models obtained by the Monte Carlo optimization for both endpoints are the represented by the following relationship:

pEC₁₀ model

$$\text{Split 1 pEC}_{10} = 1.6154476 (\pm 0.0136475) + 0.1917901 (\pm 0.0006197) \times \text{DCW}(1,10) \quad (14)$$

$$\text{Split 2 pEC}_{10} = 2.0134704 (\pm 0.0124620) + 0.1346531 (\pm 0.0005317) \times \text{DCW}(1,10) \quad (15)$$



$$\text{Split 3 pEC}_{10} = 0.3932798 (\pm 0.0145016) + 0.2487573 (\pm 0.0007835) \times \text{DCW}(1,10) \quad (16)$$

pEC₅₀ model

$$\text{Split 1 pEC}_{50} = 1.2841679 (\pm 0.0150488) + 0.1617599 (\pm 0.0007597) \times \text{DCW}(1,10) \quad (17)$$

$$\text{Split 2 pEC}_{50} = 1.2939204 (\pm 0.0113965) + 0.1531264 (\pm 0.0005331) \times \text{DCW}(1,10) \quad (18)$$

$$\text{Split 3 pEC}_{50} = 1.1720502 (\pm 0.0122202) + 0.1507783 (\pm 0.0005351) \times \text{DCW}(1,10) \quad (19)$$

3.4. Model validation

Validation of the developed models is important in evaluating the reliability and robustness of the QSTR models. Validation of the model can be examined using the: (i) cross-validation (Q^2) or internal validation (R^2). The predictive ability of the QSTR model is acceptable if the numerical value of Q^2 and R^2 is greater than 0.7;³⁵ (ii) external validation, CCC (concordance correlation coefficient), Q_2F_1 , Q_2F_2 , Q_2F_3 , s (standard error of estimation), RMSE (root-mean-square error), MAE (mean absolute error), F (Fischer ratio), and metrics (R^2m and MAE based metric). In terms of external validation, the model has good predictability if CCC is greater than 0.85.³⁶ Also, if r^2m

values >0.5 and $\Delta r^2m < 0.2$, the model can be interpreted as a reliable model; (iii) Y-scrambling or data randomization.

Herein all these methods had been used for model validation. The IIC criterion was applied as a final statistical parameter to validate the developed QSTR models. The statistical characteristics calculated with eqn (14)–(19) are provided in Table 1. The mathematical equations of the applied statistical criteria are very well explained in the literature.^{32,37} All designed QSAR models were statistically reliable and the numerical values of statistical quantities were found in acceptable ranges as reported in the literature.^{33,34}

In QSTR modelling of pEC₁₀, the numerical values of $R^2_{\text{Validation}}$ and $Q^2_{\text{Validation}}$ were in the range of 0.7246–0.7849 and 0.7149–0.7776, respectively. Whereas, in the QSAR modelling of pEC₅₀, the numerical values of $R^2_{\text{Validation}}$ and $Q^2_{\text{Validation}}$ were in the range of 0.7366–0.8150 and 0.7231–0.8065, respectively. The most reliable model was presented by Split 3 for pEC₁₀ as the statistical result of the determination coefficient was the highest. The numerical values of various parameters for the validation set of split 3 were $R^2 = 0.7849$, $Q^2 = 0.7776$, CCC = 0.8648, $r^2m = 0.7612$ and $\Delta r^2m = 0.1010$ (Table 1). Similarly, for endpoint pEC₅₀, the model developed for split 3 was assigned as a prominent model. The statistical results for benchmarks for the validation set were $R^2 = 0.8150$; $Q^2 = 0.8065$; CCC = 0.9020; $r^2m = 0.7743$ and $\Delta r^2m = 0.0683$. Thus, these statistical results confirmed that the models constructed were acceptable in terms of statistics. Fig. 1 shows the plots of

Table 1 The summary of statistical characteristics and criteria of predictability of the QSTR models obtained for pEC₁₀ and pEC₅₀ of organic compounds for three random splits

| Split | Set | <i>n</i> | R^2 | CCC | IIC | Q^2 | $Q_{F_1}^2$ | $Q_{F_2}^2$ | $Q_{F_3}^2$ | R_m^2 | CR_p^2 | \bar{r}_m^2 | Δr_m^2 | <i>S</i> | MAE | <i>F</i> |
|-------------------------|--------------------|----------|--------|--------|--------|--------|-------------|-------------|-------------|---------|----------|---------------|----------------|----------|--------|----------|
| pEC₁₀ | | | | | | | | | | | | | | | | |
| 1 | Training | 118 | 0.8550 | 0.9218 | 0.8072 | 0.8504 | | | | | 0.8522 | | | 0.651 | 0.496 | 684 |
| | Invisible training | 79 | 0.8609 | 0.8856 | 0.5277 | 0.8535 | | | | | 0.8556 | | | 0.742 | 0.576 | 476 |
| | Calibration | 54 | 0.7186 | 0.8349 | 0.8389 | 0.6883 | 0.7282 | 0.7045 | 0.8212 | 0.7154 | 0.7111 | 0.6049 | 0.1210 | 0.725 | 0.592 | 133 |
| | Validation | 83 | 0.7246 | 0.8435 | 0.6846 | 0.7149 | | | | 0.7246 | | 0.6174 | 0.143 | 0.8339 | 6291 | |
| 2 | Training | 115 | 0.8855 | 0.9393 | 0.8932 | 0.8804 | | | | | 0.8793 | | | 0.533 | 0.408 | 874 |
| | Invisible training | 73 | 0.8868 | 0.9022 | 0.4317 | 0.8802 | | | | | 0.8823 | | | 0.706 | 0.553 | 553 |
| | Calibration | 63 | 0.8487 | 0.9146 | 0.9210 | 0.8391 | 0.8466 | 0.8460 | 0.8362 | 0.8160 | 0.8388 | 0.7468 | 0.1385 | 0.657 | 0.513 | 342 |
| | Validation | 83 | 0.7643 | 0.8716 | 0.7643 | 0.7731 | | | | 0.7575 | | 0.6965 | 0.1219 | 0.8779 | 0.7052 | |
| 3 | Training | 113 | 0.8866 | 0.9399 | 0.7473 | 0.8826 | | | | | 0.8796 | | | 0.545 | 0.426 | 867 |
| | Invisible training | 79 | 0.8775 | 0.9194 | 0.5672 | 0.8722 | | | | | 0.8742 | | | 0.691 | 0.517 | 551 |
| | Calibration | 59 | 0.8106 | 0.8985 | 0.8632 | 0.7970 | 0.8002 | 0.7987 | 0.8465 | 0.7260 | 0.8049 | 0.7336 | 0.0152 | 0.679 | 0.537 | 244 |
| | Validation | 83 | 0.7892 | 0.8648 | 0.8831 | 0.7776 | | | | 0.7612 | | 0.6061 | 0.1010 | 0.6765 | 0.5691 | |
| pEC₅₀ | | | | | | | | | | | | | | | | |
| 1 | Training | 114 | 0.8401 | 0.9131 | 0.7161 | 0.8331 | | | | | 0.8335 | | | 0.683 | 0.537 | 588 |
| | Invisible training | 82 | 0.8395 | 0.9006 | 0.7660 | 0.8311 | | | | | 0.8278 | | | 0.733 | 0.587 | 418 |
| | Calibration | 52 | 0.7915 | 0.8717 | 0.8839 | 0.7771 | 0.7853 | 0.7851 | 0.8433 | 0.7479 | 0.7792 | 0.6529 | 0.1900 | 0.681 | 0.533 | 190 |
| | Validation | 85 | 0.7924 | 0.8297 | 0.7490 | 0.7774 | | | | 0.6276 | | 0.5802 | 0.0949 | 0.7716 | 0.6247 | |
| 2 | Training | 116 | 0.8341 | 0.9096 | 0.9133 | 0.8289 | | | | | 0.8297 | | | 0.655 | 0.517 | 573 |
| | Invisible training | 76 | 0.8704 | 0.9186 | 0.8496 | 0.8626 | | | | | 0.8634 | | | 0.671 | 0.529 | 497 |
| | Calibration | 59 | 0.7802 | 0.8795 | 0.8808 | 0.7623 | 0.7622 | 0.7435 | 0.7914 | 0.6309 | 0.7679 | 0.6918 | 0.1218 | 0.774 | 0.596 | 202 |
| | Validation | 83 | 0.7366 | 0.8517 | 0.8494 | 0.7231 | | | | 0.5993 | | 0.6371 | 0.0756 | 0.7696 | 0.6055 | |
| 3 | Training | 116 | 0.8665 | 0.9285 | 0.7831 | 0.8617 | | | | | 0.8568 | | | 0.617 | 0.461 | 740 |
| | Invisible training | 79 | 0.9130 | 0.9350 | 0.9123 | 0.9088 | | | | | 0.9065 | | | 0.512 | 0.409 | 808 |
| | Calibration | 56 | 0.7270 | 0.8484 | 0.8525 | 0.7031 | 0.6898 | 0.6860 | 0.7888 | 0.5823 | 0.7205 | 0.6237 | 0.0829 | 0.756 | 0.606 | 144 |
| | Validation | 83 | 0.8150 | 0.9020 | 0.8320 | 0.8065 | | | | 0.7743 | | 0.7402 | 0.0683 | 0.7245 | 0.6110 | |



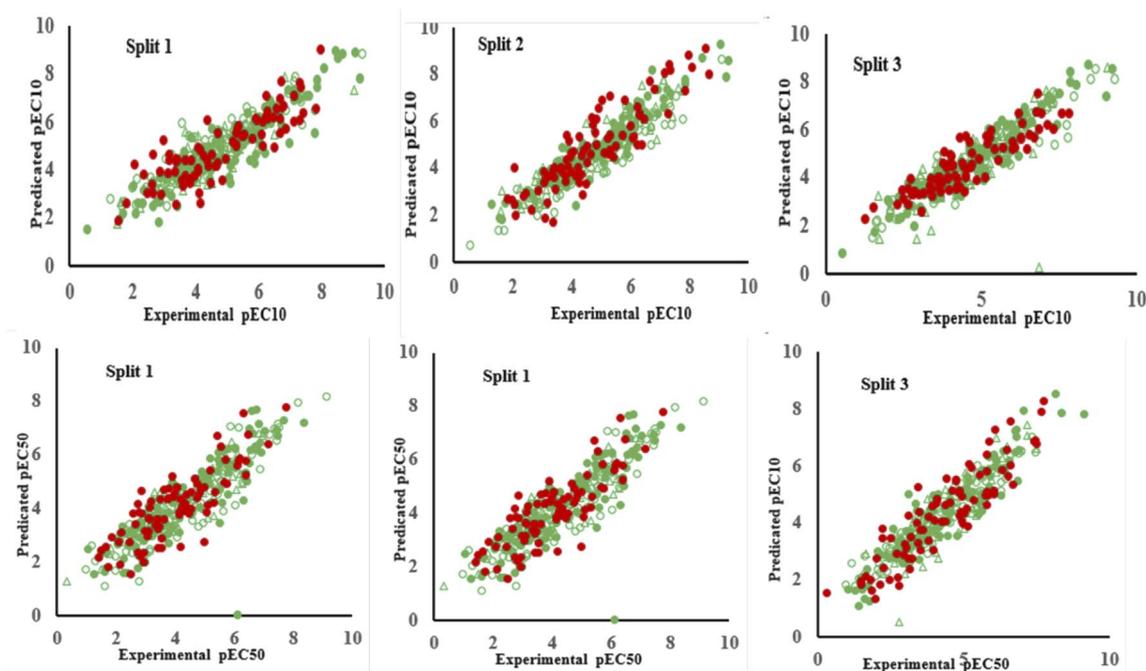


Fig. 1 Graphical display of QSTR models for pEC₁₀ and pEC₅₀ of organic compounds obtained for three splits.

experimental pEC₁₀/pEC₅₀ versus predicted pEC₁₀/pEC₅₀ for all splits and it displays that the predicted pEC₁₀/pEC₅₀ have a good correlation with its experimental data. Also, Fig. 1 indicates the uniform data distribution of pEC₁₀/pEC₅₀ for training, invisible training, calibration and validation sets across all runs. The plots of residual pEC₁₀/pEC₅₀ versus predicted pEC₁₀/pEC₅₀ for all QSTR models are represented in Fig. 2. Residual scattering of pEC₁₀ and pEC₅₀ was found near the horizontal line centred around zero, implying that all constructed QSTR models were well fitted. The Kolmogorov–Smirnov test for normality (at the 95% confidence level) was done by SPSS version 26. It confirmed a normal distribution of residuals for all proposed models for pEC₁₀ and pEC₅₀ (Table S2†).

3.5. Mechanistic interpretation

“Mechanistic interpretation if possible” is the 5th principle of OECD. The objective of mechanistic interpretation is to explore a mechanistic relationship between the descriptors employed in a model and the endpoint being predicted.

Monte Carlo optimization may be used numerous times to get a mechanistic explanation for CORAL models. If a molecular characteristic has acquired a positive correlation weight in all runs, its existence is likely to promote an increase in endpoint magnitude. If a molecular characteristic has a negative correlation weight in all of the preceding runs, its existence is more likely to decrease the intensity of the endpoint. The relevance of the molecular characteristic is unclear if the weights alternate (some positive, some negative). It is also necessary to consider the frequencies of molecular characteristics in the training and control sets.

In the present research, the structural attributes (SAK) extracted from SMILES and HSG attributes were employed to explore a relationship between the DCW and pEC₁₀ or pEC₅₀.

The SAK extracted from at last three or more independent runs of the Monte Carlo optimization were chosen for mechanistic interpretation. The SAK having the positive or negative CW values in all runs were kept in the category of a promoter of increase or decrease endpoint (pEC₁₀ or pEC₅₀). Table 2 illustrates the list of structural attributes of pEC₁₀ and pEC₅₀ with their CWs for three independent runs.

Based on the results summarized in Table 2, the promoters of pEC₁₀ increase were: C5...0..., c...c...c..., c...(c...c..., C...C...C..., N...(c...c..., C...(c...c... and the promoters of pEC₅₀ increase were: C5...0..., C...(c...c..., c...c...c..., C6...A...1..., C...C...C... On the other hand, the promoters of pEC₁₀ decrease were: c...n...c..., S...(c...c..., +++S...B2==, S...(c...=..., and +++Cl...S====; whereas promoters of pEC₅₀ decrease were: 1...n...(c..., S...(c...=..., [...-...Cl..., and +++O...S====. The results of mechanistic interpretation are illustrated in Fig. 3. Hence, The hydrophilic attributes like 1...n...(c... and S...(c...=... exert positive contributions to controlling the aquatic toxicity and reducing algal toxicity, whereas attributes such as c...c...c..., C...C...C... enhance lipophilicity of the molecules and consequently enhance algal toxicity (see Fig. 3). The hydrophilic attributes like 1...n...(c... and S...(c...=... attributes and lipophilic attributes such as c...c...c..., C...C...C... influences the bioavailability of organic compounds and regulates their passage across biological membranes. A chemical with a greater lipophilicity may be more hazardous.



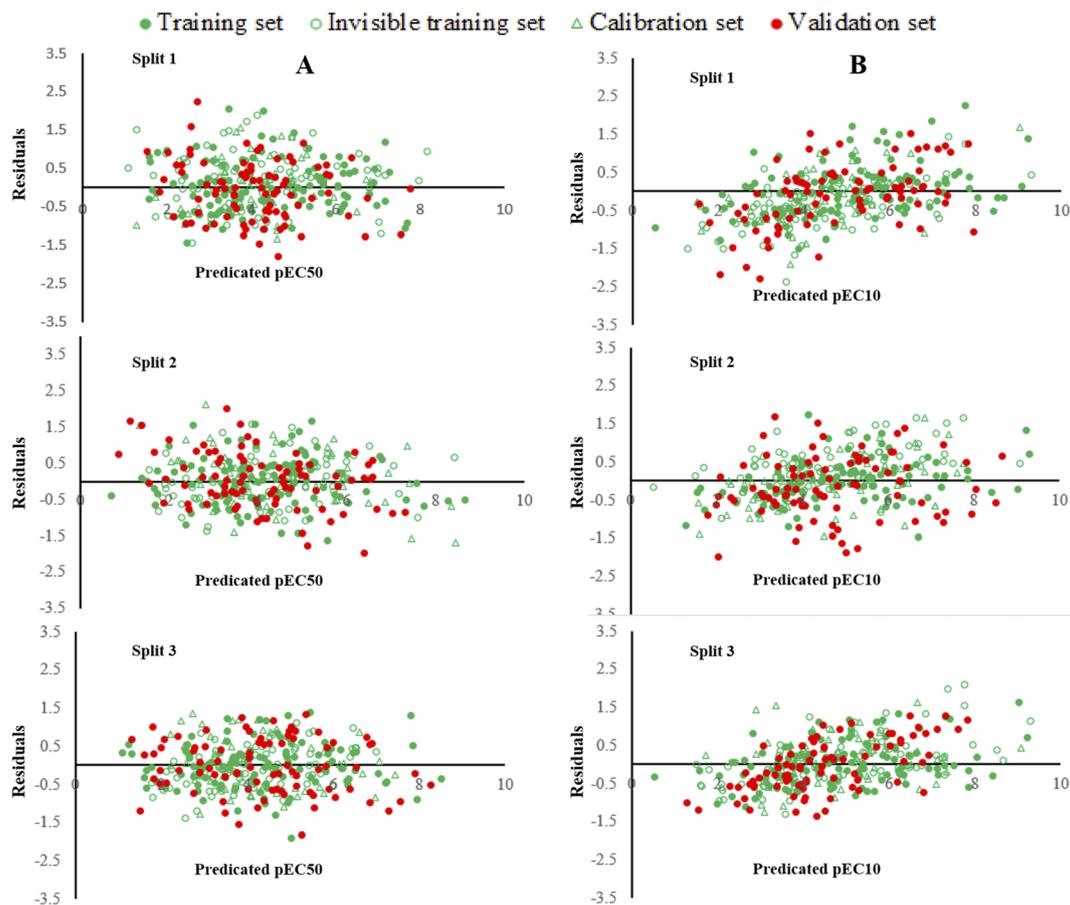


Fig. 2 A graphical presentation of residual pEC₁₀ versus predicted pEC₁₀ (A) and residual pEC₅₀ versus predicted pEC₅₀ (B) for all constructed QSTR models.

3.6. Comparison with the previously reported models

Previously several QSAR/QSTR Models to predict pEC_x of organic chemicals against *P. subcapitata* have been reported and some statistical characteristics of the reported QSTR models are summarized in Table 3.

Lee and Chen³⁸ reported two QSAR models for the prediction of the pEC₅₀ of 20 benzoic acids to *P. subcapitata*. The R^2 of the models were 0.921 and 0.965. Aruoja *et al.*^{39,40} predicted the toxicity in terms of pEC₅₀ of 58 polar narcotic chemicals ($R^2 = 0.6$) and 50 non-polar narcotic chemicals ($R^2 = 0.9469$) in two independent QSTR studies. They also built a combined model for 108 polar and nonpolar narcotic compounds based on three descriptors including $\log K_{ow}$, molecular weight (MW) and $\Delta H_f / \#_{atoms}$ (R^2 of 0.9149).

Khan and Roy^{29,41,42} reported several QSTR models for the toxicity pEC₅₀ of 30 cosmetics, 69 pharmaceuticals and 334 organic compounds (pEC₁₀ and pEC₅₀) against *P. subcapitata* in three independent studies. The dataset for the developed model of cosmetics was divided into 20 compounds of the training set and 10 compounds of the test set with R^2 of 0.885 and 0.712, respectively. For QSTR modelling of 69 pharmaceutical compounds, the total data set was partitioned into sets of training (53 compounds) and test (16 compounds). The

suggested models for pharmaceuticals, respectively, have R^2 of 0.69 and 0.71 for the training and test sets. In the next study, they constructed several six descriptors-based QSTR models for pEC₅₀ (24 h) and eight descriptors-based QSTR models for pEC₁₀ using 334 organic chemicals against *P. subcapitata*. The dataset was divided into the training set (251 compounds, $R^2 = 0.72$) and the test set (83 compounds, $R^2 = 0.70$).

Later, Yu⁴³ developed six descriptors QSTR models for 334 chemicals against *P. subcapitata*, by employing SVM (Support Vector Machine) and genetic algorithm. The dataset for the endpoint pEC₁₀ was divided into training ($n = 167$ compounds, $R^2 = 0.76$ and MAE = 0.60) and test sets ($n = 167$ compounds, $R^2 = 0.75$ and MAE = 0.61) in the ratio of 1 : 1 (training set and 167 compounds test set). Also, the QSTR models were developed for endpoint pEC₅₀ with 167 compounds for training ($R^2 = 0.75$ and MAE = 0.60) and 167 compounds for test sets ($R^2 = 0.74$ and MAE = 0.61).

Recently, Masand *et al.*⁶ developed a QSTR model to estimate the EC₅₀ for 72 h based assay for the microalga *Pseudo-kirchneriella subcapitata* utilising a data collection of 271 compounds from various chemical classes. The Genetic Algorithm Multi-linear regression (GA-MLR) was employed to develop models. The dataset was divided into two sets, *i.e.*



Table 2 The structural attribute as promoters of endpoint increase/decrease, their correlation weights, the number of each attribute in each set and instances of interpretation attributes

| Endpoint | SAK | Split | CWs run 1 | CWs run 2 | CWs run 3 | N1 | N2 | N3 | Defect | Comments |
|-----------------------------|-------------|-------|-----------|-----------|-----------|-----|----|----|--------|--|
| Promoter of increase | | | | | | | | | | |
| pEC ₁₀ | C5.....0... | 1 | 0.0518 | 0.65382 | 0.39759 | 113 | 73 | 54 | 0.0003 | Absence of five-member rings |
| | | 2 | 0.65986 | 1.29285 | 0.5346 | 108 | 67 | 62 | 0.0003 | |
| | | 3 | 1.07757 | 1.25744 | 0.48657 | 107 | 78 | 56 | 0 | |
| c...c...c... | 1 | 1 | 0.60173 | 0.70843 | 0.06133 | 57 | 23 | 24 | 0.0005 | Presence of three consecutive aromatic carbons |
| | | 2 | 0.05675 | 0.25722 | 0.44496 | 50 | 27 | 30 | 0.0005 | |
| | | 3 | 0.12996 | 0.47028 | 0.75404 | 49 | 32 | 26 | 0.0001 | |
| c...(...c... | 1 | 1 | 0.70809 | 0.0575 | 1.34021 | 56 | 22 | 25 | 0.0001 | Presence of two aromatic carbon with branching |
| | | 2 | 0.38614 | 0.33907 | 0.1424 | 42 | 29 | 26 | 0.0007 | |
| | | 3 | 0.11744 | 0.51584 | 1.00395 | 44 | 37 | 22 | 0.0002 | |
| C...C...C... | 1 | 1 | 0.5463 | 1.04019 | 0.43998 | 27 | 22 | 18 | 0.0023 | Presence of three consecutive aliphatic carbons |
| | | 2 | 1.06593 | 1.00475 | 0.65444 | 33 | 13 | 20 | 0.0006 | |
| | | 3 | 0.6226 | 0.74328 | 0.93265 | 27 | 24 | 14 | 0 | |
| N...(...C... | 1 | 1 | 0.40394 | 0.48781 | 1.00039 | 23 | 19 | 13 | 0.0013 | Presence of aliphatic nitrogen and aliphatic carbon with branching |
| | | 2 | 1.07368 | 0.89617 | 0.01453 | 22 | 11 | 15 | 0.0013 | |
| | | 3 | 0.3214 | 0.5839 | 0.59097 | 21 | 23 | 12 | 0.0005 | |
| C...(...C... | 1 | 1 | 0.44453 | 0.59506 | 1.29729 | 43 | 45 | 31 | 0.0028 | Presence of two aliphatic carbon with branching |
| | | 2 | 0.30408 | 0.72512 | 0.53272 | 55 | 36 | 29 | 0.0002 | |
| | | 3 | 0.07648 | 0.49085 | 0.56336 | 52 | 40 | 31 | 0.0008 | |
| Promoter of decrease | | | | | | | | | | |
| c...n...c... | 1 | 1 | -0.0287 | -0.44782 | -1.06545 | 3 | 5 | 2 | 0.0023 | Presence of aromatic nitrogen between two aromatic carbon |
| | | 2 | -1.23621 | -0.75303 | -0.19895 | 4 | 3 | 2 | 0.0005 | |
| | | 3 | -1.56359 | -1.69078 | -0.99982 | 4 | 2 | 2 | 0.0002 | |
| S...(...C... | 1 | 1 | -0.49469 | -2.01565 | -0.11843 | 3 | 2 | 2 | 0.0023 | Presence of sulphur with branching with carbon double bond |
| | | 2 | -0.56135 | -0.0549 | -0.38003 | 13 | 9 | 7 | 0.0001 | |
| | | 3 | -1.33917 | -1.08063 | -1.01206 | 4 | 7 | 3 | 0.0022 | |
| ++++S...B2=== | 2 | 1 | -0.56135 | -0.0549 | -0.38003 | 13 | 9 | 7 | 0.0001 | Presence of sulphur with branching and double bond |
| | | 2 | -0.56135 | -0.0549 | -0.38003 | 13 | 9 | 7 | 0.0001 | |
| | | 3 | -0.56135 | -0.0549 | -0.38003 | 13 | 9 | 7 | 0.0001 | |
| ++++Cl...S=== | 1 | 1 | -0.57654 | -0.35935 | -0.62229 | 3 | 1 | 1 | 0.0017 | Presence of chlorine with sulphur |
| | | 2 | -0.57654 | -0.35935 | -0.62229 | 3 | 1 | 1 | 0.0017 | |
| | | 3 | -0.57654 | -0.35935 | -0.62229 | 3 | 1 | 1 | 0.0017 | |
| Promoter of increase | | | | | | | | | | |
| pEC ₅₀ | C5.....0... | 1 | 0.21385 | 0.49259 | 2.08764 | 111 | 77 | 46 | 0.0006 | Absence of five-member rings |
| | | 2 | 2.21049 | 0.704 | 1.70235 | 110 | 74 | 56 | 0 | |
| | | 3 | 1.43226 | 1.5737 | 2.15936 | 110 | 75 | 54 | 0.0001 | |
| C...(...C... | 1 | 1 | 0.25343 | 0.18014 | 0.42824 | 58 | 36 | 27 | 0.0001 | Presence of two aliphatic carbon with branching |
| | | 2 | 1.24886 | 0.47209 | 1.01774 | 60 | 35 | 28 | 0.0005 | |
| | | 3 | 1.25593 | 0.18762 | 1.24105 | 59 | 34 | 23 | 0.0012 | |
| c...c...c... | 1 | 1 | 0.30441 | 0.73197 | 0.19607 | 47 | 38 | 17 | 0.0013 | Presence of three consecutive aromatic carbons |
| | | 2 | 0.42912 | 0.29232 | 0.60473 | 46 | 31 | 27 | 0.0008 | |
| | | 3 | 1.09812 | 0.29491 | 0.08848 | 45 | 36 | 24 | 0.0006 | |
| C6...A...1... | 1 | 1 | 1.2658 | 0.05442 | 0.26358 | 38 | 21 | 15 | 0.0008 | Presence of one six-member aromatic ring |
| | | 2 | 0.40875 | 0.26677 | 0.20824 | 32 | 22 | 21 | 0.0015 | |
| | | 3 | 0.89128 | 1.05867 | 0.39511 | 30 | 28 | 21 | 0.0023 | |
| C...C...C... | 1 | 1 | 0.91697 | 0.94405 | 0.63035 | 32 | 21 | 14 | 0.0002 | Presence of three consecutive aliphatic carbons |
| | | 2 | 1.21722 | 1.07992 | 1.33949 | 29 | 24 | 16 | 0.0005 | |
| | | 3 | 1.08398 | 1.16517 | 0.89018 | 29 | 17 | 15 | 0.0004 | |
| Promoter of decrease | | | | | | | | | | |
| 1...n...(... | 1 | 1 | -0.82145 | -1.58394 | -0.45675 | 7 | 6 | 3 | 0.0004 | Presence of aromatic nitrogen on the first ring with branching |
| | | 2 | -0.84679 | -1.04423 | -0.83943 | 6 | 3 | 4 | 0.0016 | |
| | | 3 | -0.94912 | -0.6517 | -0.22174 | 6 | 5 | 1 | 0.0048 | |
| S...(...=... | 2 | 1 | -0.75816 | -0.71358 | -1.06783 | 8 | 3 | 2 | 0.0035 | Presence of sulphur with branching and double bond |
| | | 2 | -0.75816 | -0.71358 | -1.06783 | 8 | 3 | 2 | 0.0035 | |
| | | 3 | -0.75816 | -0.71358 | -1.06783 | 8 | 3 | 2 | 0.0035 | |
| ++++O...S=== | 3 | 1 | -0.84696 | -0.5927 | -0.1503 | 14 | 2 | 5 | 0.0017 | Presence of oxygen with sulphur |
| | | 2 | -0.84696 | -0.5927 | -0.1503 | 14 | 2 | 5 | 0.0017 | |
| | | 3 | -0.84696 | -0.5927 | -0.1503 | 14 | 2 | 5 | 0.0017 | |
| [...-...Cl... | 2 | 1 | -0.95022 | -0.29786 | -0.57338 | 4 | 2 | 2 | 0.0001 | Presence of chloride ion |
| | | 3 | -0.92707 | -0.72223 | -0.89901 | 5 | 3 | 0 | 1 | |



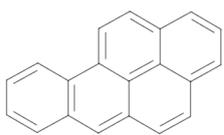
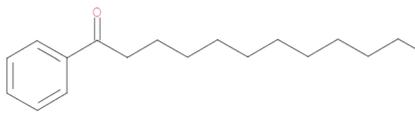
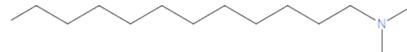
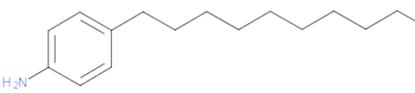
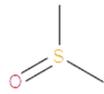
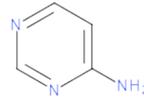
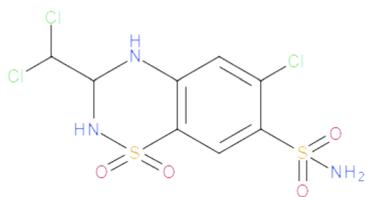
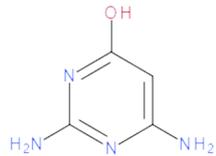
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|--|---|
| <p>Compound 223</p>  <p><chem>c12c3e4ccc1c1c(cc2ccc3ccc4)ccccc1</chem></p> <p>c...c...c... Enhancing toxicity pEC₅₀=8.16 and pEC₁₀=8.42</p> | <p>Compound 117</p>  <p><chem>c1(C(=O)CCCCCCCCCCC)ccccc1</chem></p> <p>c...c...c... and C...C...C... Enhancing toxicity pEC₅₀=7.04 and pEC₁₀=7.83</p> |
| <p>Compound 348</p>  <p><chem>C(CCCCCN)CCCCC</chem></p> <p>C...C...C... and N...(...C... Enhancing toxicity pEC₅₀=6.87 and pEC₁₀=7.36</p> | <p>Compound 185</p>  <p><chem>c1(ccc(cc1)N)CCCCCCCCC</chem></p> <p>c...c...c..., C...C...C... and N...(...C... Enhancing toxicity pEC₅₀=6.60 and pEC₁₀=7.12</p> |
| <p>Compound 333</p>  <p><chem>S(=O)(C)C</chem></p> <p>S...(...=..., ++++O---S== and S...(...C... Reducing toxicity pEC₅₀=0.31 and pEC₁₀=0.54</p> | <p>Compound 278</p>  <p><chem>c1(cccn1)N</chem></p> <p>1...n...(... Reducing toxicity pEC₅₀=1.96 and pEC₁₀=2.35</p> |
| <p>Compound 92</p>  <p><chem>c12c(NC(NS2(=O)=O)C(Cl)Cl)cc(c(c1)S(=O)(=O)N)Cl</chem></p> <p>S...(...=..., ++++O---S==, ++++Cl--S== and ++++S--- B2== Reducing toxicity pEC₅₀=2.80 and pEC₁₀=3.52</p> | <p>Compound 260</p>  <p><chem>c1(cc(nc(n1)N)O)N</chem></p> <p>1...n...(... Reducing toxicity pEC₅₀=2.39 and pEC₁₀=2.65</p> |

Fig. 3 Some examples in organic chemicals responsible for enhancing and reducing algal toxicity based on model interpretation.

a training set and a prediction set with a ratio of 80 : 20 (217 : 54 compounds). The numerical values of R^2 , Q_{LOO}^2 and MAE for the suggested QSTR model were 0.72, 0.70 and 0.524, respectively. Seven descriptors were employed to develop QSTR models.

If the QSTR models were judged based on the results of the MAE of the test or validation set, then the present QSTR modelling was also preferred over the reported QSTR models. The numerical value of MAE of the validation set for the pEC₅₀ endpoint was 0.461 which was lower than the other reported results except for the work reported by Khan *et al.*⁴¹ and Masanad *et al.*⁶ But, only 69 chemicals were employed by Khan *et al.* to build the QSTR model. Whereas, 271 organic compounds were used by Masanad *et al.* to develop the QSTR models.

However, In the earlier published work, two sets were applied to build up QSTR models (training and test set), but in the present research, the QSTR models were developed using four sets (training, invisible training, calibration and validation set). All previously reported models used a single split, but in the present research, 3 splits were used to generate three QSTR models using the target function TF₂. Various validation parameters for the assessment of the model were employed in the earlier reported works, but, the index of ideality correlation (IIC) was never used and is employed in the present work. In the present study only one descriptor, DCW, was used to generate QSTR models, while previously reported methods were developed by utilizing more than one descriptor. The mechanistic interpretation in terms of SMILES fragment was reported first



Table 3 The comparison between some of the earlier published models and the present study for the prediction pEC10 and pEC50

| S. no. | X% effective concentrations | Chemical class | h (test duration in h) | No of descriptor | Total number of components | Data set size | | R ² | | MAE | | Ref. |
|--------|-----------------------------|---------------------------------------|------------------------|------------------|----------------------------|---------------|-----------------|----------------|------------|---------------|---------------|--------------------|
| | | | | | | Training | Inv. train. | Cal | Test model | Training Test | Training Test | |
| 1 | pEC50 | Benzoic acids | 48 | 2 | 20 | 20 | 0.965 and 0.921 | | | | | 38 |
| 2 | pEC50 | -Polar narcotic chemicals | 72 | 2 | 58 | 58 | 0.6 | | | | | 39 |
| 3 | pEC50 | Non-polar narcotic chemicals | 72 | 2 | 50 | 50 | 0.9469 | | | | | 40 |
| 4 | pEC50 | Polar and nonpolar narcotic chemicals | 72 | 3 | 108 | 87 | 21 | 0.9149 | | | | |
| 5 | pEC50 | Cosmetics | 96 | 4 | 30 | 20 | | 0.885 | 0.712 | | | 0.328 41 |
| 6 | pEC50 | Pharmaceuticals | 96 | 5 | 69 | 53 | 16 | 0.69 | | 0.73 | | 0.55 42 |
| 7 | pEC50 | Pharmaceuticals | 96 | 5 | 69 | 53 | 16 | 0.71 | | 0.64 | | 0.57 |
| 8 | pEC50 | Organic compounds | 24 | 6 | 334 | 251 | 83 | | 0.72 | 0.7 | 0.69 | 0.67 29 |
| 9 | pEC10 | Organic compounds | 24 | 8 | 334 | 251 | 83 | | 0.7 | 0.77 | 0.7 | 0.61 |
| 10 | pEC10 | Organic chemicals | 24 | 6 | 334 | 167 | 167 | | 0.76 | 0.75 | 0.60 | 0.61 43 |
| 11 | pEC50 | Organic chemicals | 24 | 6 | 334 | 167 | 167 | | 0.75 | 0.74 | 0.6 | 0.61 |
| 12 | pEC50 | Organic chemicals | 72 | 7 | 271 | 217 | 54 | 0.72 | 0.718 | 0.693 | 0.506 | 0.432 6 |
| 13 | pEC50 | Organic chemicals | 24 | 1 | 334 | 113 | 79 | | 0.8150 | 0.8665 | 0.6110 | 0.461 Present work |
| 14 | pEC10 | Organic chemicals | 24 | 1 | 334 | 116 | 79 | | 0.7892 | 0.8866 | 0.5691 | 0.426 |

time in the present research. By using the results of mechanistic interpretation, one may predict the toxicity of unknown molecules. Hence, the QSTR models developed herein are more reliable and have better statistical quality and predictability.

4. Conclusion

Using a hybrid optimal descriptor, which was obtained by a combination of SMILES and HSG attributes, QSTR models were generated to predict the toxicity (EC₁₀ and EC₅₀) of 334 different organic chemicals against *P. subcapitata* based on the Monte Carlo optimization method. The balance of correlation method with IIC was used to establish QSTR models. The IIC was employed to construct the QSTR models which improve the robustness and predictability of the generated models, particularly for the validation set. Also, the developed QSTR models were monoparametric. To establish the reliability of QSTR models, three random splits and four sets of a single split (active training, invisible training, calibration, and validation sets) were employed. The reliability and predictability of the suggested QSTR models were evaluated using internal validation, external validation and data randomization including R², CCC, IIC, Q², Q²F₁, Q²F₂, Q²F₃, s, MAE, F, RMSE, R²m, ΔR²m, CR²P, and Y test. The structural attributes responsible for the toxicity were also identified. The hydrophilic attributes like 1...n...(...) and S...(...=... exert positive contributions to controlling the aquatic toxicity and reducing algal toxicity, whereas attributes such as c...c...c..., C...C...C... enhance lipophilicity of the molecules and consequently enhance algal toxicity. However, all of the designed QSTR models were suitable to estimate the EC₁₀ and EC₅₀ of diverse chemicals.

Conflicts of interest

The authors declare no conflicts of interest.

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