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Exploring the role of quantum chemical descriptors in modeling acute toxicity of diverse chemicals to *Daphnia magna*

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ABSTRACT

Various quantum-mechanically computed molecular and thermodynamic descriptors along with physico-chemical, electrostatic and topological descriptors are compared while developing quantitative structure–activity relationships (QSARs) for the acute toxicity of 252 diverse organic chemicals towards *Daphnia magna*. QSAR models based on the quantum-chemical descriptors, computed with routinely employed advanced semi-empirical and *ab-initio* methods, along with the electron-correlation contribution (CORR) of the descriptors, are analyzed for the external predictivity of the acute toxicity. The models with reliable internal stability and external predictivity are found to be based on the HOMO energy along with the physico-chemical, electrostatic and topological descriptors. Besides this, the total energy and electron-correlation energy are also observed as highly reliable descriptors, suggesting that the intra-molecular interactions between the electrons play an important role in the origin of the acute toxicity, which is in fact an unexplored phenomenon. The models based on quantum-chemical descriptors such as chemical hardness, absolute electronegativity, standard Gibbs free energy and enthalpy are also observed to be reliable. A comparison of the robust models based on the quantum-chemical descriptors computed with various quantum-mechanical methods suggests that the advanced semi-empirical methods such as PM7 can be more reliable than the *ab-initio* methods which are computationally more expensive.

1. Introduction

Daphnia magna, a type of water-flea, is a widely used laboratory animal for the testing of ecotoxicity, and it has been a subject for modeling the toxic effects of diverse chemicals through quantitative structure–activity relationships (QSARs) [1–4]. The toxicity in water leads to the demise of daphnids, and this perturbs the food chain because daphnids serve as food for many aquatic organisms. In fact, chemicals which are toxic to *D. magna* can, directly or indirectly, cause toxicological effects at all the trophic levels. Therefore, the thirst for the externally predictive QSAR models for the acute toxicity of diverse chemicals towards the *D. magna* had been continued since many years [5–8]. In the literature [4,9–12], the toxic effects of various hazardous chemicals towards *D. magna*, have been modeled mainly through the physico-chemical descriptors like octanol/water partition coefficient (log *P*) representing the hydrophobicity, besides several topological and quantum-chemical descriptors particularly the energy of highest occupied molecular orbital (HOMO) [9].

It should be noted that the present work assesses the QSAR models based on regression to make quantitative prediction for the toxicity of diverse chemicals towards *D. magna*. However, there have been equally important advanced and sophisticated QSAR approaches based on the classification method [13] which can effectively predict whether a chemical is biologically active or inactive. Recently [14–21], such classification based QSAR models have been reported for predicting the toxicity, of large and heterogeneous datasets of compounds, against many organisms, besides assessing multiple toxicological profiles under diverse experimental conditions. For example, Tenorio-Borroto et al. [14–16], had proposed multi-target quantitative structure–activity/property relationships (mt-QSAR/QSPR) models along with the flow cytometry analysis for the prediction of cytotoxicity and immunotoxicity, which can effectively models the drug-target interactions and effects of organic compounds over the cellular and molecular targets of immune system. Moreover, such techniques can be important for the high throughput screening of drugs to elucidate the drug discovery processes. Besides this, a topological and structure based approach commonly referred as TOPS-MODE approach [17] has also gained popularity to develop the mt-QSARs for the identification of compounds as a drug, pesticide, herbicide *etc.*

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Recently, this approach has been applied for developing the mt-QSAR for tyrosine kinase inhibitors [18].

In fact, an increasing interest of research groups towards the development of toxicity models has added significant tools to the field of computational modeling. In a recent study, Kleandrova et al. [19] has reviewed significant advancements in the QSAR modeling for the prediction of acute toxicity, and has also introduced a multitasking toxicity model. Besides this, Furuhama et al. [20] has proposed quantitative structure–activity–activity relationships (QSAAR) for modeling the species-specific acute aquatic toxicity of aromatic amine and phenols, whereas Speck-Planche et al. [21] had predicted multiple ecotoxicological effects of agrochemical fungicides through multi-species QSAR models. Moreover, QSAR approaches based on molecular docking and simulation techniques have also been quite promising [22,23].

In the present study, QSAR models are proposed and analyzed for predicting the acute toxicity of diverse hazardous chemicals towards the *D. magna*. The models are mainly developed using the physico-chemical, electrostatic and topological descriptors along with the quantum-chemical molecular and thermodynamic descriptors computed using advanced semi-empirical methods like PM7 [24], and *ab-initio* methods such as the Hartree–Fock (HF) [25,26] and the density functional theory (DFT) [26,27]. Notably, the quantum-chemical descriptors formulated through the electron-correlation contribution (CORR) [28–33] are also employed to see the role of instantaneous electron–electron interactions in the modeling of the acute toxicity at the level of electron-dynamics. In our recent studies, the electron-correlation contribution of a quantum-chemical descriptor was observed to be highly significant while modeling the externally predictive QSAR models for the biological activities [28,29,32] and physico-chemical properties [30,31,33] of different chemicals. For example, the contribution of electron-correlation to the total energy of a molecule, energy of the HOMO, and to the electrophilicity are found to be highly significant while modeling the mutagenic activity of nitrated-PAHs [28,29,32] Besides this, the correlation energy is also observed as a robust descriptor while developing single-parameter based externally predictive quantitative structure–property relationship (QSPR) models for the super cooled vapor pressure of polychlorinated-naphthalenes [30], and also for the aqueous solubility, subcooled liquid vapor pressure, *n*-octanol/water and *n*-octanol/air partition coefficients of the polychlorinated-dibenzo-*p*-dioxins (PCDDs) and -dibenzo-furans (PCDFs) [31] and polychlorinated naphthalenes [33].

In our recent work [32], we had also analyzed the performance of various exchange-correlation functionals of the DFT while developing externally predictive QSARs for the mutagenicity of nitrated-PAHs. In this study, it was observed that the quantum-mechanical exchange interactions can be quite critical along with the electron-correlation in modeling the mutagenicity, however, the incorporation of electron-correlation is found to be highly significant in the QSAR models in order to have low errors in the external prediction. However, modeling toxicity of a data set constituting diverse chemicals is difficult since it involves multiple mechanisms [34]. Therefore, modeling of the toxicity with quantum-chemical descriptors and their electron-correlation contribution may pave new insights into the mechanisms of toxicity besides the existing knowledge from the models based on the topological descriptors and physico-chemical properties which are though known to be quite useful while developing QSAR models since partition coefficients can be quite crucial factor in the determination of a chemical's absorption through the cellular membranes.

Fig. 1 depicts the chemicals under investigation in the present study, which are also listed in Supporting information Table S1. These chemicals are comprised of a wide range of organic functionalities such as linear hydrocarbons, benzene, substituted benzenes, chemicals with —OH, —NO_2, —NH_2, —C═O, —C═S, R—O—R' functional groups, ring system of C, H atoms, with and without hetero atoms such as N, O, S *etc.*, chemicals containing Cl atom(s) and chemicals having P atom *etc.* Most of these chemicals are present as major pollutants in the environment, and are frequently used as pesticides. Many of these chemicals, particularly those having functionalities like —OH, —NO_2, —NH_2 *etc.*, are capable of extensive biotransformation. Moreover, their metabolite or degradation product(s) are also hazardous in nature. Further, some of the chemicals included in the present study namely, the halogen substituted hydrocarbons, amines, phenols are associated with skin, liver and kidney diseases [35–37], chemicals like alcohols are central nervous system depressants and are responsible for neurological disorders [38]. Carcinogenity is well known hazard of most of these chemicals, mainly due to the benzene derivatives such as nitrobenzenes, poly-aromatic- and heteroaromatic hydrocarbons [35,39]. The dangerous effects of these chemicals raise the need for modeling the toxicity associated with them, to regulate the use of such chemicals by industries and organizations for the purpose of risk assessment.

This paper is organized as follows: Section 2 provides the detailed theoretical strategy employed for the computation of various quantum-chemical descriptors utilized in this work and for the estimation of their electron-correlation contribution. This is followed by Section 3 on materials and methods, which provide information on the chemical data set and toxicity under investigation, and on the development and statistical validation of the QSAR models. The developed models are further analyzed in Section 4 on results and discussion where the internal and external predictivity of the present models is explored and compared with the robust models available in the literature [9]. Finally, the last section makes few concluding remarks.

2. Theoretical and computational details

In the present study, a number of quantum-chemical molecular descriptors are employed such as the total electronic energy of molecule (E), energies of the highest occupied and lowest unoccupied molecular orbital (E_{HOMO} and E_{LUMO}), absolute electronegativity (χ), chemical hardness (η), electrophilicity index (ω), and dipole moment (d) [28–30]. Besides these, the widely accepted thermodynamic descriptors, namely, standard Gibbs free energy (G) and enthalpy (H) computed using the quantum-mechanical methods are also employed for the model development. Since these descriptors account for most of the electronic properties associated with the chemical structure [40], therefore, when the toxicity of a chemical results mainly due to the covalent interactions of it with the cellular target at the atomic level, these descriptors are expected to be influential for the ADME (absorption, distribution, metabolism and excretion) properties of a chemical [41]. In such a scenario, the energies of frontier orbitals (E_{HOMO} and E_{LUMO}) and Gibbs free-energy of a chemical could prove to be reliable descriptors as they can account for the charge transfer interactions between a chemical and cellular target.

The energy of the HOMO/LUMO represents the chemical's ability of accepting or donating electrons, whereas the Gibbs free-energy represents the capacity of a chemical to do work during a chemical transformation. Similarly, the total energy of a chemical can be a measure of its covalent and non-covalent interaction with the target. Furthermore, the electron density based descriptors such as absolute electronegativity, chemical hardness and electrophilicity index could also be advantageous for the same purpose [42]. The chemical hardness, which in fact is representative of the energy-gap between HOMO and LUMO, is an indicator for the stability. A chemical with large HOMO–LUMO energy-gap is likely to be less active

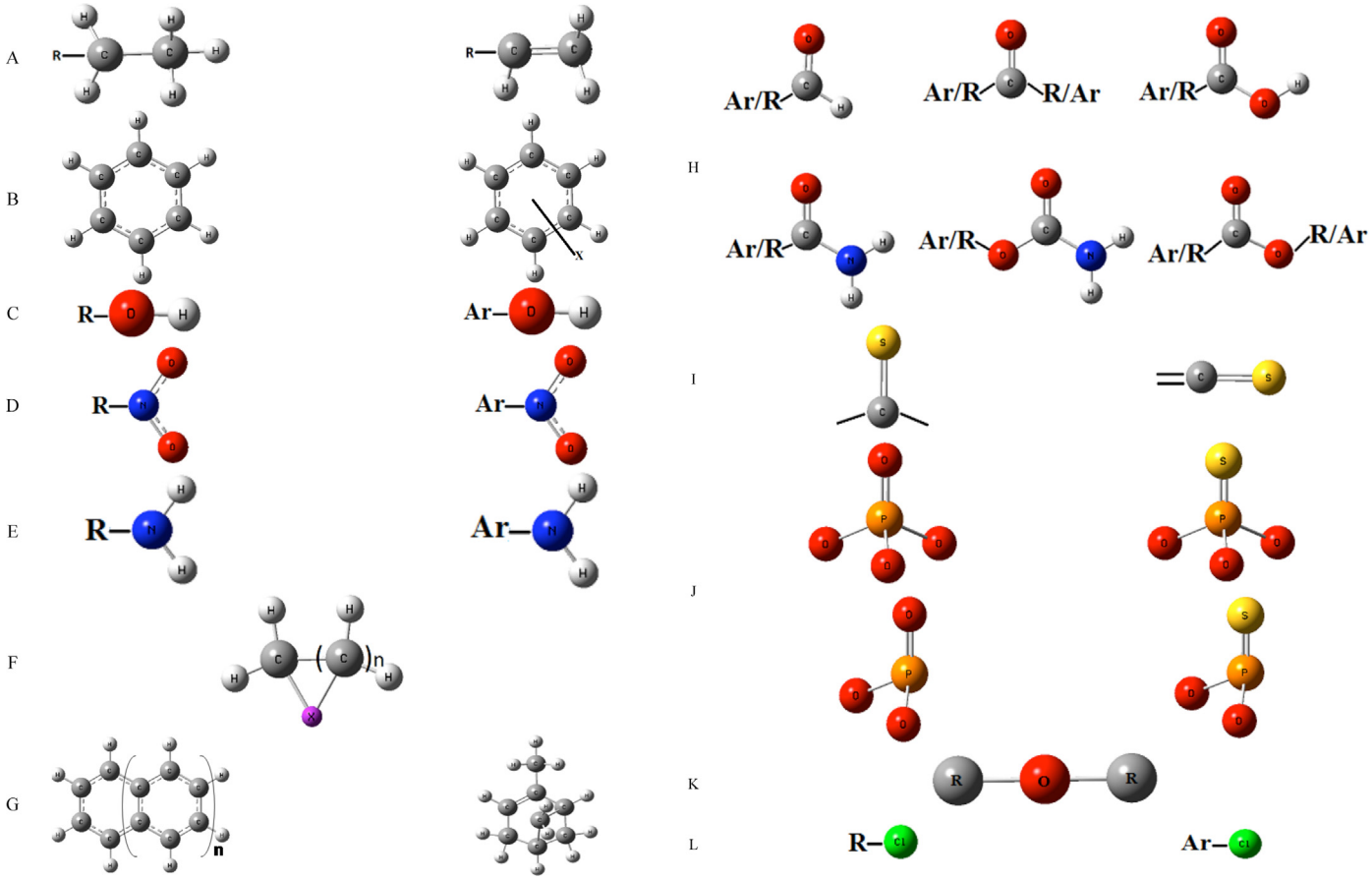

Fig. 1. Various structural functionalities of the data set comprising 252 chemicals: (A) linear hydrocarbons, (B) benzene & substituted benzene, (C) chemicals with —OH group, (D) chemicals with —NO_2 group, (E) chemicals with —NH_2 group, (F) ring system of C & H with an hetero atom (X = N, O, S), (G) ring system of C & H without an hetero atom, (H) chemicals with —C=O group, (I) chemicals with —C=S group, (J) chemicals containing a phosphorus atom, (K) chemicals with R—O—R' group, (L) chemicals containing Cl atom. (R represents an alkyl group and Ar represents an aryl group).

since it requires more amount of energy for excitation of electrons. Similarly, the electronegativity which represents the ability of a chemical to attract electrons towards it also plays a significant role in the covalent interactions of a chemical.

However, an accurate computation of all the aforementioned quantum-chemical descriptors is critically dependent on the amount of electron-correlation captured by the quantum-mechanical method employed to compute the descriptors. Therefore, the descriptors accounting for the electron-correlation contribution of the molecular descriptors are also employed for the model development, as used in our recent studies [28–33]. The electron-correlation contribution (CORR) of a molecular descriptor (D) was estimated using [28–33,43],

$$D_{CORR} = D_{DFT} - D_{HF}, \quad (1)$$

where D represents one of the descriptors such as E, E_{HOMO}, E_{LUMO} *etc.*, computed through *ab-initio* methods such as HF and DFT. It should be noted that the DFT incorporates electron-correlation through exchange-correlation (XC) energy density functional, however, the HF method ignores a significant part of dynamic electron-correlations though it accounts for quantum-mechanical exchange interactions [25,26]. In fact, molecular energy computed through the DFT can be in strict analogy with the exact energy if an exact XC functional to capture the electron-correlation is employed in the computation, which is though unknown. In the present work, a widely used hybrid B3LYP [44,45] XC energy density functional is employed for the estimation of electron-correlation. The methodology chosen here is highly viable though it approximately estimates the electron-correlation, but it is computationally more economical over other advanced quantum mechanical approaches like the full-configuration interaction method [46–49].

For computing the quantum-chemical molecular and thermodynamic descriptors, geometry of each of the 252 chemicals (listed in Supporting information Tables S1), is optimized with the advanced semi-empirical method namely PM7, and also at the HF and DFT/B3LYP levels of the theory using 6-311G(d,p) basis set [49,50]. The optimized geometry of each of the chemical is further analyzed through the vibrational frequency analysis to ensure that the structure computed has only real frequencies and it corresponds to true global minimum. It should, however, be noted that the Gibbs free energy is not computed using the PM7 method because the equilibrium energy estimated using semi-empirical methods may be erroneous. All the semi-empirical computations are performed with the semi-empirical molecular orbital package (MOPAC) 2012 [51], whereas the *ab-initio* HF and DFT computations are performed with Gaussian 03 [52] suite of quantum-chemistry software package.

Furthermore, in addition to the above mentioned quantum-chemical descriptors, physico-chemical ($\log P_{mix}$), topological (^1BIC) and electrostatic ($WNSA$-1) descriptors were also employed. The values for the descriptors $\log P_{mix}$, ^1BIC and $WNSA$-1, are taken from a previous study by Moosus and Maran [9]. The detailed calculations of these descriptors along with the corresponding relations are described in Moosus and Maran [9]. It should be noted that $\log P_{mix}$ refers to 1-octanol-water partition coefficient determined either experimentally or using atom/fragment contribution

method implemented in KOWWIN estimation program of EPI suite [53]. However, in the literature reported on QSPRs, the notation, $\log P_{mix}$, generally refers to the partition coefficient of binary mixtures [54,55]. The choice of these descriptors is based on the robust models available in the literature [Ref. [9] and references therein].

3. Material and methods

3.1. Test chemicals and biological activity

The data set for 252 organic chemicals, listed in Supporting information Table S1, having aliphatic, aromatic and cyclic structures covering diverse organic functionalities, is taken from the existing literature [9] on the acute toxicity towards *D. magna*. The toxicity is reported as logarithmic scale of the lethal concentration (LC_{50} value in mol/L), that is, the dose responsible for the mortality of 50% of a group of test animals in a specified period, 48-h in this case. The data-set used in the present study though may be quite small compared to the models available in the literature, for example, the data set of 1365 compounds used by Kühne et al. [56]. However, it should be noted that the data set employed by Kühne et al. [56] in a study on Read-Across prediction of acute toxicity is based on an atom-centered fragment method, is taken from a variety of databases available in the literature including ECOTOX database as also used in the present work, which is comprised of chemicals including organic and inorganic compounds (both charged and uncharged), but it is heterogeneous in terms of endpoints and experimental conditions. The data set of Moosus and Maran [9] employed in the present work is actually retrieved from the ECOTOX database following rigorous procedure such that the data set is not only homogeneous in terms of end points but it is also structurally very diverse, though it excludes inorganic and charged compounds. Moreover, similar to the data set of Kühne et al., the experimental value of the toxicity, $\log LC_{50}$ (48 h) ranges between −0.10 and −10.01 in the present data-set compared to −0.26 to −10.67 in that of Kühne et al., though it is always desirable to include as many compound as possible in the data set. Besides this, it should also be noted that the main objective of the present study is not simply to develop models for the toxicity of chemicals towards the *D. magna*, but also to find any role of the advanced quantum-chemical descriptors computed at the higher level of quantum-mechanical theories.

Moreover, interesting variations in the toxicity of the chemicals in the present data-set were observed with the amount of electron-correlation energy and the value of partition-coefficient. Two chemicals, namely, 4-amido-1-(nitrsoaminoamidino)-1-tetrazene and diisotridecylamine (chemical ID numbers 66 and 229, respectively, in Supporting information Table S1), which are observed as extreme outliers, possess, respectively, very low and high value, for the electron-correlation energy as well as for the partition-coefficient. Further, since the partition coefficient is an indicator for the ability of a chemical to penetrate the cellular membrane, and hence its toxicity, therefore, highly toxic chemicals are found to be associated with significantly high electron-correlation energy as also evident from E_{CORR} and $\log P_{mix}$ values of chemicals (for example, chemicals with ID number 1, 2, 243 and 246, provided in Supporting information Table S1).

It should further be noted that one chemical, azadirachtin, belonging to the category of insecticides, is not involved in the model development in the present study. Due to huge complex structure of this chemical and a number of functional groups present in it, the computation for its optimized geometry failed using *ab-initio* methods applied at the chosen level of the theory and computational resources employed for the present study. In fact, even computationally inexpensive advanced semi-empirical method, PM7, is found to be unsuccessful. Moreover, this chemical was also not considered in the training and/or validation set in a previous study by Moosus and Maran, where actual data set was comprised of 253 chemicals [9]. The present data set of 252 chemicals is further subjected to model development and validation process, as described in the next section.

3.2. QSAR analysis

In the present work, mainly three- to six-descriptor based regression models are developed through multi-linear regression analysis by employing genetic algorithm for the variable selection (GA–VS) [57]. For the internal and external validation of the models, the whole data set is split into a training set and an external prediction set through ordered response splitting where prediction set constitute of 30% and 50% of the whole data set. Besides this, the splitting of the data set is also performed with 45% random splitting (for details, see Supporting information Table S2). Further, a reliable model must have a well-defined domain of applicability because a model can show reliable predictions only for the chemicals which falls into the space defined by the descriptors chosen and the modeled response. Here also, the applicability domain of the developed models is determined through the leverage approach using the Williams plot [58], where chemicals with high leverage values were detected as structural outliers, and chemicals with standardized residuals values more than three units were considered as response outliers. These structural and response outliers are omitted from the final models since such outliers can erroneously influence the reliability of the model.

To determine the internal reliability of the developed models, coefficient of determination (R^2), and cross-validated R^2 (Q^2_{LOO}) obtained through leave one out (LOO) procedure are employed. Generally, models with $R^2 > 0.60$ and $Q^2_{LOO} > 0.50$ are considered as acceptable regression models [59,60]. However, these validation measures may not be sufficient to decide the reliability of the developed models, since due to the chance of over-fitting of the data, these parameters may correspond to higher values. Therefore, to ensure that the R^2 and Q^2_{LOO} values in models are significant, and not due to chance correlation, more efficient and stable procedure are employed which include cross-validated leave many out (Q^2_{LMO}) method leaving 30% of the chemicals from the training set, Y-scrambling (Q^2_{Yscr}) and randomization (Q^2_{Yrand}) procedures with 1000 iterations. A closer value of Q^2_{LMO} parameter to that of R^2 and Q^2_{LOO}, ensures the robustness of a model, whereas lower value of parameters obtained through Y-scrambling and randomization procedures suggests that the proposed model excludes chance-correlation. Further, a criterion of difference, that is $|R^2 - Q^2_{LOO}| < 0.1$, is also employed to judge the performance of model in fitting and internal predictivity [61]. The descriptor collinearity is verified using QUIK rule [62]. The *F*-values from *F* statistic are also examined to represent the statistical significance of the regression models.

Further, the external predictivity of a QSAR models is an important aspect for its applicability. Most of the QSAR models reported in the literature for the toxicity of chemicals towards *D. magna* are externally validated mainly through the R^2_{EXT} parameter, with an acceptable value $R^2_{EXT} > 0.60$. However, R^2_{EXT} is dependent on the data-set size and distribution, therefore, it may not be sufficient to judge the external predictivity of the models. A more appropriate external validation parameter, namely, Q^2_{F3} of Consonni et al. [63] is employed here to determine the external predictivity of the models. The significance of using this parameter lies in the fact that it is independent of the data-set size and also of the data-distribution in the training as well as external validation set. Along with this, a more rigorous external validation parameter, that is, the concordance correlation coefficient (CCC_{EXT}) proposed by Lin [64], is also

Table 1
Comparison of key internal and external validation parameters of the QSAR models based on different quantum-chemical molecular, quantum-mechanically computed thermodynamic descriptors along with physico-chemical, electrostatic and topological descriptors, with model known in literature [Ref. [9]] for the acute toxicity of chemicals towards the *Daphnia magna*.

Sr. no.	Descriptors	No. of chemicals in training/prediction set	R^2	Q^2_{LOO}	R^2_{LMO}	Q^2_{LMO}	R^2_{EXT}	Q^2_{F3}	CCC_{EXT}	$RMSE_{CV}$	$RMSE_{EXT}$
1.	E^{HOMO}_{AM1}, $\log P_{mix}$, *WNSA*-1, ^1BIC[a]	118/117	0.740	0.714	0.741	0.711	0.554[c]	0.594	0.731	0.820	0.978
2.	E^{HOMO}_{PM7}, $\log P_{mix}$, *WNSA*-1, ^1BIC[b]	118/117	0.752	0.726	0.753	0.722	0.561	0.598	0.733	0.802	0.972
3.	E_{PM7}, E^{HOMO}_{PM7}, $\log P_{mix}$, *WNSA*-1, ^1BIC[b]	118/117	0.760	0.731	0.764	0.727	0.582	0.617	0.749	0.796	0.949
4.	H_{PM7}, E^{HOMO}_{PM7}, $\log P_{mix}$, *WNSA*-1, ^1B[b]	118/117	0.756	0.726	0.760	0.720	0.569	0.604	0.740	0.802	0.966
5.	E_{CORR}, E^{HOMO}_{PM7}, $\log P_{mix}$, *WNSA*-1, ^1BIC[b]	118/117	0.760	0.731	0.764	0.727	0.561	0.592	0.738	0.796	0.979
6.	G_{CORR}, E^{HOMO}_{PM7}, $\log P_{mix}$, *WNSA*-1, ^1BIC[b]	118/117	0.760	0.731	0.762	0.730	0.562	0.593	0.738	0.796	0.978
7.	H_{CORR}, E^{HOMO}_{PM7}, $\log P_{mix}$, *WNSA*-1, ^1BIC[b]	118/117	0.760	0.730	0.763	0.727	0.562	0.593	0.738	0.796	0.978
8.	E^{HOMO}_{PM7}, d_{CORR}, $\log P_{mix}$, *WNSA*-1, ^1BIC[b]	118/117	0.760	0.729	0.763	0.730	0.544	0.580	0.727	0.798	0.994
9.	E^{HOMO}_{PM7}, X_{CORR}, $\log P_{mix}$, *WNSA*-1, ^1BIC[b]	118/117	0.752	0.725	0.753	0.720	0.564	0.602	0.735	0.805	0.968
10.	E^{HOMO}_{PM7}, E^{LUMO}_{CORR}, $\log P_{mix}$, *WNSA*-1, ^1BIC[b]	118/117	0.752	0.725	0.755	0.715	0.564	0.601	0.735	0.805	0.969
11.	E^{HOMO}_{PM7}, η_{CORR}, $\log P_{mix}$, *WNSA*-1, ^1BIC[b]	118/117	0.752	0.724	0.753	0.722	0.561	0.599	0.734	0.806	0.971
12.	E_{CORR}, d_{CORR}, E^{HOMO}_{PM7}, $\log P_{mix}$, *WNSA*-1, ^1BIC[b]	118/117	0.768	0.734	0.771	0.740	0.545	0.573	0.731	0.792	1.003
13.	E_{PM7}, E^{HOMO}_{PM7}, d_{CORR}, $\log P_{mix}$, *WNSA*-1, ^1BIC[b]	118/117	0.768	0.733	0.772	0.735	0.565	0.599	0.742	0.793	0.971

[a] Model reproduced with QSARINS software in present work using identical descriptors and data set as employed by Moosus and Maran [Ref. [9]].
[b] Models obtained employing genetic algorithm on quantum chemical and thermodynamic descriptors computed in the present work along with the physicochemical, electrostatic and topological descriptors of Ref. [9], using identical splitting as employed by Moosus and Maran [Ref. [9]].
[c] Vaule for R^2_{EXT} parameter obtained with QSARINS software in the present work, whereas reported value in Ref. [9] is 0.634

analyzed for the developed models. Besides these, the parameters based on r^2_m metrics (average $\bar{r}^2_m$, and differential Δr^2_m) proposed by Ojha et al. [65] were also employed. Further, the reliability of the internal and external predictivity of the developed models is also examined through the root mean square error (cross-validated: $RMSE_{CV}$, and external: $RMSE_{EXT}$) values, which should be as low as possible and have minimum variation in the internal and external validation. Finally, the scatter plots between the experimental and predicted response were also analyzed to examine the degree of scattering between the observed and predicted responses. The variable selection, splitting of the data set, and further model development and validation was performed through QSARINS [66,67] software for QSAR model development and validation.

4. Results and discussion

The key internal and external validation parameters of relevant models are listed in Tables 1–3, while all the parameters described in the previous section are further provided in Supporting information Tables S3–S19. The reported models in these tables are developed employing various quantum-chemical molecular and thermodynamic descriptors along with their electron-correlation contribution (CORR) computed in the present study, in addition to the physico-chemical ($\log P_{mix}$), topological (^1BIC) and electrostatic (*WNSA*-1) descriptors taken from the study by Moosus and Maran [9] which are also provided in Supporting information Table S1. It should be noted that Moosus and Maran [9] observed the HOMO energy, computed using AM1 semi-empirical method, to be the most reliable quantum-chemical descriptor, whereas the present work employs advanced semi-empirical PM7 method along with the *ab-initio* HF and DFT methods, besides the correlation contribution of the quantum-chemical descriptors, namely, E, E_{HOMO}, E_{LUMO}, d, χ, η, and ω described in the previous sections.

In the present study, we also reproduced the statistical validation parameters of the best model reported in the previous study by Moosus and Maran [9] (entry 1 in Table 1). Though the proposed four-descriptor model based on the HOMO energy suggested by Moosus and Maran, has reliable internal and good external validation parameters, however, the model contains large number of response and structural outliers as evident from the Williams plot (Fig. 2). Moreover, it is observed here that the values of the parameter for external validation parameter (R^2_{EXT}) employed by Moosus and Maran, do not match with the actual R^2_{EXT} value obtained with QSARINS software as evident in Table 1, the value of actual parameter is <0.60. Observing this, we compared the QSAR models developed using quantum-chemical molecular and thermodynamic descriptors computed in the present study in combination with the $\log P_{mix}$, *WNSA*-1, and ^1BIC descriptors used by Moosus and Maran, in order to investigate the significance of the quantum-chemical descriptors computed at the higher level of the theory, in modeling the acute toxicity of diverse chemicals. This choice of descriptor selection in the present study is supported by the genetic algorithm employed to select the useful and precise combination of most robust descriptors for developing the multilinear regression models for the acute toxicity.

4.1. Robust models (present and literature)

Table 1 lists the most relevant statistical parameters for the four-, five- and six-descriptor models obtained though GA–VS (also see Supporting information Table S3). It is evident from entry 2 in Table 1 that the four-descriptor model developed using the HOMO energy computed with the advanced semi-empirical PM7 method has higher internal stability than the same four-parameter model (entry 1 in Table 1) reported by Moosus and Maran [9] but based on the AM1 computed HOMO energy. However, the incorporation of one more quantum-chemical descriptor, for example, the total energy (E) or enthalpy (H) computed at PM7 level as well as electron-correlation contribution to the total energy (E_{CORR}), Gibbs free energy (G_{CORR}) and enthalpy (H_{CORR}) seems to improve the internal stability of the model as evident from the entries 3 to 11 in Table 1. However, further addition of another quantum-chemical descriptor to the five descriptor models does not show any significant improvement in the stability of the models as evident in entries 12 and 13 in Table 1. It should be noted that in these models, structural outliers were detected using standardized residual value of 2.5 as employed by Moosus and Maran [9]. The statistical parameters of models listed in Table 1 suggest that the quantum-mechanically computed molecular and thermodynamic descriptors can also be significant in modeling the toxicity of diverse chemicals towards *D. magna*.

Following the suggestions from the above comparison of the present and existing models, and observing that models developed with the identical training and prediction set of previously reported model [9] contains large number of outliers, further model development was carried out using various combinations of quantum-chemical molecular and thermodynamic descriptors computed in the present work along with the

Table 2
Important internal and external validation parameters of the four-descriptors based QSAR models developed with the quantum-chemical descriptors computed with PM7, HF, DFT methods and with the CORR descriptors along with the physicochemical, electrostatic and topological descriptors employed by Moosus and Maran [Ref. [9]]. The model indicated in the bold are among the best models observed.

Model	Descriptors	No. of chemicals in training/prediction set	Splitting employed	R^2	Q^2_{LOO}	$R^2 - Q^2_{LOO}$	Q^2_{LMO}	Q^2_{F3}	CCC_{EXT}	$RMSE_{CV}$	$RMSE_{EXT}$
1 (Quantum-chemical descriptor) + 3 (physicochemical, electrostatic and topological descriptors as used in Ref. [9])											
Ia	E_{PM7}, $\log P_{mix}$, *WNSA*-1, ^1BIC	113/111	50% (ordered response)	0.596	0.559	0.037	0.556	0.659	0.720	0.989	0.869
Ib	E_{HF}, $\log P_{mix}$, *WNSA*-1, ^1BIC	113/111	50% (ordered response)	0.608	0.541	0.067	0.566	0.621	0.691	1.009	0.917
Ic	E_{DFT}, $\log P_{mix}$, *WNSA*-1, ^1BIC	113/111	50% (ordered response)	0.608	0.541	0.067	0.563	0.621	0.691	1.009	0.917
Id	E_{CORR}, $\log P_{mix}$, *WNSA*-1, ^1BIC	113/111	50% (ordered response)	0.602	0.563	0.040	0.570	0.648	0.707	0.985	0.883
IIa	E^{HOMO}_{PM7}, $\log P_{mix}$, *WNSA*-1, ^1BIC	113/111	50% (ordered response)	**0.677**	**0.647**	**0.030**	**0.646**	**0.749**	**0.803**	**0.886**	**0.746**
IIb	E^{HOMO}_{HF}, $\log P_{mix}$, *WNSA*-1, ^1BIC	113/111	50% (ordered response)	**0.653**	**0.620**	**0.033**	**0.614**	**0.737**	**0.794**	**0.918**	**0.764**
IIc	E^{HOMO}_{DFT}, $\log P_{mix}$, *WNSA*-1, ^1BIC	113/111	50% (ordered response)	**0.654**	**0.622**	**0.033**	**0.615**	**0.735**	**0.790**	**0.916**	**0.767**
IId	E^{HOMO}_{CORR}, $\log P_{mix}$, *WNSA*-1, ^1BIC	113/111	50% (ordered response)	**0.619**	**0.582**	**0.036**	**0.576**	**0.702**	**0.760**	**0.963**	**0.813**
2 (quantum-chemical descriptor) + 2 (physicochemical, electrostatic or topological descriptors as used in Ref. [9])											
IIIa	E_{PM7}, E^{HOMO}_{PM7}, $\log P_{mix}$, ^1BIC	113/111	50% (ordered response)	**0.636**	**0.601**	**0.035**	**0.599**	**0.742**	**0.783**	**0.941**	**0.757**
IIIb	E_{HF}, E^{HOMO}_{HF}, $\log P_{mix}$, ^1BIC	113/111	50% (ordered response)	0.660	0.629	0.030	0.622	0.682	0.747	0.907	0.840
IIIc	E_{DFT}, E^{HOMO}_{DFT}, $\log P_{mix}$, ^1BIC	113/111	50% (ordered response)	0.651	0.620	0.031	0.615	0.678	0.738	0.919	0.845
IIId	E_{CORR}, E^{HOMO}_{CORR}, $\log P_{mix}$, ^1BIC	113/111	50% (ordered response)	0.628	0.591	0.037	0.583	0.687	0.744	0.953	0.833
IVa	E_{PM7}, E^{HOMO}_{PM7}, $\log P_{mix}$, *WNSA*-1	113/111	50% (ordered response)	0.625	0.591	0.034	0.627	0.712	0.773	0.952	0.800
IVb	E_{HF}, E^{HOMO}_{HF}, $\log P_{mix}$, *WNSA*-1	113/111	50% (ordered response)	0.650	0.614	0.036	0.616	0.674	0.746	0.926	0.850
IV c	E_{DFT}, E^{HOMO}_{DFT}, $\log P_{mix}$, *WNSA*-1	113/111	50% (ordered response)	0.631	0.589	0.043	0.593	0.662	0.744	0.955	0.867
IVd	E_{CORR}, E^{HOMO}_{CORR}, $\log P_{mix}$, *WNSA*-1	113/111	50% (ordered response)	0.600	0.556	0.044	0.562	0.666	0.727	0.993	0.861

aforementioned physico-chemical, electrostatic and topological descriptors, employing different internal training and external prediction set compositions. Though in the present study, all the possible combinations of descriptors for models were explored (as provided in Supporting information Tables S4–S19), however, as evident in Tables 2 and 3, the models incorporating the total energy (E) and the energy of HOMO (E_{HOMO}) are observed to have robust statistical parameters and a generalized domain of applicability, suggesting these models to be more reliable and hence, taken for further discussions.

For the models reported in Tables 2 and 3, a total of 28 chemicals were detected as structural and response outliers as depicted in Fig. 3(a–d), which are excluded finally taking 113 compounds in the training set, compared to 17 chemicals excluded for the models reported in the Table 1 while taking 118 chemicals in the training set (for details, see Supporting information Table S2).

Table 3
Important internal and external validation parameters of the three-descriptors based QSAR models developed with th quantum chemical descriptors computed with PM7, HF, DFT methods and with the CORR descriptors along with descriptors employed by Moosus and Maran [Ref. [9]]. The models indicated in bold are among the best models observed.

Model	Descriptors	No. of chemicals in training/prediction set	Splitting employed	R^2	Q^2_{LOO}	$R^2 - Q^2_{LOO}$	Q^2_{LMO}	Q^2_{F3}	CCC_{EXT}	$RMSE_{CV}$	$RMSE_{EXT}$
1 (Quantum-chemical descriptor) + 2 (physicochemical, electrostatic and topological descriptors as used in Ref. [9])											
Va	E_{PM7}, $\log P_{mix}$, ^1BIC	113/111	50% (ordered response)	0.577	0.544	0.033	0.549	0.662	0.708	1.006	0.866
Vb	E_{HF}, $\log P_{mix}$, ^1BIC	113/111	50% (ordered response)	0.596	0.565	0.032	0.565	0.601	0.671	0.983	0.939
Vc	E_{DFT}, $\log P_{mix}$, ^1BIC	113/111	50% (ordered response)	0.596	0.535	0.032	0.550	0.602	0.671	0.983	0.939
Vd	E_{CORR}, $\log P_{mix}$, ^1BIC	113/111	50% (ordered response)	0.601	0.569	0.032	0.565	0.645	0.701	0.978	0.888
VIa	E_{PM7}, $\log P_{mix}$, *WNSA*-1	113/111	50% (ordered response)	0.548	0.515	0.033	0.513	0.627	0.686	1.037	0.910
VIb	E_{HF}, $\log P_{mix}$, *WNSA*-1	113/111	50% (ordered response)	0.569	0.509	0.060	0.521	0.586	0.657	1.044	0.959
VIc	E_{DFT}, $\log P_{mix}$, *WNSA*-1	113/111	50% (ordered response)	0.569	0.509	0.060	0.517	0.586	0.657	1.044	0.959
VId	E_{CORR}, $\log P_{mix}$, *WNSA*-1	113/111	50% (ordered response)	0.559	0.521	0.038	0.510	0.612	0.670	1.031	0.928
VIIa	E^{HOMO}_{PM7}, $\log P_{mix}$, ^1BIC	113/111	50% (ordered response)	**0.625**	**0.596**	**0.029**	**0.591**	**0.733**	**0.777**	**0.947**	**0.769**
VIIb	E^{HOMO}_{HF}, $\log P_{mix}$, ^1BIC	113/111	50% (ordered response)	**0.603**	**0.572**	**0.031**	**0.565**	**0.718**	**0.764**	**0.975**	**0.791**
VIIc	E^{HOMO}_{DFT}, $\log P_{mix}$, ^1BIC	113/111	50% (ordered response)	**0.600**	**0.568**	**0.032**	**0.559**	**0.717**	**0.760**	**0.979**	**0.792**
VIId	E^{HOMO}_{CORR}, $\log P_{mix}$, ^1BIC	113/111	50% (ordered response)	0.585	0.552	0.033	0.536	0.688	0.738	0.997	0.832
VIIIa	E^{HOMO}_{PM7}, $\log P_{mix}$, *WNSA*-1	113/111	50% (ordered response)	**0.621**	**0.592**	**0.028**	**0.590**	**0.706**	**0.765**	**0.951**	**0.808**
VIIIb	E^{HOMO}_{HF}, $\log P_{mix}$, *WNSA*-1	113/111	50% (ordered response)	**0.611**	**0.581**	**0.029**	**0.569**	**0.702**	**0.764**	**0.964**	**0.813**
VIIIc	E^{HOMO}_{DFT}, $\log P_{mix}$, *WNSA*-1	113/111	50% (ordered response)	0.599	0.570	0.029	0.568	0.691	0.750	0.977	0.829
VIIId	E^{HOMO}_{CORR}, $\log P_{mix}$, *WNSA*-1	113/111	50% (ordered response)	0.586	0.553	0.033	0.547	0.680	0.741	0.996	0.842
2 (Quantum-chemical descriptor) + 1 (physicochemical, electrostatic and topological descriptors as used in Ref. [9])											
IXa	E_{PM7}, E^{HOMO}_{PM7}, $\log P_{mix}$	113/111	50% (ordered response)	0.562	0.526	0.035	0.526	0.682	0.721	1.025	0.841
IXb	E_{HF}, E^{HOMO}_{HF}, $\log P_{mix}$	113/111	50% (ordered response)	0.619	0.589	0.030	0.583	0.646	0.712	0.955	0.886
IXc	E_{DFT}, E^{HOMO}_{DFT}, $\log P_{mix}$	113/111	50% (ordered response)	0.594	0.565	0.029	0.564	0.629	0.687	0.982	0.908
IXd	E_{CORR}, E^{HOMO}_{CORR}, $\log P_{mix}$	113/111	50% (ordered response)	0.598	0.564	0.034	0.555	0.660	0.719	0.984	0.869
Xa	E_{PM7}, E^{HOMO}_{PM7}, ^1BIC	113/111	50% (ordered response)	0.364	0.317	0.047	0.322	0.485	0.493	1.231	1.069
Xb	E_{HF}, E^{HOMO}_{HF}, ^1BIC	113/111	50% (ordered response)	0.484	0.407	0.077	0.438	0.497	0.565	1.147	1.057
Xc	E_{DFT}, E^{HOMO}_{CORR}, ^1BIC	113/111	50% (ordered response)	0.378	0.287	0.091	0.335	0.388	0.421	1.258	1.166
Xd	E_{CORR}, E^{HOMO}_{CORR}, ^1BIC	113/111	50% (ordered response)	0.553	0.515	0.039	0.505	0.601	0.666	1.038	0.941
XIa	E_{PM7}, E^{HOMO}_{PM7}, *WNSA*-1	113/111	50% (ordered response)	0.519	0.481	0.038	0.481	0.632	0.694	1.073	0.903
XIb	E_{HF}, E^{HOMO}_{HF}, *WNSA*-1	113/111	50% (ordered response)	0.595	0.567	0.028	0.558	0.615	0.698	0.980	0.924
XIc	E_{DFT}, E^{HOMO}_{DFT}, *WNSA*-1	113/111	50% (ordered response)	0.542	0.510	0.032	0.502	0.563	0.644	1.043	0.985
XId	E_{CORR}, E^{HOMO}_{CORR}, *WNSA*-1	113/111	50% (ordered response)	0.554	0.510	0.044	0.509	0.613	0.679	1.042	0.927

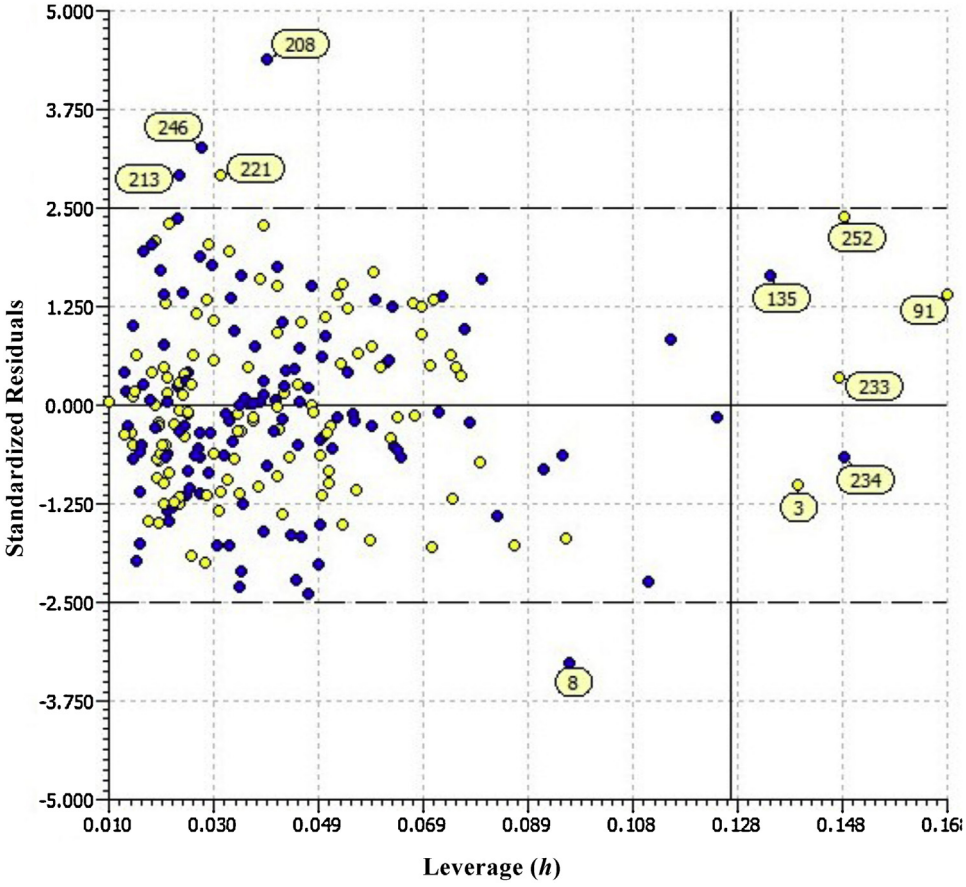

Fig. 2. Williams plot of the standardized residuals *vs* leverage (h) for the model based on E_{AM1}^{HOMO}, log P_{mix}, *WNSA*-1, ^{1}*BIC* given by Moosus and Maran [Ref. [9]] (entry 1 in Table 1). Training and prediction set chemicals are represented with open (yellow) and filled (blue) circles, respectively. Encircled number represents the ID number of chemicals detected as structural and response outliers (for details, refer to Supporting information Table S1). The vertical (solid) line indicates warning leverage h*, whereas the horizontal (dashed) line specifies the standardized residual value of 2.5. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

An analysis of the chemical structure of outliers observed for various models reveals that they possess high molecular weight (>300 amu) except 2-propenal (chemical ID number 208) which is though not observed as strong outlier. Most of the outliers possess complex structure with more than one bulky chemical functionalities, for example, PO_4 group along with the ethyl, butyl and benzene present as substituent as in case of octicizer (chemical ID number 226), cubane like structure in chlordecone (chemical ID number 233), PSO_3 and NO_2 functionalities present on the benzene ring as in case of fenitrothion (chemical ID number 235), parathion-methyl (chemical ID number 240) and parathion (chemical ID number 244) *etc*. Notably, two chemicals, 4-amido-1-(nitrsoaminoamidino)-1-tetrazene (chemical ID number 66) and diisotridecylamine (chemical ID number 229), having extended chain of N- and C-atoms, respectively, are observed as very strong response and structural outliers. Furthermore, most of the chemicals having phosphate (PO_4) or thiophosphate (PSO_3) groups are observed as structural outliers. The structural complexity and/or higher molecular weight might be the reason for these chemicals to be observed as structural outliers. It should also be noted that the model development and validation was also performed excluding only the structural outliers while retaining the response outliers and *vice-versa*, however, no significant improvement is observed in the statistical validation parameters. Therefore, 28 chemicals including structural and response outliers are excluded, and model development is performed on the reduced data set of 224 chemicals, however, these models may not have a more generalized applicability domain as those reported in Table 1.

4.2. Models based on four-descriptors

Table 2 lists the statistical validation parameters for four-descriptor models, developed using a combination of one quantum-chemical descriptor with three aforementioned descriptors which are taken from a study by Moosus and Maran [9]. Here, most significant statistical validation parameters are observed in case of models II(a–d) which are based on the HOMO energy along with log P_{mix}, *WNSA*-1, and ^{1}*BIC*, and these models also have more generalized domain of applicability as depicted in Fig. 4(a–d). It should be noted that the different models (a–d) in Table 2 are based on the quantum-chemical descriptor computed using four different quantum-mechanical methods: (a) PM7 (b) HF (c) DFT (d) CORR, but employing the same log P_{mix}, ^{1}*BIC* and *WNSA*-1 descriptors. Analysis of statistical validation parameters of models suggests that the model incorporating the HOMO energy descriptor computed with the PM7 method to be the best. Moreover, this result is consistent with the Moosus and Maran study, where the HOMO energy descriptor was computed with AM1 method. Further, the models I(a–d) based on the total energy are also observed to be reliable though somewhat less predictive since the value for external validation parameter Q^2_{F3} does not satisfy the threshold value ($\geq$0.70) as suggested by Chirico and Gramatica [68]. However, in case of models based on the PM7 and CORR computed descriptors, an interesting feature observed here is that the value of external validation parameters, Q^2_{F3} and CCC_{EXT} is higher as compared to those obtained for the model given by Moosus and Maran [9]. The reliability of models Id and IId indicates that the electron-correlation

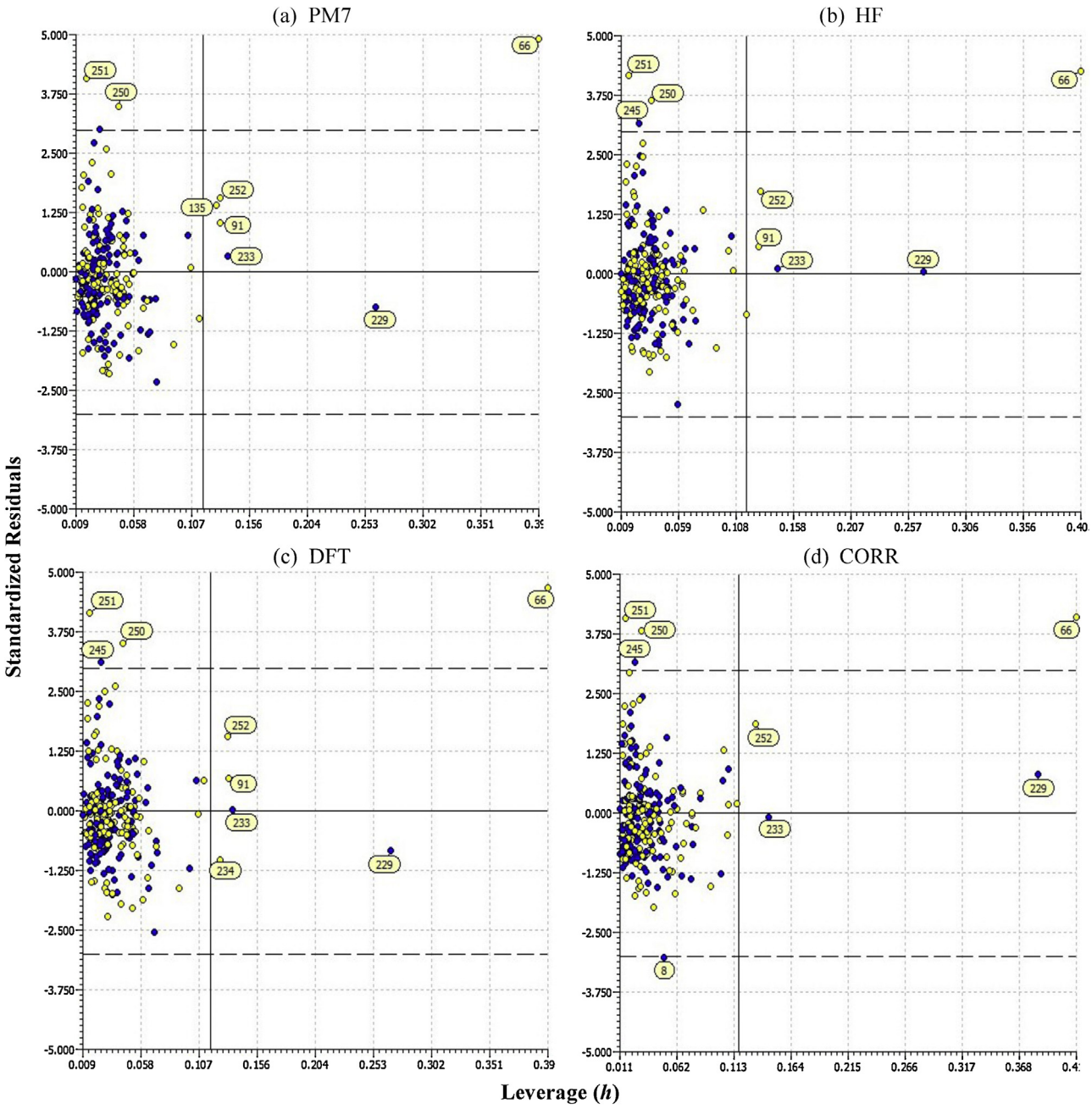

Fig. 3. Williams plot of the standardized residuals *vs* leverage (h) for model II(a-d) (without excluding outliers), based on E_{HOMO}, $\log P_{mix}$, *WNSA*-1, ^1BIC, with descriptor E_{HOMO} computed using (a) PM7 method (b) HF method (c) DFT method and (d) CORR method. Training and prediction set chemicals, using activity sampling splitting, are represented with open (yellow) and filled (blue) circles, respectively. Encircled number represents the ID number of chemicals detected as structural and response outliers (for details, refer to Supporting information Table S1). The vertical (solid) line indicates warning leverage h*, whereas the horizontal (dashed) line specifies standardized residual value of 3.0. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

contribution to the total energy (E_{CORR}) and the HOMO energy (E^{HOMO}_{CORR}) can also be quite important. Since electron-correlation contribution represents instantaneous interactions between the electrons in a molecule, therefore, these intra-molecular interactions play an important role in the origin of the acute toxicity of chemicals.

Furthermore, among the four-descriptor models, models based on the absolute electronegativity computed with the PM7, HF and DFT methods also show reliable internal and external validation parameters as evident in Supporting information Tables S4–S6. Similar trend is observed for the chemical hardness, except in case of DFT method, which shows somewhat less external reliability. However, similar models developed with the CORR based descriptors found to be less reliable (see Supporting information Tables S4–S15). Among the models III and IV listed in Table 2, the model incorporating PM7 computed descriptors is also observed to be reliable and predictive, however, model IIIa based on E_{PM7}, E^{HOMO}_{PM7}, $\log P_{mix}$, and ^1BIC descriptors, is internally more reliable and has smaller error values than model IVa which incorporates *WNSA*-1 instead of $\log P_{mix}$. The models III(b–d) and IV(b–d) based on the HF, DFT and CORR computed descriptors are observed to be internally robust, however, they are externally less reliable since Q^2_{F3} value for these models do not satisfy the accepted thresholds. However, again these models are externally more predictive than the model proposed by Moosus and Maran. An interesting feature observed here is that the removal of electrostatic descriptor,

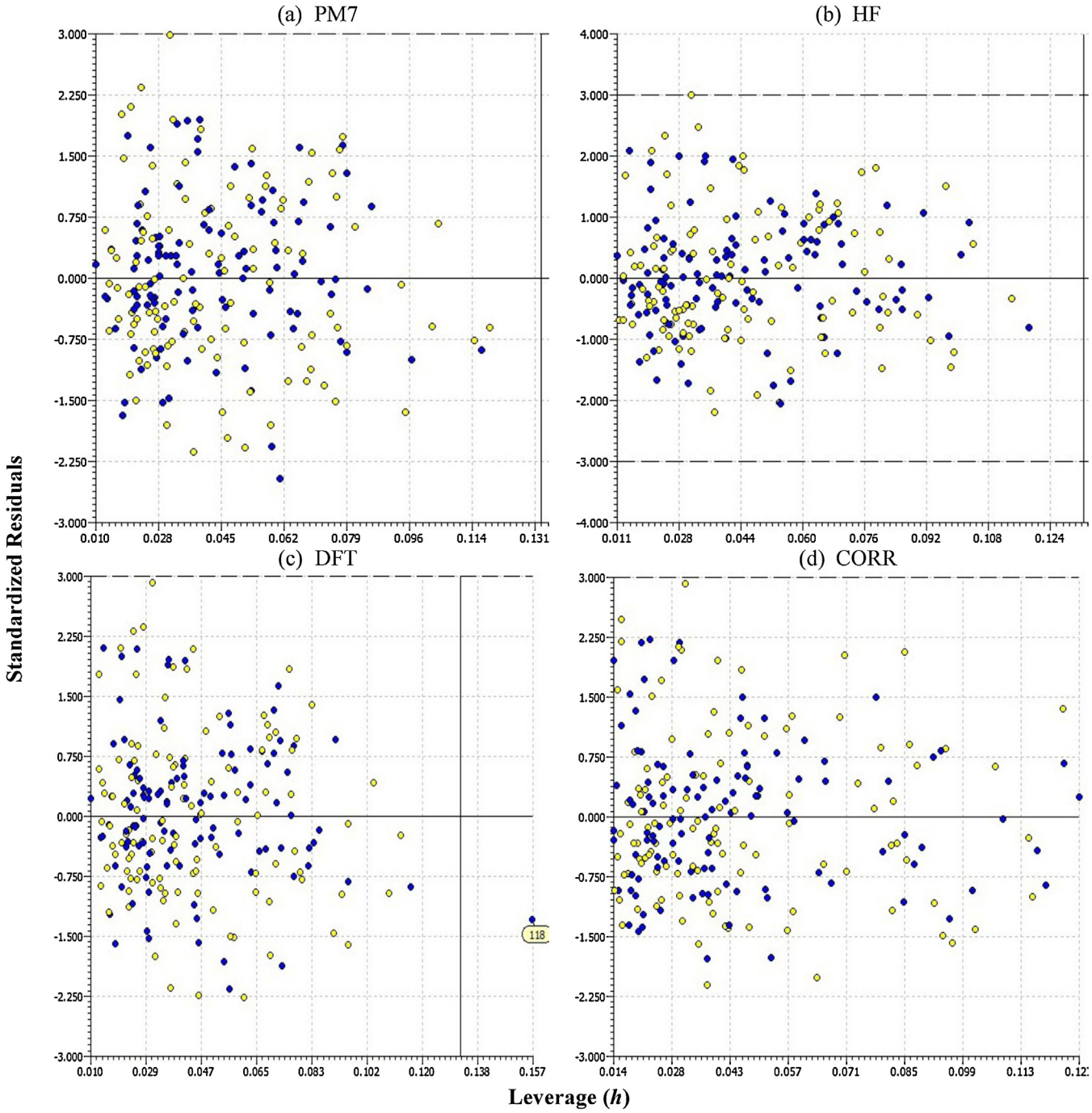

Fig. 4. Williams plot of the standardized residuals *vs* leverage (h) for the models II(a–d) (listed in Table 2), based on E_{HOMO}, $\log P_{mix}$, *WNSA*-1, ^1BIC, after excluding the outliers which are depicted in Fig. 5, with descriptor E_{HOMO} computed using (a) PM7 method (b) HF method (c) DFT method and (d) CORR method. Training and prediction set chemicals, using ordered response 50% splitting, are represented with open (yellow) and filled (blue) circles, respectively. Encircled number represents the ID number of chemical with high leverage value (for details, refer to Supporting information Table S1). The vertical (solid) line indicates warning leverage h*, whereas the horizontal (dashed) line specifies the standardized residual value of 3.0. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

namely, *WNSA*-1, and addition of one quantum-chemical descriptor, namely, E_{PM7} to model IIa (based on the PM7) results in a model IIIa with reliable internal and external stability as evident from the statistical parameters listed in Table 2. However, this is not observed if the topological descriptor is removed from model IIa as evident from the parameters observed for the model IVa based on the PM7 method. Similar trend is observed in the models incorporating the HF, DFT, and CORR computed descriptors as in case of models II(b–d), III(b–d) and IV(b–d). A comparison of these models suggests that the topological descriptor (^1BIC) and quantum-chemical descriptors particularly the total energy and the HOMO energy are quite significant for developing the externally predictive models for the acute toxicity of organic chemicals towards *D. magna*.

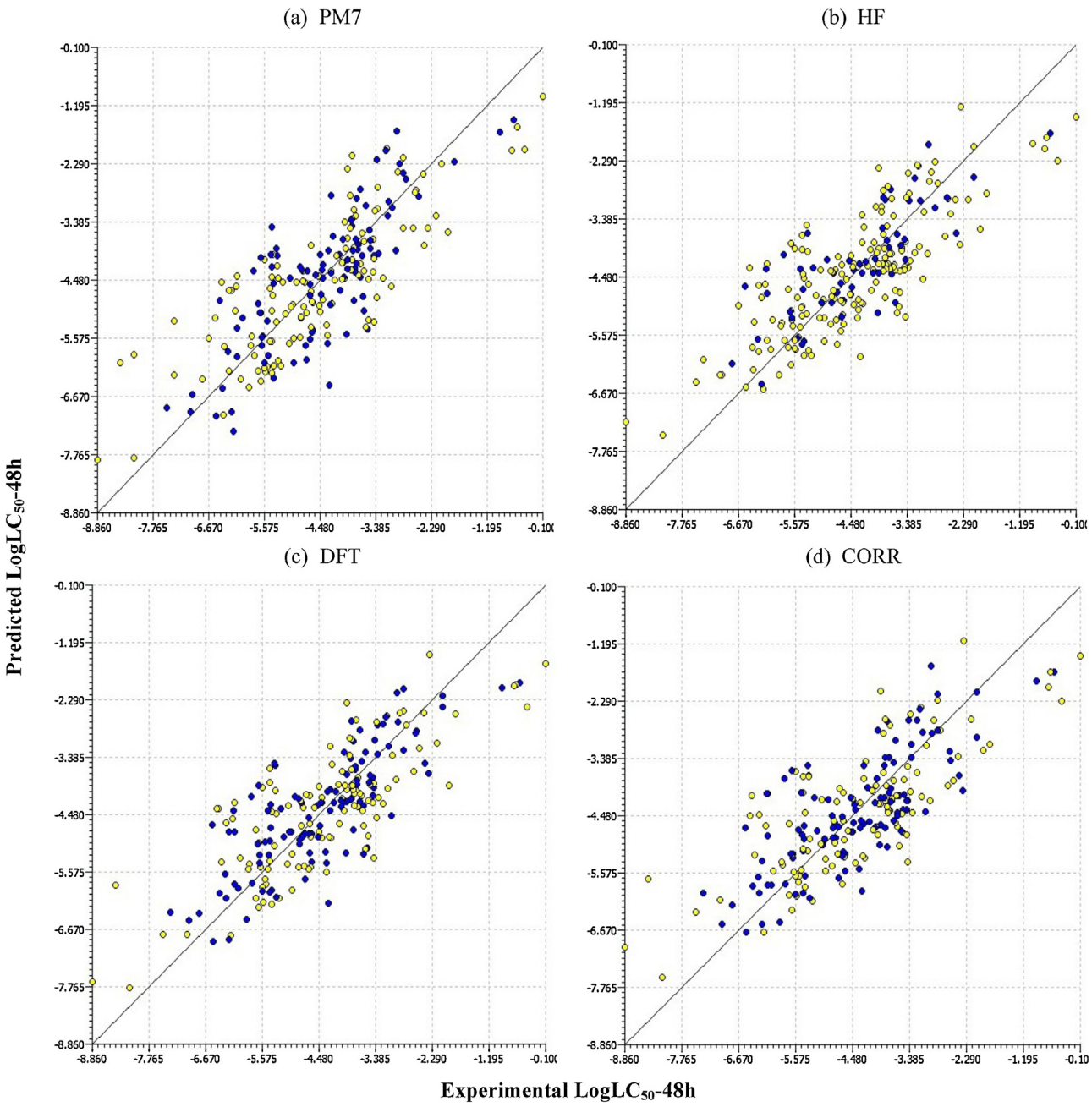

Fig. 5. Scatter plot of experimental *vs* predicted log LC_{50}-48 h for the models II(a–d) (listed in Table 2), based on E_{HOMO}, log P_{mix}, *WNSA*-1, ^{1}*BIC* with E_{HOMO} descriptor computed using (a) PM7 method (b) HF method (c) DFT method and (d) CORR method. Training and prediction set chemicals using activity sampling splitting are represented with open (yellow) and filled (blue) circles, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.3. Models based on three-descriptors

Table 3 lists the statistical validation parameters for the three-descriptor models. Analyzing the validation parameters of models V(a–d) to XI(a–d), the three-descriptor models are observed to be relatively less robust than the four-descriptor models. Moreover, models IX(a–d) to XI(a–d) having two quantum-chemical descriptors and only one physico-chemical or topological descriptor are neither found to be internally stable nor externally predictive, except model IXb incorporating the HF computed descriptors, which is internally stable. However, most significant validation parameters are observed for model VIIa incorporating PM7 computed descriptor. The stability of this model is close to that of models IIa and IIIa which incorporates the quantum-chemical descriptors computed using PM7 method. Further, it is evident from Table 3 that the models incorporating the total energy and two of the physico-chemical, electrostatic and topological descriptors, as in models V(a–d) and VI(a–d), are found to be unreliable since they do not meet the threshold values of validation parameters, except for the models Vd based on the CORR descriptors, which is internally stable. However, the three-descriptor based models VId–XId incorporating the CORR descriptors are also observed to be unreliable. Furthermore, analyzing the robustness of the models incorporating HF and DFT computed descriptors, as in models VII(b–c) and VIII(b–c), the models based on ^{1}*BIC* are found to be more reliable than those based on *WNSA*-1. Further, an interesting feature observed here is that among the three-descriptor models, the models incorporating the chemical hardness are also observed

to be statistically significant particularly in case of PM7 and HF methods (as provided in Supporting information Tables S4 and S5, S8 and S9, and S12 and S13), suggesting the chemical hardness to be an important aspect of a chemical while modeling the toxicity. It should be noted that in Tables 2–3, the models incorporating the quantum-mechanically computed thermodynamic descriptors are not listed because the parameters for the models incorporating Gibbs free energy and enthalpy are nearly similar to the models which incorporates the total energy (see Supporting information Tables S4–S19). This suggests that the quantum-mechanically computed thermodynamic descriptors are also as important as quantum-chemical molecular descriptors while developing QSAR models for the acute toxicity of chemicals.

However, it should be noted from Tables 1–3 that none of the developed model satisfies the threshold value of 0.85 for the external validation parameter, CCC_{EXT} proposed by Lin [64], and as suggested by Chirico and Gramatica [61] to implement for QSAR studies to access the external predictability of the models, though in the models II(a–d), CCC_{EXT} value is close to the threshold value suggesting models have nearly 80% agreement between the experimental and predicted response. Further, most of the models, for example, II(a–d), III(a–d) and IV(a–d), based on the total energy and HOMO energy, satisfy the threshold value for Q^2_{F3} parameter. It should further be noted that the models which do not meet the threshold values of the external validation parameters as those of suggested by Chirico and Gramatica [68] should not be neglected, because these models satisfy the commonly acceptable threshold for $Q^2_{F3} \geq 0.60$ as utilized by Roy et al. [69]. However, to judge the real external predictivity of the models, the scatter plots are further analyzed, since it provides the ocular and effortless check to the quality of the model. In the scatter plots for models II(a–d) depicted in Fig. 5(a–d), the prediction data set points are located diagonally, neither too upward nor too downward from the line of scattering between the experimental and predicted toxicity. Further, most of the models developed in the present study satisfy another stringent validation parameter, $\Delta r^2_m \leq 0.20$ [65] (as evident in Supporting information Tables S4–S19). Moreover, the difference criteria of internal stability *i.e.*, $|R^2 - Q^2_{LOO}| < 0.1$ is followed by all the models as evident from Tables 2–3 and Supporting information Tables S4–S19. Besides these, the proposed models in the present work have good validation parameters even if the model development and validation is performed with the complete set of compounds taken in the training set without any splitting (as provided in Supporting information Tables S16–S19).

From overall analysis of the models developed in the present study, only the model based on the PM7 computed HOMO energy along with log*Pmix*, ^{1}*BIC*, *WNSA*-1, descriptors is found to be better than the model proposed in a previous study by Moosus and Maran [9] which differs from the present model only in the computation of the HOMO energy using AM1 semi-empirical method. This could be expected since PM7 is a significantly improved advanced semi-empirical method over AM1 which has now undergone significant revisions in form of the PM7 method. Moreover, this four-descriptor model from the present and previous studies suggests that the HOMO energy is a critical quantum-chemical descriptor in determining the acute toxicity of diverse chemicals. However, in the present study, the models based on quantum-mechanically computed total energy are also found to be equally reliable as those based on the HOMO energy. Besides this, the addition of electron-correlation energy and correlation contribution of Gibbs free-energy to the HOMO energy based four-descriptor models is found to increase the robustness and external predictivity of the models, though the addition of another descriptor may improve statistical quality of the models but it is not always the case. Moreover, this is important considering the fact that in the previous study by Moosus and Maran [9], the addition of further descriptors to the aforementioned four-descriptor model based on the HOMO energy, is observed to decrease the performance of the model.

5. Conclusions

In conclusion, the models proposed, in this work, for the acute toxicity of diverse chemicals towards *D. magna*, are more reliable for the external prediction than the similar model available in the literature as evident from the satisfactory external validation parameters of the models II(a–d) and III(a) in Table 2 compared to the literature model (entry 1 in Table 1). It is, however, surprising that the more complex and advanced quantum-chemical descriptors, as employed in the present study while modeling the acute toxicity for a diverse set of chemicals, are unable to deliver as robust results as they provide for the modeling of the biological activities and physico-chemical properties of a congeneric set of compounds such as polyclic aromatic hydrocarbons, polychloronaphthalenes, polychlorinated-dibenzo-*p*-dioxins and -dibenzo-furans as evident in our recent studies [28–33]. This inability of the advanced quantum-chemical descriptors, particularly those based on the electron-correlation, may partly be ascribed to the heuristic method adopted for their computation in the present study. However, any advanced computation of these descriptors requires huge computational resources, making the descriptors practically unviable.

Finally, the advanced semi-empirical methods like PM7 are more successful than the *ab-initio* quantum mechanical methods in the quantitative modeling because the quantum-mechanical parameters deployed in the semi-empirical methods are already calibrated using the experimentally determined thermo-chemical and spectroscopic data of a diverse set of known chemicals which may be quite similar to the chemical for which a QSAR model is being developed. However, *ab-initio* methods compute the descriptors from the first-principles without using any experimental input. Moreover, the reliability of the models II(b–d) based on the HOMO energy computed with the HF, DFT and CORR methods, is comparable to that of the PM7 based model (IIa). In the view of the structural complexity of chemicals akin to the biological and environmental sciences, the models based on the descriptors computed using semi-empirical methods are likely to make more severe predictions than those based on the descriptors computed using the *ab-initio* methods. Since the diverse set of chemicals are difficult to model and require a number of entirely different descriptors depending on the properties being modeled, the importance of advanced quantum-chemical descriptors cannot be ruled out as evident from the dependence of the acute toxicity on the electron-correlation contribution to the HOMO energy, which is largely an unexplored phenomenon.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jmgm.2015.06.009

References

[1] R. Todeschini, M. Vighi, R. Provenzani, A. Finizio, P. Gramatica, Modeling and prediction by using whim descriptors in QSAR studies: toxicity of heterogeneous chemicals on *Daphnia Magna*, Chemosphere 32 (1996) 1527–1545.

[2] Y.G. Lee, S.H. Hwang, S.D. Kim, Predicting the toxicity of substituted phenols to aquatic species and its changes in the stream and effluent waters, Arch. Environ. Contam. Toxicol. 50 (2006) 213–219.

[3] P.C. Von der Ohe, R. Kühne, R.U. Ebert, R. Altenburger, M. Liess, G. Schüürmann, Structural alerts – a new classification model to discriminate excess toxicity from narcotic effect levels of organic compounds in the acute daphnid assay, Chem. Res. Toxicol. 18 (2005) 536–555.

[4] M.T.D. Cronin, Y.H. Zhao, R.L. Yu, pH-dependence and QSAR analysis of the toxicity of phenols and anilines to *Daphnia magna*, Environ. Toxicol. 15 (2000) 140–148.

[5] S. Marchini, L. Passerini, M.D. Hoglund, A. Pino, M. Nendza, Toxicity of aryl- and benzylhalides to Daphnia magna and classification of their mode of action based on quantitative structure–activity relationship, Environ. Toxicol. Chem. 18 (1999) 2759–2766.

[6] S. Tao, X. Xi, F. Xu, B. Li, J. Cao, R. Dawson, A fragment constant QSAR model for evaluation the EC50 values of organic chemicals to *Daphnia magna*, Environ. Pollut. 116 (2002) 57–64.

[7] M.A. Lampi, J. Gurska, X.D. Huang, D.G. Dixon, B.M. Greenberg, A predictive quantitative structure–activity relationship model for the photoinduced toxicity of polycyclic aromatic hydrocarbons to *Daphnia magna* with the use of factors for photosensitization and photomodification, Environ. Toxicol. Chem. 26 (2007) 406–415.

[8] S. Cassani, S. Kovarich, E. Papa, P.P. Roy, L. van der Wal, P. Gramatica, Daphnia and fish toxicity of (benzo) triazoles: validated QSAR model, and interspecies quantitative activity–activity modeling, J. Hazard. Mat. 258–259 (2013) 50–60.

[9] M. Moosus, U. Maran, Quantitative strucutre–activity relationship analysis of acute toxicity of diverse chemicals to *Daphniam Magna* with whole molecular descriptor, SAR QSAR Environ. Res. 22 (2011) 757–774.

[10] G. Hodges, D.W. Roberts, S.J. Marshall, J.C. Dearden, The aquatic toxicity of anionic surfactants to Daphnia magna – a comparative QSAR study of linear alkylbenzene sulphonates and ester sulphonates, Chemosphere 63 (2006) 1443–1450.

[11] S. Kar, K. Roy, QSAR modeling of toxicity of diverse organic chemicals to *Daphnia* magna using 2D and 3D descriptors, J. Hazard. Mater. 177 (2010) 344–351.

[12] A.A. Toropov, E. Benfenati, QSAR models for *Daphnia* toxicity of pesticides based on combination of topological parameters of molecular structures, Bioorg. Med. Chem. 14 (2006) 2779–2788.

[13] R. Guha, P.C. Jurs, Determining the validity of a QSAR model – a classification approach, J. Chem. Inf. Model. 45 (2005) 65–73.

[14] E. Tenorio-Borroto, F.R. Ramirez, A. Speck-Planche, M.N.D.S. Cordeiro, F. Luan, H. Gonzalez-Diaz, QSPR and flow cytometry analysis (QSPR-FCA): review and new findings on parallel study of multiple interactions of chemical compounds with immune cellular and molecular targets, Curr. Drug Metab. 15 (2014) 414–428.

[15] E. Tenorio-Borroto, C.G. Penuelas-Rivas, J.C. Vasquez-Chagoyan, N. Castanedo, F.J. Prado-Prado, X. Garcia-Mera, H. Gonzalez-Diaz, Model for high-throughput screening of drug immunotoxicity – study of the anti-microbial G1 over peritoneal macrophages using flow cytometry, Eur. J. Med. Chem. 72 (2014) 206–220.

[16] E. Tenorio-Borroto, C.G. Penuelas Rivas, J.C. Vasquez Chagoyan, N. Castanedo, F.J. Prado-Prado, X. Garcia-Mera, H. Gonzalez-Diaz, ANN multiplexing model of drugs effect on macrophages; theoretical and flow cytometry study on the cytotoxicity of the anti-microbial drug G1 in spleen, Bioorg. Med. Chem. 20 (2012) 6181–6194.

[17] P.G. Maykel, G.-D. Humberto, M.R. Reinaldo, A.C. Miguel, R.A. de Ronal, TOPS-MODE based QSARs derived from heterogeneous series of compounds. Application to the design of new herbicides, J. Chem. Inf. Comput. Sci. 43 (2003) 1192–1199.

[18] M. Giovanni, C. Adriana, G. Adriano, U. Eugenio, B. Paola, C. Ignazio, T. Francesca, G.-D. Humberto, Using the TOPS-MODE approach to fit multi-target QSAR models for tyrosine kinase inhibitors, Eur. J. Med. Chem. 46 (2011) 2185–2192.

[19] V.V. Kleandrova, F. Luan, A. Speck-Planche, M.N. Cordeiro, In silico assessment of the acute toxicity of chemicals: recent advances and new model for multitasking prediction of toxic effect, Mini Rev. Med. Chem. 15 (2015) 677–686.

[20] A. Furuhama, K. Hasunuma, Y. Aoki, Interspecies quantitative structure–activity–activity relationships (QSAARs) for prediction of acute aquatic toxicity of aromatic amines and phenols, SAR QSAR Environ. Res. 26 (2015) 301–323.

[21] A. Speck-Planche, V.V. Kleandrova, F. Luan, M.N.D.S. Cordeiro, Predicting multiple ecotoxicological profiles in agrochemical fungicides: a multi-species chemoinformatic approach, Ecotoxicol. Environ. Saf. 80 (2012) 308–313.

[22] L. Minini, G. Álvarez, M. González, H. Cerecetto, A. Merlino, Molecular docking and molecular dynamics simulation studies of *Trypanosoma cruzi* triosephosphate isomerase inhibitors. Insights into the inhibition mechanism and selectivity, J. Mol. Graph. Model. 58 (2015) 40–49.

[23] A. Balupuri, P.K. Balasubramanian, C.G. Gadhe, S.J. Cho, Docking based 3D-QSAR studies of pyridyl aminothiazole derivatives as checkpoint kinase 1 inhibitors, SAR QSAR Environ. Res. 25 (2014) 651–671.

[24] J.J.P. Stewart, Optimization of parameters for semiempirical methods VI: more modifications to NDDO approximations and re-optimization of parameters, J. Mol. Model. 19 (2013) 1–32.

[25] A. Szabo, N.S. Ostlund, Modern Quantum Chemistry: Introduction to Advanced Electronic Strucutre Theory, MacMillian, New York, 1982.

[26] E.G. Lewars, Computational Chemistry: Introduction to the Theory and Applications of Molecular and Quantum Mechanics, 2nd ed., Heidelberg, Springer, 2011.

[27] R.G. Parr, W. Yang, Density Functional Theory of Atoms and Molecules, Oxford University Press, New York, 1989.

[28] Vikas, Reenu, Chayawan, Does electron-correlation has any role in the quantitative structure–activity relationships? J. Mol. Graph. Model. 42 (2013) 7–16.

[29] Reenu, Vikas, Electron-correlation based externally predictive QSARs for mutagenicity of nitrated-PAHs in *Salmonella typhimurium* TA100, Ecotoxicol. Environ. Saf. 101 (2014) 42–50.

[30] Vikas, Chayawan, Externally predictive quantitative modelling of super cooled liquids vapour pressure of polychlorinated-naphthalene through electron-correlation based quantum-mechanical descriptors, Chemosphere 95 (2014) 448–454.

[31] Vikas, Chayawan, Single-descriptor based quantum-chemical QSPRs for physico-chemical properties of polychlorinated-dibenzo-*p*-dioxins and -dibenzo-furans (PCDD/Fs): exploring the role of electron-correlation, Chemosphere 118 (2015) 239–245.

[32] Reenu, Vikas, Role of exchange and correlation in the real external prediction of mutagenicity: performance of hybrid and meta-hybrid exchange-correlation functionals, RSC Adv. 5 (2015) 29238–29251.

[33] Chayawan, Vikas, Externally predictive single-descriptor based QSPRs for physico-chemical properties of polychlorinated-naphthalenes: exploring relationships of $\log S_W$, $\log K_{OA}$, and $\log K_{OW}$ with electron-correlation, J. Hazadr. Mat. 296 (2015) 68–81.

[34] L.R. Christine, P.B. Steven, J.B. Steven, E.H. Dean, A.D. Robert, Predicting modes of toxic action from chemical structure: acute toxicity in the fathead minnow (*Pimephales promelas*), Environ. Toxicol. Chem. 16 (1997) 948–967.

[35] O.V. Monsanto, 2001, *96-CV-440, Exhibit 3A03F*, Alabama, USA, cited in Chemical Industry Archives, Anniston Case, by Environmental Working Group, Washington, DC, 2002.

[36] World Health Organization/International Labour Organization: International Chemical Safety Cards, http://www.inchem.org/documents/icsc/icsc/eics0070htm (accessed 14.11.14).

[37] NIOSH (National Institute of Occupational Safety and Health), Pocket Health Guide to Chemical Hazards, U.S. Department of Health and Human Services, Washington, DC, 1997.

[38] National Institute on Alcohol Abuse and Alcoholism (NIAAA), Health risks and benefits of alcohol consumption, Alcohol. Res. Health 24 (2000) 5–11.

[39] Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Nitrobenzene. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA, 1990.

[40] L. Pauling, E.B. Wilson, Introduction to Quantum Mechanics: With Applications to Chemistry, Courier Dover Publications, 1985.

[41] D. Butina, M.D. Segall, K. Frankcombe, Predicting ADME properties *in silico*: methods and models, Inf. Biotechol. 7 (Supp. DDT) (2002) S83–S88.

[42] R. Vijayaraj, V. Subramanian, P.K. Chattaraj, Comparison of global reactivity calculated using various density functionals: a QSAR perspective, J. Chem. Theory Comput. 5 (2009) 2744–2753.

[43] Vikas, P. Sangwan, Role of electron-correlation in polydeprotonation of benzene to form trianions, J. Phys. Org. Chem. 27 (2014) 565–582.

[44] A.D. Becke, Density-functional thermochemistry, III. The role of exact exchange, J. Chem. Phys 98 (1993) 5648–5652.

[45] P.J. Stephens, F.J. Devlin, C.F. Chabalowski, M.J. Frisch, Ab initio calculations of vibrational absorption and circular dichroism spectra using density functional force fields, J. Phys. Chem. 98 (11) (1994) 623–11627.

[46] R.J. Bartlett, Many-body perturbation theory and coupled cluster theory for electron correlation in molecules, Annu. Rev. Phys. Chem. 32 (1981) 359–401.

[47] C.D. Sherrill, H.F. Schaefer III, The configuration interaction method: advances in highly correlated approaches, Adv. Quantum Chem. 34 (1999) 143–269.

[48] C.D. Sherrill, An Introduction to Configuration Interaction Theory, School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, 1995.

[49] F. Jensen, Introduction to Computational Chemistry, John Wiley and Sons, Chichester, England, 2007, pp. 133–158.

[50] E.R. Davidson, D. Feller, Basis set selection for molecular calculations, Chem. Rev. 86 (1986) 681–696.

[51] J.J.P. Stewart, MOPAC version 12.239W, Stewart Comput. Chem. (2012) (http://OpenMOPAC.net).

[52] Gaussian 03, Revision D.01, M.J. Frisch et al., Gaussian, Inc., Wallingford CT, 2004.

[53] US Environmental Protection Agency, Estimation Programs Interface SuiteTM for Mictrosoft® Windows, v 4.00, US EPA, Washington DC, 2009.

[54] A. Leo, C. Hansch, D. Elkins, Partition coefficients and their use, Chem. Rev. 71 (1971) 525–615.

[55] W.H.J. Vaes, E.U. Ramos, H.J.M. Verhaar, J.L.M. Hermens, Acute toxicity of nonpolar versus polar narcosis: is there a difference, Environ. Toxicol. Chem. 17 (1998) 1380–1384.

[56] R. Kühne, R.-U. Ebert, P.C. von der Ohe, N. Ulrich, W. Brack, G. Schüürmann, Read-across prediction of the acute toxicity of organic compounds towards the water flea *Daphnia magna*, Mol. Inf. 32 (2013) 108–120.
[57] R.L. Haupt, S.E. Haupt, Practical Genetic Algorithms, 2nd ed., Wiley-Interscience, New Jersey, United States, 2004.
[58] A. Tropsha, P. Gramatica, V.K. Gombar, The importance of being earnest: validation is the absolute essential for successful application and interpretation of QSPR models, QSAR Comb. Sci. 22 (2003) 69–77.
[59] A. Tropsha, Best practices for QSAR model development, validation and explitation, Mol. Inf. 29 (2010) 476–488.
[60] A. Golbraikh, A. Tropsha, Beware of q^2, J. Mol. Graph. Model. 20 (2002) 269–276.
[61] N. Chirico, P. Gramatica, Real external predictivity of qsar models: how to evaluate it? Comparison of different validation criteria and proposal of using the concordance correlation coefficient, J. Chem. Inf. Model. 21 (2011) 2320–2335.
[62] R. Todeschini, A. Maiocchi, V. Consonni, The *K* correlation index: theory development and its application in chemometrics, Chemom. Intell. Lab. Syst. 46 (1999) 13–29.
[63] V. Consonni, D. Ballabio, R. Todeschini, Comments on the definition of the Q^2 parameter for QSAR validation, J. Chem. Inf. Model. 49 (2009) 1669–1678.
[64] L.I.A. Lin, Concordance correlation coefficient to evaluate reproducibility, Biometrics 45 (1989) 255–268.
[65] P.K. Ojha, I. Mitra, R.N. Das, K. Roy, Further exploring r^2_m metrics for validation of QSPR models dataset, Chemometr. Intell. Lab. Syst. 107 (2011) 194–205.
[66] N. Chirico, E. Papa, S. Kovarich, S. Cassani, P. Gramatica, QSARINS, software for QSAR MLR model development and validation, QSAR Res. Unit Environ. Chem. Ecotoxicol. (2012), University of Insubria, Varese, Italy (http://www.qsar.it).
[67] P. Gramatica, N. Chirico, E. Papa, S. Cassani, S. Kovarich, QSARINS: a new software for the development, analysis and validation of QSAR MLR model software news and updates, J. Comput. Chem. 34 (2013) 2121–2132.
[68] N. Chirico, P. Gramatica, Real external predictivity of QSAR models. Part 2. New intercomparable thresholds for different validation criteria and the need for scatter plot inspection, J. Chem. Inf. Model. 52 (2012) 2044–2058.
[69] K. Roy, I. Mitra, S. Kar, P.K. Ojha, R.N. Das, H. Kabir, Comparative studies on some metrics for external validation of QSPR models, J. Chem. Inf. Model. 52 (2012) 396–408.