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# Toxicity of Selected Imidazolium-based Ionic Liquids on *Caenorhabditis elegans*: A Quantitative Structure-Activity Relationship Study

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Due to the large number of ionic liquids (ILs) and their potential environmental risk, assessing the toxicity of ILs by ecotoxicological experiment only is insufficient. Quantitative structure-activity relationship (QSAR) has been proven to be a quick and effective method to estimate the viscosity, melting points, and even toxicity of ILs. In this work, the $LC_{50}$ values of 30 imidazolium-based ILs were determined with *Caenorhabditis elegans* as a model animal. Four suitable molecular descriptors were selected on the basis of genetic function approximation algorithm to construct a QSAR model with an $R^2$ value of 0.938. The predicted $lgLC_{50}$ in this work are in agreement with the experimental values, indicating that the model has good stability and predictive ability. Our study provides a valuable model to predict the potential toxicity of ILs with different sub-structures to the environment and human health.

**Key words:** Imidazolium-based ionic liquids, *Caenorhabditis elegans*, Toxicity, Quantitative structure-activity relationship

## I. INTRODUCTION

Ionic liquids (ILs) are a class of compounds consisting of two oppositely charged ions [1]. To date, ILs are considered as promising alternatives to traditional organic solvents due to their beneficial and tunable physicochemical properties such as weak volatility, low melting points (<100 °C), broad solvation capacity, wide range of fluidity, thermal and electrochemical stabilities and the designability of ionic liquids [2−4]. Owing to the superiority that ILs can combine various cations and anions to freely manipulate their characteristics, there are well over one million ILs that can be synthesized to meet specific requirements for different applications, such as gas compression, sensors, lithium-ion batteries, dye-sensitized solar cells or potential pharmaceutical ingredients [5, 6]. However, the poor biodegradability and high water solubility imply the potential environmental risks of ILs, especially to aquatic ecosystem [7, 8]. At present, there have been numerous reports on the toxicity of ILs to bacteria, cells, enzyme systems, plants and aquatic organisms, like *Vibrio fischeri*, *Daphnia magna and algae*, *etc.* [9−12]. Furthermore, the huge quantity and variety of ILs make it a great significance to estimate their environmental effects by building a rapid and effective method instead of the time- and material-consuming ecotoxicological assays [13].

Quantitative structure-activity relationship (QSAR) is a model that is used to establish a correlation between the physicochemical, biological activity of a given molecular, and a set of molecular properties [14]. In recent years, there have been a few QSAR models based on the acute toxicity data. A review article regarding different QSAR studies performed on the toxicity of ILs was published recently [1]. Luis *et al.* [13] established a QSAR model ($R^2$=0.925) by using a novel group contribution method and *Vibrio fischeri* as a model organism to evaluate ecotoxicity of 43 ionic liquids, and toxicity contributions of anion, cation and alkyl substitutions were calculated. In another study, Alvarez-Guerra and Irabien [15] developed a QSAR model using partial least squares-discriminant analysis (PLS-DA) to assess toxicity of 148 ionic liquids comprising of a varying combination of different cations and anions species. A QSAR model ($R^2_{pred}$=0.739) was developed by Das and Roy [16] to evaluate toxicity of ionic liquids on bacteria by using several approaches, such as multiple linear regression (MLR) and partial least squares (PLS). The developed model underwent extensive validation and was acceptable in terms of robustness and predictivity.

Some studies also applied on other test organisms like green algae and *Daphnia magna* [6, 17, 18]. Due to its small size, rapid life cycle and ease of cultivation, *Caenorhabditis elegans* (*C. elegans*) as a multicellular animal has been widely used as a model organism in the field of developmental biology, genetics, biomedical and environmental toxicology [19, 20]. With at least 40% of the genes in *C. elegans* have orthologs in the human genome [21], it is of great significance that *C. elegans*

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is applied in the risk assessment of ILs integrated with QSAR analysis.

In this study, the 50% effective concentration of selected imidazolium-based ionic liquids on *C. elegans* was determined using 24-h acute toxicity bioassays. Then these experimental data were used to build a predictive toxicity model based upon quantitative structure-activity relationship modeling (QSAR) methods with genetic function approximation (GFA) used for feature selection and MLR for model construction. Valuable information can be obtained from this model to help designing ILs with minimal toxicity to the environment and human health.

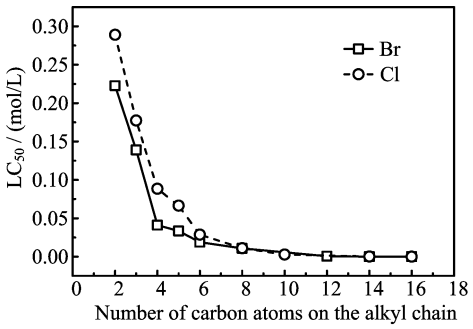


FIG. 1 Experimental $LC_{50}$ values of the ILs composed of halide anions and the imidazolium cation with different linear alkyl chain length.

## II. EXPERIMENTS

### A. Ionic liquids

The 30 imidazolium-based ionic liquids used in the experimental study are presented in Table I. The cations of these ionic liquids were imidazole rings with different alkyl side chain length. The anions were common anions, such as bromide, chloride, acetate, nitrate, and some uncommon anions like tetrafluoroborateion and thiocyanate. These ILs (more than 99% purity) were purchased from Lanzhou Zhong Ke Kai Te Co., China. Stock solutions were prepared by dissolving the ILs in sterile water at appropriate concentrations followed by passing through 0.22 μm pore-size filters for sterilization.

### B. Toxicity tests

Wild-type $N_2$ worms were cultured according to Brenner [22] at 20 °C. Age-synchronized L4 larvae worms were prepared and 20±1 L4 larvae were transferred to a well in 24-well costar plates. Each ionic liquid was diluted with K medium (containing 52 mmol/L NaCl and 32 mmol/L KCl) at a proper concentration range and 1 mL of the solution was added into a well. One mL of K medium was used as a negative control. After 24 h exposure, dead worms were scored under a dissecting microscope (Olympus SZX7, Japan). The mortality data of each ionic liquid were subjected to probit analysis to estimate the median of lethal concentration ($LC_{50}$). Two independent trails were tested for each ionic liquid. In each trail, at least three replicates were tested for each dilution.

### C. QSAR studies

The $LC_{50}$ values of ILs on *C. elegans* were log-tansformed ($lgLC_{50}$) and used for the following QSAR modelling (Table I) which contains several steps, including alignment of molecular structure, the calculation of descriptors, initial data analysis, and generation and validation of structure-activity relationship or model. The geometry structures of ILs were constructed and optimized based on density functional theory (DFT). The DFT calculations were performed with the Perdew, Burke, and Ernzerhof (PBE) functional [23] of generalized gradient approximation (GGA) [24] for the exchange-correlation term implemented in the $DMol^3$ code [25, 26]. Double precision numerical basis sets combined with p polarization (DNP) were adopted. The training set contains 26 ILs as shown in Table I. As all the cations of ILs in the dataset have the same imidazolium core, this core is aligned to a specific axis, and then all the cations are superposed over the core. Molecular descriptors, including conformational, electrotopological, electronic, information-content, quantum mechanical, spatial, structural, thermodynamic, and topological information, were calculated after the optimization. Four molecular descriptors that most closely related to the $LC_{50}$ values were screened by using GFA [27]. Another 4 ILs tested in this work and 5 data from our previous work [28] were used to validate the QSAR model of IL toxicity.

## III. RESULTS AND DISCUSSION

This study investigated the acute lethal toxicity of selected imidazolium based ILs with different alkyl chain length and anion type. The $LC_{50}$ values are shown in Table I, and differences of more than four orders of magnitude were observed. The $LC_{50}$ data ranged from $2.35\times10^{-5}$ mol/L to $2.89\times10^{-1}$ mol/L. As shown in FIG. 1, the $LC_{50}$ values, for both ionic liquids with chloride and bromide anions, decreased with the increase of linear alkyl chain length, indicating that ILs with longer alkyl side chains exhibit higher toxicity, which is consistent with the results reported previously [29]. ILs with longer alkyl chain are generally more lipophilic and can be easily incorporated into and ultimately disrupt the cell membranes. Some studies have demonstrated that enhanced membrane permeability may lead to increased

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TABLE I Experimental and predicted toxicity results for selected imidazolium ionic liquids.

| No. | ILs | $LC_{50expt.}$ | $lgLC_{50expt.}$ | $lgLC_{50pred.}$ | Residual value |
|---|---|---|---|---|---|
| | Training set | | | | |
| 1 | 1-Ethyl-3-methylimidazolium bromide | $2.23\times10^{-1}$ | −0.65 | −0.53 | −0.12 |
| 2 | 1-Butyl-3-methylimidazolium bromide | $4.10\times10^{-2}$ | −1.39 | −1.12 | −0.27 |
| 3 | 1-Hexyl-3-methylimidazolium bromide | $1.88\times10^{-2}$ | −1.73 | −1.58 | −0.15 |
| 4 | 1-Octyl-3-methylimidazolium bromide | $1.08\times10^{-2}$ | −1.97 | −1.95 | −0.02 |
| 5 | 1-Dodecyl-3-methylimidazolium bromide | $5.31\times10^{-4}$ | −3.28 | −3.26 | −0.02 |
| 6 | 1-Tetradecyl-3-methylimidazolium bromide | $6.48\times10^{-5}$ | −4.19 | −3.72 | −0.47 |
| 7 | 1-Hexadecyl-3-methylimidazolium bromide | $4.85\times10^{-5}$ | −4.31 | −4.28 | −0.03 |
| 8 | 1-Propenyl-3-methylimidazolium chloride | $1.77\times10^{-1}$ | −0.75 | −0.85 | 0.10 |
| 9 | 1-Butyl-3-methylimidazolium chloride | $8.85\times10^{-2}$ | −1.05 | −1.15 | 0.10 |
| 10 | 1-Pentyl-3-methylimidazolium chloride | $6.65\times10^{-2}$ | −1.18 | −1.42 | 0.24 |
| 11 | 1-Octyl-3-methylimidazolium chloride | $1.09\times10^{-2}$ | −1.96 | −2.26 | 0.30 |
| 12 | 1-Decyl-3-methylimidazolium chloride | $2.68\times10^{-3}$ | −2.57 | −2.75 | 0.18 |
| 13 | 1-Tetradecyl-3-methylimidazolium chloride | $1.06\times10^{-4}$ | −3.98 | −3.95 | −0.03 |
| 14 | 1-Hexadecyl-3-methylimidazolium chloride | $2.35\times10^{-5}$ | −4.63 | −4.29 | −0.34 |
| 15 | 1-Butyl-3-methylimidazolium acetate | $8.38\times10^{-2}$ | −1.08 | −1.36 | 0.28 |
| 16 | 1-Butyl-3-methylimidazolium dibutyl phosphate | $2.71\times10^{-3}$ | −2.57 | −2.30 | −0.27 |
| 17 | 1-Butyl-3-methylimidazolium nitrate | $5.27\times10^{-2}$ | −1.28 | −1.35 | 0.07 |
| 18 | 1-Butyl-3-methylimidazolium iodide | $8.71\times10^{-2}$ | −1.06 | −1.12 | 0.06 |
| 19 | 1-Butyl-3-methylimidazolium tetrafluoroborate | $1.13\times10^{-3}$ | −2.95 | −2.15 | −0.80 |
| 20 | 1-Butyl-3-methylimidazolium dicyanamide | $3.54\times10^{-2}$ | −1.45 | −1.74 | 0.29 |
| 21 | 1-Butyl-3-methylimidazolium trifluoromethanesulfonate | $2.79\times10^{-2}$ | −1.55 | −2.09 | 0.54 |
| 22 | 1-Butyl-3-methylimidazolium tosylate | $9.97\times10^{-2}$ | −1.00 | −1.43 | 0.43 |
| 23 | 1-Butyl-3-methylimidazolium thiocyanate | $5.33\times10^{-2}$ | −1.27 | −1.74 | 0.47 |
| 24 | 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide | $1.68\times10^{-2}$ | −1.77 | −1.68 | −0.09 |
| 25 | 1-Butyl-3-methylimidazolium perchlorate | $4.44\times10^{-2}$ | −1.35 | −1.36 | 0.01 |
| 26 | 1-Butyl-3-methylimidazolium trifluoroacetate | $3.11\times10^{-2}$ | −1.51 | −1.98 | 0.47 |
| | Test set | | | | |
| 1 | 1-Propenyl-3-methylimidazolium bromide | $1.39\times10^{-1}$ | −0.86 | −0.82 | −0.04 |
| 2 | 1-Pentyl-3-methylimidazolium bromide | $3.35\times10^{-2}$ | −1.48 | −1.40 | −0.08 |
| 3 | 1-Ethyl-3-methylimidazolium chloride | $2.89\times10^{-1}$ | −0.54 | −0.56 | 0.02 |
| 4 | 1-Hexyl-3-methylimidazolium chloride | $2.87\times10^{-2}$ | −1.54 | −1.60 | 0.06 |
| 5[a] | 1-Decyl-3-methylimidazolium bromide | $1.39\times10^{-3}$ | −2.86 | −2.73 | −0.13 |
| 6[a] | 1-Butyl-2-methyl-3-methyl-imidazolium bromine | $2.86\times10^{-2}$ | −1.54 | −1.09 | −0.46 |
| 7[a] | 1-Octyl-2-methyl-3-methyl-imidazolium bromine | $5.34\times10^{-3}$ | −2.27 | −2.13 | −0.15 |
| 8[a] | 1-Decyl-2-methyl-3-methyl-imidazolium bromine | $1.23\times10^{-3}$ | −2.91 | −2.51 | −0.40 |
| 9[a] | 1-Dodecyl-2-methyl-3-methyl-imidazolium bromine | $2.61\times10^{-4}$ | −3.58 | −2.91 | −0.68 |

[a] The data are from Ref.[31].

toxicity of longer ILs [30, 31].

FIG. 1 also shows that the bromide moiety was more active than the chloride moiety in acute lethal toxicity for the ILs with shorter alkyl chain. The influence of the anion moiety gets weaker as the alkyl chain length increases, suggesting the dominant intrinsic effect of the imidazolium cation moiety [32]. Cho *et al.* [33] have also shown that the halide anions have only a little effect. The $lgLC_{50}$ was used as the dependent variable and molecular descriptor of IL structure as independent variables to construct the QSAR equation. Four molecular descriptors, including Chi-1, IC, Q, and Dipole-$Y$, that most closely related to the $LC_{50}$ values were screened by using GFA. The multiple linear equation is as follows:

$$\begin{aligned} lgLC_{50} = &-0.665(\text{Chi-1}) - 1.722(\text{IC}) + 0.001(\text{Q}) + \\ &0.094(\text{Dipole-}Y) + 7.130 \end{aligned} \quad (1)$$

$N_{training}$=26, $N_{test}$=9, $R^2$=0.938, adjusted $R^2$=0.926, $R^2_{test}$=0.9916, $Q^2_{LOO}$=0.9047, $F$-value=79.381.

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Comparing these validation parameters with those of the QSAR study on IL toxicity to other organisms [34, 35], the values of $R^2$, adjusted $R^2$, $R^2_{\text{test}}$ and $F$-value for external validation are high indicating that Eq.(1) fits the training set data very well and contains additional molecular characteristics and their physicochemical properties which can help to elucidate the important features responsible for toxicity. $Q^2_{\text{Loo}}$ is the leave-one-out (LOO) cross-validation squared correlation coefficient and was used to internally validate the developed model. The $Q^2_{\text{LOO}}$ value was close to 1, indicating that the model had very good stability and predictive ability. The $p$ values ($<0.05$) of the descriptors in the multiple linear equation including Chi-1, IC, Q, and Dipole-$Y$ are 0.000, 0.004, 0.006, and 0.000, respectively, which indicate that the selected molecular descriptors play important roles in predicting the IL toxicity to *C. elegans*.

One of the molecular descriptor, Chi-1, is an atomic connectivity index (order 1) [36]. This is a topological descriptor which helps to differentiate molecules according to their overall shape, degree of branching, size, and flexibility.

$$\text{Chi-1} = \sum (\delta_i \delta_j)^{-0.5} \tag{2}$$

$$\delta = \sigma - h \tag{3}$$

The property $\delta$ is the number of its electrons in sigma bonds to skeletal neighbors. The property of $\sigma$ is the number of electrons in $\sigma$ bonds to all neighbors and $h$ is the number of H atoms bonded to atom $i$. In this case, the value of Chi-1 depends on the length of the alkyl chain substituted on the imidazolium in the cation of ILs. The Chi-1 becomes larger with the longer alkyl chain for more carbon atoms connected. This descriptor is preceded by a negative coefficient, indicating that the ILs with larger Chi-1 values lead to higher toxicity.

To determine IC, the information-content descriptors, molecules are viewed as structures that can be partitioned into subsets of equivalent elements. The modifications of IC are shown as bonding information content (BIC), structural information content (SIC) and information content (CIC) [37]. This indicates that IC is related to the number of bonds and vertices, which also depends on the alkyl chain length in the cation. The longer alkyl chain contains more bonds and vertices. The coefficient of IC with the largest absolute value has the greatest impact on the toxicity, suggesting that the cation is a major factor determining the toxicity of ILs, which is consistent with the results of other reports [38, 39].

Q is the heat of formation descriptor (kcal/mol) calculated from the VAMP electrostatics model, indicating conformational stability or the energy required to ionize the valence electrons of the atoms in the cation and anion of ILs. The positive coefficient of Q indicates that the stable ILs will have high toxicity, which is reasonable. This indicates that the model based on Eq.(1) is suitable to describe the toxicity of ILs to *C. elegans*.

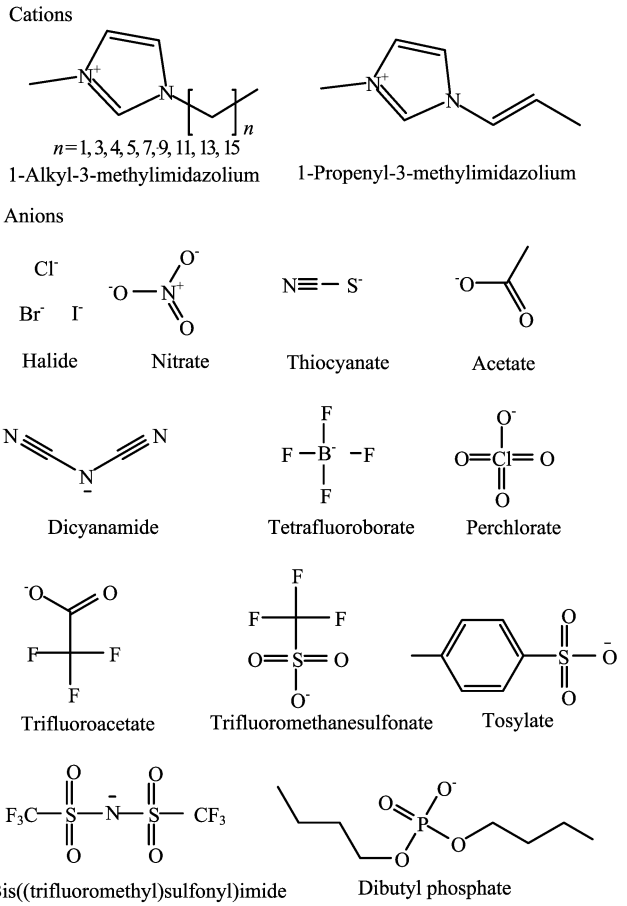


FIG. 2 Chemical structures of cations and anions of imidazolium-based ionic liquids.

Dipole is the dipole moment descriptor, a 3D electronic descriptor related to the strength and orientation behavior of cation and anion in an electrostatic field. The magnitudes of dipole along $x$, $y$, and $z$ axes are calculated, and the toxicity of IL is related to Dipole-$Y$. The attraction between cations and anions is predicted by utilizing partial atomic charges and atomic coordinates. The descriptor uses Debyes units.

The $LC_{50}$ of the ILs with the same cation and different anion were also investigated, and the structures of anions are shown in FIG. 2. For the same cation of 1-butyl-3-methylimidazolium, the ILs with tetrafluoroborate ($BF_4^-$) and dibutyl-phosphate have much smaller $lgLC_{50}$ than others from the experimental measurements (FIG. 3). This indicates that the Dipole for these two ILs are smaller than the other ILs when the cation is 1-butyl-3-methyl-imidazolium, and the toxicity of these two ILs is higher than the other 1-butyl-3-methylimidazolium ILs. The lower Dipole reveals the lower interaction strength between cation and anion. For the anion of $BF_4^-$, the lower Dipole may result from the hydrolyzation, which causes less number of $LC_{50}$ than cation. And the hydrolysis products may also increase the toxicity [40]. For the anion of

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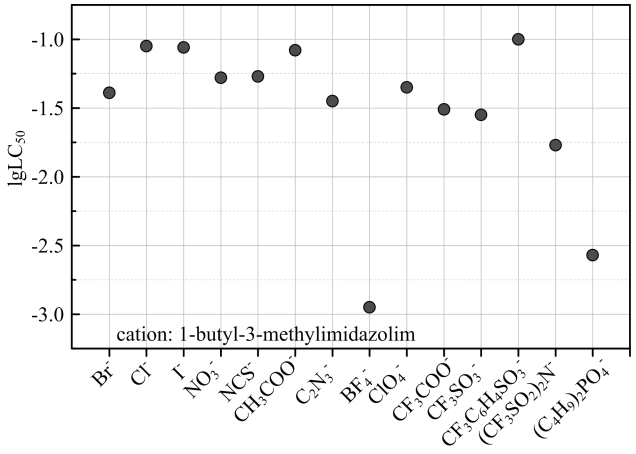


FIG. 3 Experimental $lgLC_{50}$ values of the ILs with the same cation (1-butyl-3-methylimidazolium) but different anions, IL with the anion of $BF_4^-$ and dibutyl-phosphate presents the lower $lgLC_{50}$.

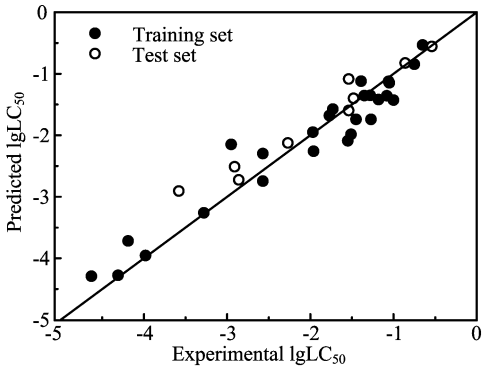


FIG. 4 Comparison of the experimental data of *C. elegans* and the predicted ones from the QSAR model based on Eq.(1).

dibutyl-phosphate, the lower interaction with cation is owing to the steric effect of the dibutyl chain (FIG. 2). The dipole-dipole attraction as one of the nondispersive forces among cation and anion might be responsible for the surface tension of liquids [40]. ILs with low surface tension will easily penetrate through the cell membrane, which might result in the high toxicity to *C. elegans*. Thus, the Dipole descriptor is related to the ability of membrane penetration of ILs, and the positive coefficient indicates that low Dipole causes high toxicity.

External validation was performed by using the data of the test set. The predicted $lgLC_{50}$ could be obtained from the above QSAR model (Eq.(1)). As shown in FIG. 4, both the values of the training and test sets are located around the diagonal of the chart, indicating that the calculated values obtained from the QSAR model are very close to the experimental data. This model has appropriate reliability and good predictive capability.

Application of predictive toxicology model permits us to estimate the potential toxicity of ILs with different sub-structures. In recent years, considerable models have been developed based on the toxicity data of different test organisms and have provided valuable information on the structural features that are important for the toxicity of ILs [15, 17, 18, 35]. Toxicity assays in *C. elegans* are fast and inexpensive, and previous studies have shown that assay results in *C. elegans* could be successfully used in predicting chemical activities in mammals [41, 42]. This study used the nematode *C. elegans* as an *in vivo* animal model for toxicity assay and a QSAR model was constructed based on four molecular descriptors selected by GFA algorithm. The results in our work suggest that rigorous control of different assembling from various cation and anion species is demanded to maintain the IL products classified as green solvents. For instance, the adoption of the anions with the characteristics of facile hydrolysis and steric effect should be minimized in the practical applications. The possible reason is that ILs with weak interionic attraction between cation and anion might result in higher toxicity. It is also important to pay attention to the possible ripple effect from the toxicological interactions of ILs with other environmental pollutants [43]. Precautions should be taken in all the fields of applications during handling the ILs for their potential health threat over flora and fauna, especially for modulating effect at a genetic level [44]. Our results may provide useful information for predicting environmental and human health toxicity of existing and potential ILs.

## IV. CONCLUSION

In this work, a QSAR model was successfully developed to predict the toxicity of ILs on *C. elegans*. On the basis of the toxicity data of ILs covering different cation alkyl chain length and diverse anions, four molecular descriptors were selected by GFA method. The descriptors in this model reflect the alkyl chain of the cation, heat of formation, and the strength and orientation behavior of cation and anion (or the surface tension of ILs). The external and internal validations for this model by the test set demonstrate that the predicted toxicity is consistent with the experimental data. The results prove that this QSAR model has the reliable ability to predict the toxicity of ILs on *C. elegans*.

## V. ACKNOWLEDGMENTS

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