Toxicity Study for Halogenated Aromatics to Mixed Bacteria in River Water

G. H. Lu*a, Y. M. Lib and J. C. Liua

Abstract

The bacterial growth inhibition test was used to determine toxicity of the halogenated aromatics on the mixed bacteria from the Yangtze River. The concentration values causing 50% reduction of bacteria growth for 24 hr (IC50) and the maximum tolerance concentration for 7 d (MATC) were gained according to the concentration-response curves. An obvious linear relationship was found between the two toxicity descriptors. The following quantitative structure-activity relationships were developed by using the valence molecular connectivity index method: -lg IC50 = 0.8266 Xp - 0.2363 Xv + 3.811 (R² = 0.833) and -lgMATC = 1.2016 Xp - 0.3783 Xv + 4.614 (R² = 0.900). The two equations were found to fit well. It suggested that the models derived from the molecular connectivity index could be used successfully to predict the toxicity of halogenated aromatic compounds.

Keywords: Halogenated aromatics; acute toxicity; chronic toxicity; molecular connectivity index; QSAR

1. Introduction

The Yangtze River Delta is one of the most densely urbanized areas in China, with a series of big, medium and small cities and towns along the river. In recent years, water pollution in the lower reaches of the Yangtze River has become more serious due to the release of industrial and agricultural pollutants.

Halogenated aromatic compounds are widely used as solvents, herbicides, antiseptics, and pesticides, and have a high environmental persistence. They have been reported to be present in the water and/or
sediment of the Yangtze River, but the concentration of these compounds is still considered to be relatively low[1,2].

Information on the extent of organic pollutant toxicity is important for risk assessment of chemicals in the environment and for regulating manufacture and use. Quantitative structure-activity relationships (QSARs) play an important role in ecological risk assessment of organic chemicals[3]. In view of this, we have determined the acute toxicity and the chronic toxicity of partial halogenated aromatics to mixed bacteria from the Yangtze River, and developed quantitative structure-activity relationship (QSAR) models.

2. Materials and Methods

The bacterial growth inhibition test was used to determine the acute and chronic toxicity of the 17 halogenated aromatics[4]. Chlorinated chemicals were purchased from Shanghai Chemical Reagent Co., China, with chemical purity more than 95%. Chemicals were not re-purified prior to use. Water samples were gathered from the Nanjing section in the Yangtze River (Jiangsu Province, China). The samples were obtained at a depth of 0.5 m and 50 m away from the bank. There were no large industry enterprises or new pollutant sources in the vicinity of this section of the river. The pollutants from the upper reaches of the river have been admixed equably, and the concentration of most halogenated aromatic hydrocarbons is at the ng/L level[1]. The water samples were stored at 4 °C when not being used. Bacterial counts were determined by standard plate count techniques and was determined to be 4.6×10⁵-6.1×10⁷ CFU/ml.

The culture was maintained in liquid medium containing beef extract, 3 g; peptone, 10 g; NaCl, 5 g; distilled water, 1 L. The pH of the culture medium was adjusted to 7.2-7.4 and the medium was sterilized for 20 min at 121°C. For each compound studied, 5-7 concentrations were tested with a logarithmic difference of 0.2 mol/L for the acute toxicity test and 8-10 concentrations were tested with a logarithmic difference of 0.05-0.1 mol/L for the chronic toxicity test. A stock solution of test chemical was prepared in ethanol for each test concentration to be investigated. 1 ml of test chemical stock solution and 1 ml of river water were added to 50 ml of culture medium in 250 ml conical flasks. 1 ml of 95% ethanol solution and 1 ml of sterilized river water added to 50 ml of culture medium was used as a blank control, and 1 ml of river water and 1 ml of 95% ethanol solution added to 50 ml of culture medium was used as a seed control. All flasks were capped to avoid volatility loss. For each concentration and control, experiments were performed in triplicate. All samples were incubated for 24 hr for the acute toxicity and 7 d for the chronic toxicity at 20±1°C, respectively. The turbidities were measured using a spectrophotometer (UV-1201) at 530 nm against a blank control.

The six-order valence molecular path/cluster index \(^6\chi_{pcv}\) and the three-order valence molecular path index \(^3\chi_{pv}\) were calculated based on the concept of the molecular connectivity index method[5] The multiple regression analysis procedure of SPSS statistical package (ver. 11.0, SPSS Company, Chicago, IL, USA) was employed for QSAR analysis. The toxic potency, -\(\text{lg}IC_{50}\) and -\(\text{lgMATC}\) were employed as the dependent variables, respectively, and \(^6\chi_{pcv}\) and \(^3\chi_{pv}\) as the independent variables. Model quality was characterized by the number of observations (\(n\)), the square of correlation coefficient (\(R^2\)); the adjusted square of correlation coefficient (\(R^2_{adj}\)), the standard error (\(S\)); the mean square ratio (\(F\)), the significance level (\(Pr\)).

3. Results and Discussion

The concentration-response curves of the logarithm of the toxicant concentration against the absorbance rate for 1,4-dichlorobenzene (1,4-DCB) are shown in Figure 1-2. Logarithms of the inverse median
inhibition concentration after 24 hr exposure, expressed as -lgIC$_{50}$ (mol/L), were gained through one variable linear regression analysis of the negative logarithm of compound concentrations and the inhibition rates as the relative toxic potency. The no observed effect concentration (NOEC, mol/L) and the lowest observed effect concentration (LOEC, mol/L) values were calculated using the concentration-response curves for 7 d. The chronic toxicity of the chemicals was expressed as the geometric mean of NOEC and LOEC, i.e. the maximum tolerance concentration (MATC). The toxicity data are listed in Table 1.

The experimental results in Table 1 show that the -lgIC$_{50}$ values ranged from 4.36 for 1,2,4-trichlorobenzene to 3.54 for 2-chloroaniline, whereas the -lgMATC values ranged from 5.25 for 1,2,4-trichlorobenzene to 4.31 for chlorobenzene. The toxicity of halogenated benzenes to the river bacteria is mainly related to the kinds, amount and relative position of the groups in benzene ring. When comparing the acute toxicity values of 17 compounds to mixed bacteria with those chronic toxicity data, the two toxicity descriptors gave consistent results for most chemicals studied. Their correlation equation is -lgMATC=1.178(-lgIC$_{50}$)+0.080, and the correlation relationship is 0.903.

It is well known that non-specific toxicity of chemicals can be described by two kinds of actions: non-polar narcosis (type I narcosis) and polar narcosis (type II narcosis). Non-polar narcotic chemicals are considered baseline toxicants. Their toxicity is proportional to their concentrations at the site of action and is caused solely by membrane perturbation[6,7]. Polar narcotic chemicals, typified by most phenols and anilines, exhibit toxic potency higher than that estimated by their hydrophobicity due to the existence of polar substituents in the molecules[8]. Ren et al. observed the toxicity of the twenty narcotic compounds (including eleven non-polar narcotics and nine polar narcotics) to the activated sludge bacteria, and found the toxicity of the polar narcotics was higher than the corresponding baseline toxicity. They developed QSARs by using the logarithm of each n-octanol/water partition coefficient (lgP) for non-polar narcotics and polar narcotics, respectively. The quality of model fit and prediction of the two equations was similar with comparable $R^2$ and RMSE (root mean square error) values[9]. Lu et al. found that the toxicity of anilines and phenols to the carp (Cyprinus carpio) could be modeled well by lgP alone[10].

There is no an obvious correlation between lgP and toxicity for all of 17 compounds in this study. For 7 polar narcotic chemicals, halogenated anilines and phenols, the lgP-dependent model explains only 70.9% of variance. However, a successful lgP-dependent QSAR was obtained for non-polar
toxicity as follows:
\[ \log_{10}\frac{1}{IC_{50}} = 0.565(0.198)\log P + 2.000(0.779) \]
\( n = 10, \quad R^2 = 0.887, \quad R^2_{adj} = 0.873, \quad S = 0.070, \quad F = 62.6, \quad Pr > F = 0.000 \) (1)
where the numbers in parentheses are the standard errors on the coefficients. It is suggested that alkyl halogenated benzenes formed a non-polar narcotic group based on their baseline toxicity to river bacteria.

There is an extensive literature describing QSARs between toxicity data and the molecular connectivity indices. Zhang et al. developed QSAR models for the toxicity data of chlorinated aromatic compounds to algae using the molecular connectivity index \( 0X_p \) and \( \Delta^0X \). The authors thought that the toxicity of chemicals studied appeared to be positively correlated with molecular volume, and to be negatively correlated with molecular polarity[11]. Liu et al. investigated the toxicity of chlorinated phenols to \( \textit{Vibrio fischeri} \) using one-order valence molecular connectivity index \( 1X_v \) and the average polarizability as the structural parameters, and concluded the toxicity related to molecular volume[12]. In addition, \( 1X \) was also found to be positive correlation with the toxicity of chlorophenols on L929 cells[13]. Jing et al. calculated the second order valence molecular connectivity index \( 2X_v \) and found the increase of \( 2X_v \) could lead to the toxicity enhancement of substituted aromatic compounds to \( \textit{Photobacterium Phosphoreum} \)[14].

The following QSAR models based on the valence molecular connectivity index \( 6X_{pc} \) and \( 3X^2_p \) were obtained from multivariable regression analyses:

\[
-\log_{10}\frac{1}{IC_{50}} = 0.808(0.210)6X_{pc}+0.262(0.151)X^2_p+3.875(0.132)
\]
\( n=17, \quad R^2 = 0.674, \quad R^2_{adj} = 0.628, \quad S = 0.113, \quad F = 14.5, \quad Pr > F = 0.000 \) (2)

\[
-\log_{10}\text{MATC} = 1.201(0.151)6X_{pc}-0.378(0.109)X^2_p+4.614(0.095)
\]
\( n=17, \quad R^2 = 0.900, \quad R^2_{adj} = 0.886, \quad S = 0.082, \quad F = 63.0, \quad Pr > F = 0.000 \) (3)

Eq. (3) explains most of the variance (90%), with maximum \( F \) values (63) and minimum standard error.

### Table 1. The molecular connectivity indices and toxicity data

<table>
<thead>
<tr>
<th>Compounds</th>
<th>( \log P )</th>
<th>( 6X_{pc} )</th>
<th>( 3X^2_p )</th>
<th>( -\log_{10}\frac{1}{IC_{50}} )</th>
<th>( -\log_{10}\text{MATC} )</th>
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<tbody>
<tr>
<td>Chlorobenzene</td>
<td>2.84</td>
<td>0.154</td>
<td>1.008</td>
<td>3.65</td>
<td>3.70</td>
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<td>1,2-Dichlorobenzene</td>
<td>3.43</td>
<td>0.546</td>
<td>1.665</td>
<td>4.01</td>
<td>3.87</td>
</tr>
<tr>
<td>1,3-Dichlorobenzene</td>
<td>3.53</td>
<td>0.477</td>
<td>1.300</td>
<td>3.96</td>
<td>3.90</td>
</tr>
<tr>
<td>1,4-Dichlorobenzene</td>
<td>3.44</td>
<td>0.546</td>
<td>1.354</td>
<td>3.87</td>
<td>3.94</td>
</tr>
<tr>
<td>2-Chlorotoluene</td>
<td>3.42</td>
<td>0.477</td>
<td>1.540</td>
<td>3.82</td>
<td>3.84</td>
</tr>
<tr>
<td>4-Chlorotoluene</td>
<td>3.33</td>
<td>0.477</td>
<td>1.286</td>
<td>3.85</td>
<td>3.90</td>
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<tr>
<td>1,2,4-Trichlorobenzene</td>
<td>4.02</td>
<td>1.166</td>
<td>1.96</td>
<td>4.32</td>
<td>4.31</td>
</tr>
<tr>
<td>Bromobenzene</td>
<td>2.99</td>
<td>0.255</td>
<td>1.269</td>
<td>3.73</td>
<td>3.72</td>
</tr>
<tr>
<td>1,2-Dibromobenzene</td>
<td>3.64</td>
<td>1.199</td>
<td>2.772</td>
<td>4.13</td>
<td>4.15</td>
</tr>
<tr>
<td>4-Bromotoluene</td>
<td>3.42</td>
<td>0.713</td>
<td>1.546</td>
<td>3.89</td>
<td>4.03</td>
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<tr>
<td>2-Chloroaniline</td>
<td>1.90</td>
<td>0.332</td>
<td>1.281</td>
<td>3.68</td>
<td>3.78</td>
</tr>
<tr>
<td>3-Chloroaniline</td>
<td>1.88</td>
<td>0.299</td>
<td>1.106</td>
<td>3.83</td>
<td>3.80</td>
</tr>
<tr>
<td>4-Chloroaniline</td>
<td>1.83</td>
<td>0.332</td>
<td>1.145</td>
<td>3.90</td>
<td>3.81</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>2.15</td>
<td>0.287</td>
<td>1.202</td>
<td>3.80</td>
<td>3.76</td>
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<tr>
<td>4-Chlorophenol</td>
<td>2.39</td>
<td>0.287</td>
<td>1.102</td>
<td>4.11</td>
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<tr>
<td>2,4-Dichlorophenol</td>
<td>3.06</td>
<td>0.809</td>
<td>1.497</td>
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<td>4.13</td>
</tr>
<tr>
<td>4-Bromoaniline</td>
<td>1.97</td>
<td>0.505</td>
<td>1.405</td>
<td>3.88</td>
<td>3.90</td>
</tr>
</tbody>
</table>

*a* \( \log P \) was obtained from ClogP for Window software (ver. 3.55, Biabyte Company, Claremont, CA, USA).

*b* The observed \( -\log_{10}\frac{1}{IC_{50}} \).

*c* The predicted \( -\log_{10}\frac{1}{IC_{50}} \) by Eq.(4).

*d* The observed \( -\log_{10}\text{MATC} \).

*e* The predicted \( -\log_{10}\text{MATC} \) by Eq.(3).
of estimate (0.082) and with neither statistical nor obvious visual outliers observed.

However, from a predictive standpoint Eq. (2) was not satisfactory. When residual errors were studied for Eq. (2), one significant outlier could be observed. 4-chlorophenol was more toxic than predicted by Eq. (2) and its isomer. After removal of 4-chlorophenol from the sample set, an improving QSAR model was obtained:

\[-\lg C_{\text{IC50}} = 0.826(0.150)6X_{pc}^v - 0.236(0.108)3X_{pv}^v + 3.811(0.096)\]

\(n=16, R^2=0.833, R^2_{\text{adj}}=0.807, S=0.081, F=32.4, Pr>F=0.000\)

Eq. (3) and (4) were used to predict the toxicity, and the predicted values are presented in Table 1. A comparison of the predicted values from the model (Eq.3 and 4) with the observed toxicity shows that they were very close. The six-order valence molecular path/cluster index \(6X_{pc}^v\) appears to be positively correlated with the volume of a molecule and can reflect the information on benzene ring and substituents, the steric configuration of substituents and hetero atom groups. The three-order valence molecular path index can not only describe the length of molecular chain but also distinguish isomers. Besides, \(3X_{pv}^v\) contain more information of steric structure and can reflect the flexibility and folding measure of a molecule[15]. The compounds studied in this paper are narcotic chemicals. In which, halogenated benzenes and alkyl halogenated benzenes belong to non-polar narcotic compounds and halogenated phenols and anilines belong to polar narcotic compounds. The toxicity of the halogenated benzene compounds to the river bacteria is mainly related to the molecular volume and spatial shape.

4. Conclusion

The toxicity of 17 halogenated aromatics to mixed bacteria from the Yangtze River was determined by the bacterial growth inhibition test. The toxicity of compounds studied to the river bacteria is mainly related to the amount of halogen substituents in benzene ring. The median inhibition concentration for 24 hr and the maximum tolerance concentration for 7 d were obtained and an obvious linear relationship was found between the two toxicity endpoints. Two satisfying QSAR models were developed for \(-\lg IC_{50}\) and \(-\lg MATC\) using the valence molecular connectivity index \(6X_{pc}^v\) and \(3X_{pv}^v\) as structural descriptors. The obtained models can be used successfully to predict toxicity not only for non-polar halogenated benzenes but also for polar halogenated anilines and phenols.

Acknowledgements

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References


