QSAR study on the toxicity of benzene and naphalene derivatives to Bacillus subtilis subsp. subtilis

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Introduction

To predict the toxicity of organic compound to living things, n-octanol-water partition coefficients (log Pow) are used chiefly.[1] It is well known that halogenated aromatic compounds like o-chlorophenols are environmental pollutants. In recent years, the quantitative structure-activity relationship (QSAR) model for the toxicity of phenols to living things has been studied. [2-5] Nineteen kinds of chlorophenols (that are precursors of dioxin compounds) and phenol (as the basic compound) were assessed for the ability to affect the cellular growth of *Bacillus subtilis subsp. Subtilis* (NBRC3007). In this study, $1x10^4$ vegetative cells were cultured with and without the chemical substances. After 24hr at 37 ℃, the numbers of vegetative cells were counted using the colony forming unit (CFU) method. In total, 50 benzene and naphtalene derivatives were assessed in the *Bacillus* assay, and comparison of the antibacterial concentrations to the physical properties of the compounds was done. By using the hydrophobicity parameter log Pow, and the lowest unoccupied molecular orbital (LUMO), the quantitative structure-activity relationship model (QSAR) was studied. We found that this method using bacteria was very useful for the toxicity testing of these chemical substances.

Materials and Methods

The classes of chemicals used in this experiment were chlorophenols, methylphenols(cresols), chloromethylphenols (chlorocresols), naphthols, chloronaphthols, chlorobenzenes, and chloronaphthalenes. The basic structures of these chemicals are benzene, phenol, and naphthalene. In total there were 50 chemicals tested. Chemicals for toxicity testing were purchased from Aldric Chemical Co., Milwaukee, Wisconsin, USA; WAKO, Tokyo, Japan; Kantokagaku, Tokyo, Japan; Tokyoukasei, Tokyo, Japan. Chemicals had a purity of 95% or better and were not re-purified prior to use. The toxicity values were from a population growth impairment test using the bactria as *Bacillus subtilis* subsp. *subtilis* (NBRC 3007). In this study, $1x10^4$ vegetative cells were cultured with or without 0.0001% to 2% of the benzene and naphtalene derivatives. After 24hr at 37 ℃, the numbers of vegetative cells were counted using CFU method. The antibacterial concentration was obtained at a constant cell number ($1x10^3$). Toxicity was calculated as log(1/Antibacterial concentration/%). All chemicals and their toxicity are showed in Fig. 1. By using logPow and LUMO the quantitative structure-activity relationship model (QSAR) was studied.[4,6,7,8]

Results and discussion

Chlorophenols, chlorocresols and chloronaphthols showed strong toxicity. Increasing the number of chlorines on the phenol increased the toxicity. Chlorophenols which were substituted in the m-position showed strong toxicity compared to other chlorophenols. Toxicities of those chlorophenols varied with the position and the number of chlorine on the phenol. All chemicals and their toxicity are shown in Table 1.

Table1 The toxicity values to *Bacillus subtilis* subsp. *subtilis* (NBRC 3007).

Compounds	log(1/Antibacterial Concentration%)	Compounds	log(1/Antibacterial Concentration%)
Benzene	1	2,3-Dimethylphenol	1.398
Phenol	1.097	2,4-Dimethylphenol	1.301
o-Chlorophenol	1.301	2,5-Dimethylphenol	1.222
m-Chlorophenol	1.523	2,6-Dimethylphenol	1.097
p-Chlorophenol	1.301	3,4-Dimethylphenol	1.699
2,3-Dichlorophenol	1.602	3,5-Dimethylphenol	1.301
2,4-Dichlorophenol	1.77	4-Chloro-2-methylphenol	1.699
2,5-Dichlorophenol	1.824	4-Chloro-3-methylphenol	2.222
2,6-Dichlorophenol	1.398	2-Chloro-5-methylphenol	1.387
3,4-Dichlorophenol	2.523	5-Chloro-2-methylphenol	1.77
3,5-Dichlorophenol	2.523	1-Naphthol	1.854
2,3,4-Trichlorophenol	2.638	2-Naphthol	1.678
2,3,5-Trichlorophenol	3.097	4-Chloro-1-naphthol	2.951
2,3,6-Trichlorophenol	2.222	2,4-Dichloro-1-naphthol	2.678

3,4,5-Trichlorophenol	3.398	Naphthalene	0.602
2,4,5-Trichlorophenol	2.046	1-Chloronaphthalene	0.959
2,4,6-Trichlorophenol	1.745	2-Chloronaphthalene	0.301
2,3,4,5-Tetrachlorophenol	3.347	Chlorobenzene	0.398
2,3,4,6-Tetrachlorophenol	2.77	o-Dichlorobenzene	0.699
2,3,5,6-Tetrachlorophenol	1.699	m-Dichlorobenzene	0.699
Pentachlorophenol	3.155	p-Dichlorobenzene	0.155
o-methylphenol	1.097	1,2,3-Trichlorobenzene	0.222
m-methylphenol	1.097	1,2,4-Trichlorobenzene	0.398
p-methylphenol	0.699	1,3,5-Trichlorobenzene	0.523

Chlorobenzenes and chloronaphtalenes were weaker in toxicity than chlorophenols. Toxicities of 39 kinds of phenpls, naphthols, chlorophenols, chloronaphthols and chloromethylphenols were compered with logPow. Antibacterial concentrations of these compounds were strongly correlated with the partition coefficients of n-Octanol/water(Pow).

$F(x) = 7.03E\text{-}1*x + \text{-}2.84E\text{-}1 \qquad R^2 = 7.70E\text{-}1$ (1)

Toxicities of 19 kinds of chlorophenols were compered with LUMO. Antibacterial concentrations of these compounds were strongly correlated to the LUMO

$F(x) = \text{-}3.00E\text{-}1*x + 2.46E\text{-}1 \qquad R^2 = 4.55E\text{-}1$ (2)

Conclusions

Chlorophenols, chlorocresols and chloronaphthols showed strong toxicity in *Bacillus*. Increasing the number of chlorines on the phenol increased the toxicity. This was especially true for chlorophenols that were substituted in the m-position, which showed strong toxicity compared to other chlorophenols. Toxicities of the chlorophenols differed depending on the position and number of chlorines on the phenol. Comparison of the toxicities to the physical properties of these compounds was done. All of the chlorophenols, cresols, chlorocresols, naphthols and chloronaphthols have the same –OH substitution. Antibacterial concentrations of these compounds were strongly correlated to the partition coefficients of n-Octanol/water(Pow). The compounds with high Pow values had strong effect on vegetative cells. For the chlorophenols, toxicities were strongly correlated to the energy of the lowest unoccupied molecular orbital (LUMO). It is possible to estimate the toxicities of phenols by the partition coefficients of n-Octanol/water and LUMO.

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