Bull. Environ. Contam. Toxicol. (1997) 58:372–379

Environmental Contamination and Toxicology

Quantitative Structure-Activity Relationship Studies of Selected Heterocyclic Nitrogen Compounds

J. Chen,[1] L. Wang,[1] G. Lu,[2] T. Zhao[3]

[1]Department of Environmental Science and Engineering, Nanjing University, Nanjing 210093, People's Republic of China
[2]Department of Chemistry, Nanjing University, Nanjing 210093, People's Republic of China
[3]Analysis Center of Nanjing University, Nanjing 210093, People's Republic of China

Received: 1 July 1996/Accepted: 2 November 1996

As heterocyclic nitrogen compounds are used more extensively as intermediates in the manufacture of pesticides and herbicides, their toxic effects to aquatic organisms should be studied. However, there has been only limited investigation of their toxicity. Recently, thirteen heterocyclic nitrogen compounds were synthesized in our laboratory, and their acute toxicity to *Daphnia magna Straus* were determined in a previous study (Chen et al. 1996a).

However, it is impossible to comprehensively test all existing chemicals to species of concern because of the large expenditures of money and time. One technique for the efficient development of toxicity data is Quantitative Structure-Activity Relationships (QSARs). QSARs correlate and predict toxicity of chemicals from their physico-chemical descriptors (Blum and Speece 1990). In the field of aquatic toxicology, many previous studies have found very successful QSARs based on logarithm of 1-octanol/water partition coefficient (Blum and Speece 1990; Könemann 1981; Schultz et al. 1990, 1994). In addition, the descriptors derived from quantum chemical computation have shown obvious advantages: they are not restricted to closely related compounds, can be easily obtained, and describe clearly defined molecular properties. Therefore, there are quite a few more examples of the use of molecular orbital generated descriptors, such as studies of Lewis (1989), Nevalainen et al. (1994), Mekenyan et al. (1994), and Xu et al. (1994). For these reasons, we have attempted to obtain QSARs of these heterocyclic nitrogen compounds based on the 1-octanol/water partition coefficient *(logKow)* and quantum chemical descriptors.

MATERIALS AND METHODS

The structures and 24h acute toxicity for the heterocyclic nitrogen compounds are reproduced in Fig. 1 and Table 1, respectively, to aid discussion. The toxicity listed in Table 1 was expressed as negative logarithm of EC_{50}, where EC_{50} is

Correspondence to: J. Chen

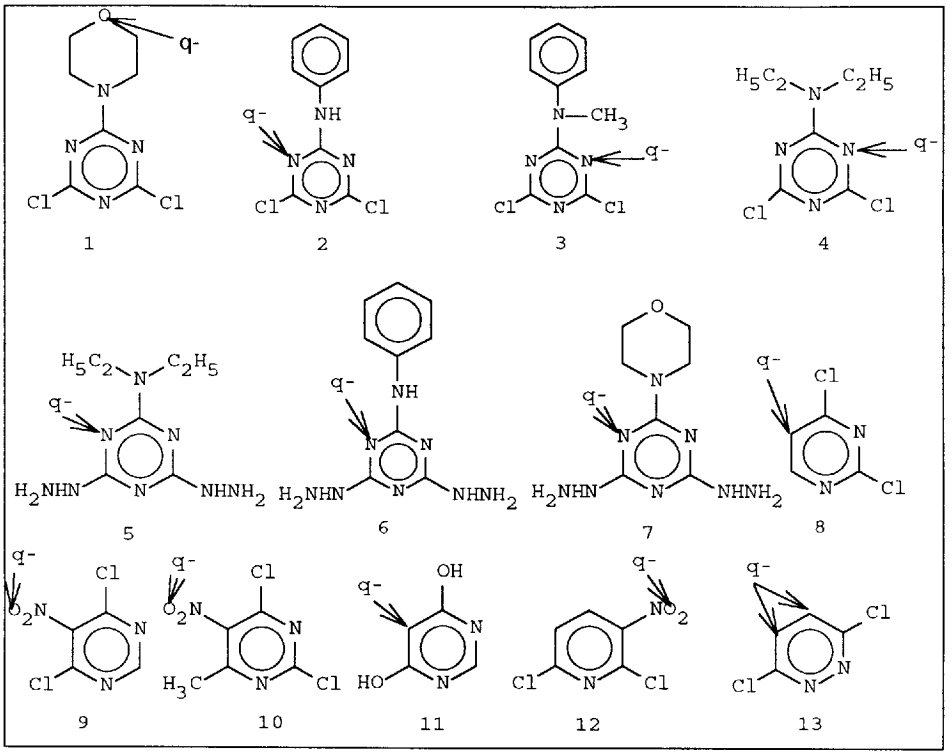

Figure 1. Structures of the Heterocyclic Nitrogen Compounds

Table 1. Toxicity and descriptors for the heterocyclic nitrogen compounds*

NO.	$-logEC_{50}$ (mol/L)			Descriptors							
	exptl.	eq.(2)	diff.	*logKow*	α	μ	E_{homo}	E_{lumo}	qH^+	q^-	q^+
1	4.83	4.33	0.50	1.25	109.848	1.004	9.707	0.692	0.138	0.271	0.214
2	4.74	5.05	-0.31	2.32	128.003	1.207	9.356	0.748	0.139	0.250	0.254
3	5.20	5.12	0.08	2.43	136.530	1.380	9.320	0.639	0.150	0.247	0.207
4	4.89	4.96	-0.07	2.17	106.239	1.541	9.622	0.548	0.114	0.254	0.213
5	4.36	4.73	-0.37	1.22	122.144	1.175	9.341	0.022	0.230	0.330	0.239
6	4.32	4.50	-0.18	1.07	143.816	0.969	9.183	0.256	0.244	0.314	0.244
7	4.22	4.21	0.01	0.62	125.707	0.764	9.487	0.131	0.234	0.325	0.234
8	3.97	3.96	0.01	1.16	58.954	1.044	10.511	0.850	0.179	0.229	0.179
9	4.13	4.02	0.11	0.45	74.821	1.122	11.508	1.758	0.218	0.318	0.586
10	3.49	3.59	-0.10	-0.16	85.881	0.849	11.167	1.717	0.127	0.327	0.583
11	3.89	3.86	0.03	0.10	52.505	0.847	10.303	0.341	0.248	0.335	0.574
12	5.63	5.31	0.32	1.96	79.664	1.557	10.677	1.505	0.176	0.327	0.248
13	2.97	3.00	-0.03	0.93	59.382	1.545	10.242	0.996	0.169	0.120	0.168

* exptl. = experimental toxicity, eq.(2) = toxicity predicted by equation(2), diff.= difference between the experimental toxicity and predicted toxicity

concentration values in unit of mol/L causing a 50% immobilization after 24h exposure.

The *logKow* of these compounds was determined by shake-flask method in the previous study (Chen et al. 1996a).

The molecular modeling package ALCHEMY II *(Tripos Associates, Inc. A molecular modeling system for the IBM PC, 1988*) was used to construct and view all molecular structures. Internal coordinates were used to write all molecular structures. Molecular geometries were optimized and quantum chemical descriptors were calculated using the AMl algorithm (Dewar et al. 1985) contained in the up-to-date version (Ver. 6.00) of the MOPAC program package *(J. J. P. Stewart, MOPAC6 Manual, Frank J. Seiler Research Laboratory, U. S. Air Force Academy, Co 80840, 1990).* The AMl algorithm was selected because it is a much more recent and common semi-empirical method, gives good estimates of molecular energies and the computational time is much shorter than needed by *ab initio* methods. In addition, AM1 algorithm is more appropriate for dealing with intermolecular interactions such as hydrogen bonds than other hamiltonians like MINDO/3, MNDO, etc. (Dewar et al. 1985). The MOPAC was run with the following keywords: AMl PRECISE, ESP, DIPOLE, POLAR. The keyword ESP (electrostatic potential calculation) was used to obtain ESP derived net atomic charges. All calculations were run on a 80586/75MHz computer equipped with 16 megabytes of internal memory and supported by Disk Operation System (DOS). The descriptors computed by the MOPAC were listed in Table 1. Units of the energy, charge, dipole and polarizability were electron volts (eV), atomic charge units (a.c.u.) and atomic units (a.u.) respectively. The molecular orbital energy and net atomic charge values listed in Table 1 are absolute values.

STATGRAPHICS (Ver. 4.0) software *(STSC, Inc. and Statistical Graphics Corporation, Serial Number: 3017435, 1985)* was used to perform regression analysis. Model adequacy was measured as the square of correlation coefficient (R^2), the standard error of estimates (SE), the F value for analysis of variance (F), and the significance level (p). The reported R^2 values were adjusted for degree of freedom (d.f.).

RESULTS AND DISCUSSION

Veith et al (1983) noted that nonreactive toxicity mechanism is most common for industrial organic compounds. Nonreactive theory hypothesizes that the toxicity is directly related to the quantity of toxicants acting upon the cell. However, the concrete toxicity mechanism and the receptor sites binding to the toxicant remain unclear. According to the current acceptable “target theory”, biological response is related to the transport of chemicals from water phase to biophase alternatively (The *logKow* can be used to describe this process) and the subsequent interaction

between the chemical and the "target molecules" (Hansch and Fujita, 1964). Many toxicants react reversibly with "target molecules", i. e.,

$$T\ (\text{toxicant}) + R\ (\text{"target molecule" in the receptor}) \rightleftharpoons T\text{-}R, \quad \text{and} \quad K = \frac{[T-R]}{[T][R]}$$

Where *T-R* is "target molecules" binding by organic toxicants, *K* is equilibrium constant between organic toxicants and "target molecules" in organism cells.

Basing on the "target theory" and aquatic toxicology, Zhao et al (1993) deduced a theoretical relationship between structure and toxicity of organic chemicals. The relationship was:

$$-\log Toxicity = a\log Kow + \log K + c \qquad (2)$$

Where *a* is a coefficient, *c* is a constant. In essence, *logK* reflects the stereo-electronic interaction between organic toxicants and the "target molecules". Therefore, the term *logK* can be described by electronic and stereo descriptors of toxicant molecules (Zhao et al. 1993). In his QSAR studies for substituted benzenes, Zhao *(Ph. D. Dissertation, Nanjing University, 1993)* once used a type of molecular connectivity index, $^1\chi^v$, to supersede the *logK*. A common criticism of the molecular connectivity indices is that the indices do not refer to any specific known physical qualities. Therefore, in present study, we try to use descriptors derived from quantum chemistry to replace the *logK*.

The interactions between "target molecules" and organic chemicals include both hydrophobic interactions (which increase with increasing molecular size) and non-hydrophobic interactions. The linear solvation energy relationship (LSER) developed by Kamlet and coworkers (Kamlet et al. 1986, 1987) is a general approach to describe these interactions between solutes and solvents. Recently, Wilson, Famini and coworkers (1991, 1992) developed a theoretical set of LSER (TLSER) descriptors that are determined solely from computation. In our previous study (Chen et al. 1996b), basing on the LSER and TLSER, we deduced a modified TLSER (MTLSER) model. In the MTLSER model, the average molecular polarizability (α) was used as a measure of hydrophobic interactions, the dipole moment (μ) was used as a measure of dipole-dipole and dipole-induced dipole interactions between solutes and solvents. In order to measure the hydrogen bonding interactions, the energy of highest occupied molecular orbital (E_{homo}) together with the most negative net atomic charges on an atom (q^-) were selected as measures of molecular ability to accept a proton, the energy of lowest unoccupied molecular orbital (E_{lumo}) together with the most positive net atomic charge on a hydrogen atom (qH^+) were selected as measures of molecular ability to donate a proton. For these reasons, in present study, the polarizability (α), dipole moment (μ), E_{homo}, q^-, E_{lumo}, qH^+ were selected as descriptors for these heterocyclic nitrogen compounds. In addition, as the most positive net

atomic charge on an atom *(q+)* in the molecules may contribute to the interactions between toxicants and "target molecules" too, q^+ was selected as a descriptor. All the descriptors are listed in Table 1.

Forward stepwise regression analysis of $-logEC_{50}$ versus *logKow* and the quantum chemical descriptors for all the compounds under study resulted in Table 2. Table showed that two descriptors, *logKow* and q^-, were selected into the QSAR model. From Table 2, we obtained the following QSAR:

$$-logEC_{50} = 1.390 + 0.8092logKow + 7.133q^- \quad (2)$$

n=13, R^2 = 0.872, SE=0.255, F=41.989, p=0.0000

Table 2. Model fitting results for toxicity of the heterocyclic nitrogen compounds

Independent variable	coefficient	SE	t-value	sig. level(p)
Constant	1.390	0.411	3.38	0.0070
LogKow	0.8092	0.0934	8.67	0.0000
q^-	7.133	1.264	5.64	0.0002

A regression equation is of no relevance when the explanatory variables applied were mutually interrelated by simple or multiple correlations. A correlation analysis between *logKow* and q^- for these compounds resulted in a correlation coefficient of -0.327, which showed that in eq. (2) no intercorrelations exist.

The F and p values of eq.(2) show the correlation is significant. Eq.(2) has small SE, and the experimental toxicity is close to the predicted toxicity (Table 1 and Fig. 2), so the equation can be used to predict toxicity of similar series of compounds to *Daphnia magna Straus.*

The importance of the descriptors in eq.(2) is indicated by the sign and magnitude of the t statistics and p values in Table 2. The significant levels listed in Table 2 show that the two variables in eq.(2) are significant ($p \leq 0.0002$). Examination of eq.(2) and Table 2 leads to the following observations: (a) The *logKow is* a most statistically significant term influencing toxicity (p=0.0000). The greater the *logKow,* the greater is the toxicity. This is reasonable since *logKow* models the relative partitioning between the aqueous phase and the more nonpolar lipid-like biophase and compounds with greater *logKow* tend to be partitioned into the organisms, resulting greater toxicity. (b) An increase in q^- leads to greater toxicity (The atoms corresponding with the q^- were marked with arrows in Figure 1). This implies the hydrogen bonding interactions between the "target molecules" and the marked atoms for these compounds in Figure 1, with the compounds provide electrons and the "target molecules" accept electrons. In detail, for compound number 1, the marked oxygen atoms provide electrons; for compounds number 2 ~ 8, the marked nitrogen atoms provide electrons; for compounds (number 9, 10 and 12)

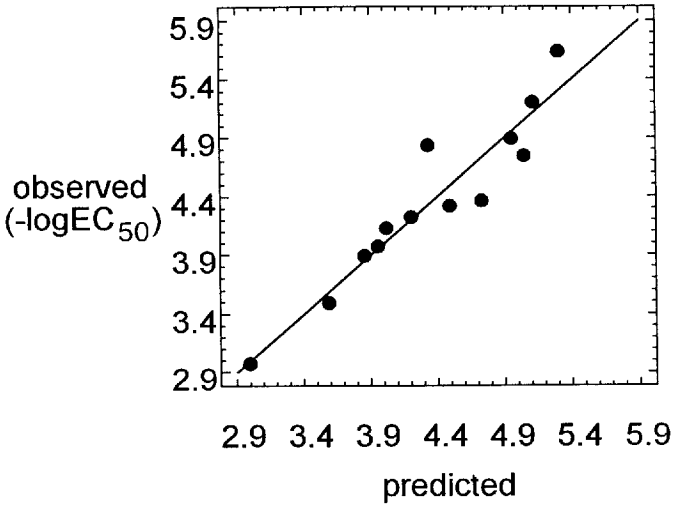

Figure 2. Plot of observed toxicity versus toxicity predicted by eq.(2)

containing nitro group ($-NO_2$), the oxygen atoms in the nitro group provide electrons; and for compounds number 11 and 13, the marked carbon atoms provide electrons.

Octanol/water partition coefficients *(logKow)* have been used successfully in hundreds of QSAR studies involving toxicological properties. The success of these correlations is inextricably linked to the extent to which molecular factors that influence *logKow* also influence the biological response in the same way. The correlation between *logKow* and toxicity for the heterocyclic nitrogen compounds obtained in the previous study (Chen et al. 1996a) was:

$$-logEC_{50} = 3.60 + 0.636 logKow \quad (3)$$

$n = 13$, $R^2 = 0.558$, $SE = 0.493$, $F = 13.9$, $p = 0.0033$

The poor correlation of eq.(3) implies there are other factors influencing toxicity of these compounds. As eq.(2) has revealed the hydrogen bonding interactions between the "target molecules" in cells of *Daphnia magna Straus* and the molecules of the heterocyclic nitrogen compounds, compared with that of eq.(3), the R^2 of eq.(2) was greatly improved, the SE of eq.(2) was greatly reduced.

Nonreactive toxicity mechanism includes two modes of action, narcosis and polar narcosis (Verhaar et al. 1992). Chemicals with narcosis type toxicity are not reactive when considering overall acute toxicity effects, and do not interact with specific receptors in an organism. In the absence of all specific mechanisms of toxicity, a chemical will, within certain boundaries, always be as toxic as its hydrophobicity (its *logKow)* indicates. It is therefore that narcosis type toxicity is

also called “baseline” toxicity or minimum toxicity. Chemicals with polar narcosis toxicity are not reactive when considering overall acute toxicity effects, but are slightly more toxic than baseline toxicity. These chemicals are usually characterized as possessing hydrogen bond donor acidity or hydrogen bond acceptor basicity. As eq.(2) showed that the nitrogen-containing compounds are slightly more toxic than baseline toxicity, and these compounds can interact with “target molecules” by hydrogen bond, the toxicity mechanism of these heterocyclic nitrogen compounds may be polar narcosis.

Our study proved the advantages of the quantum chemical descriptors. (a) They can be easily and precisely obtained by computation instead of experiment, a large amount of expenses and time can be saved; (b) They have clear physicochemical interpretations, and interpretation of the correlation equations can suggest modes of interaction between toxicants and organisms.

Acknowledgments. The project was funded by the National Natural Science Foundation of P. R. China.

REFERENCES

Blum DJW, Speece RE (1990) Determining chemical toxicity to aquatic species. Environ Sci Technol 24:284-293

Chen JW, Liao YY, Zhao YH, Wang LS, Lu GY, Zhao TN(1996a) Quantitative structure- activity relationships and mixture toxicity studies of heterocyclic nitrogen compounds. Bull Environ Contam Toxicol 57:57:77-83

Chen JW, Feng L, Liao YY, Han SK Wang LS(1996b) Using AM1 hamiltonian in quantitative structure-properties relationship studies of alkyl (l-phenylsulfonyl) cycloalkane-carboxylates. Chemosphere, 33:537 — 546

Dewar MJS, Zoebisch EG, Healy EF, Stewart JJP(1985) AM1 A new general purpose quantum mechanical molecular model. J Am Chem Soc 107: 3902 – 3909

Famini GR, Penski CA, Wilson LY(1992) Using theoretical descriptors in quantitative structure — activity relationships: some physiochemical properties. Journal of Physical Organic Chemistry 5:395–408

Hansch C, Fujita T(1964) ρ - σ - π Analysis. A method for the correlation of biological activity and chemical structure. J Am Chem Soc 86:1616 – 1626

Kamlet MJ, Doherty RM, Abboud JLM, Abraham MH, Taft RW(1986) Solubility– a new look. CHEMTECH 16:566– 576

Kamlet MJ, Doherty RM, Taft RW, Abraham MH, Veith GD, Abraham DJ(1987) Solubility properties in polymers and biological media. 8. An analysis of the factors that influence toxicities of organic nonelectrolytes to the golden orfe fish *(Leuciscus idus melanotus).* Environ Sci Technol 21: 149– 155

Könemann H (1981) Quantitative structure-activity relationships in fish toxicity studies Part 1: relationship for 50 industrial pollutants. Toxicology 19:209-221

Lewis DFV(1989) The calculation of molar polarizabilities by the CNDO/2 method: correlation with the hydrophobic parameter, logP. Journal of Computational Chemistry, 10:145–151

Mekenyan OG, Ankley GT, Veith GD, Call DJ(1994) QSARs for photoinduced toxicity: 1. Acute lethality of polycyclic hydrocarbons to Daphnia magna. Chemosphere 28: 567—582

Nevalainen T, Kolehaminen E (1994) New QSAR models for polyhalogenated aromatics. Environ Toxicol Chem 13: 1699 – 1706

Schultz TW, Wyatt NL, Lin DT(1990) Structure-activity relationships for nonpolar narcotics: A comparison of data from the *Tetrahymena*, *Photobacterium* and *Pimephales* system. Bull Environ Contam Toxicol 44:67-72

Schultz TW, Bryant SE, Lin DT (1994) Structure-toxicity relationships for *Tetrahymena:* aliphatic aldehydes. Bull Environ Contam Toxicol 52:279-285

Veith GD, Call DJ, Brooke LT (1983) Structure-toxicity relationships for the fathead minnow, *Pimephales promelas:* Narcotic industrial chemicals. Canad J Fish Aquatic Sci 40:743 —748

Verhaar HJM, Leeuwen CJV, Hermens JLM (1992) Classifying environmental pollutants. 1: Structure-activity relationships for prediction of aquatic toxicity. Chemosphere 25:471 –491

Wilson LY, Famini GR(1991) Using theoretical descriptors in quantitative-activity relationships: some toxicological indices. J Med Chem 34: 1668— 1674

Xu L, Ball SL, Dixon, Jurs PC(1994) Quantitative structure— activity relationships for toxicity of phenols using regression analysis and computational neural networks. Environ Toxicol Chem 13:841 — 851

Zhao YH, Wang LS, Gao H, Zhang Z(1993) Quantitative structure—activity relationships —Relationship between toxicity of organic chemicals to fish and to *Photobacterium Phosphoreum*. Chemosphere 26:1971—1979