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Quantitative Structure-Activity Relationships and Mixture Toxicity Studies of Heterocyclic Nitrogen Compounds

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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.

The application of Quantitative Structure-Activity Relationships(QSARs) is a valuable part of the toxic effects assessment of chemical substances. Many previous studies have found very successful QSARs based on logarithm of l-octanol/water partition coefficient(logKow) in the field of aquatic toxicology (Blum and Speece 1990; Könemann 1981a; Schultz et al. 1986, 1990, 1994).

However, many toxic effects are actually caused by mixtures of a large number of compounds, a better understanding of the joint action of chemicals is therefore necessary. Könemann(1981 b) introduced a Mixture Toxicity Index(MTI) for comparing quantitative results of mixture toxicity experiments. The MTI was defined as: MTI=1 - logM/logN, where N is the number of chemicals in a mixture, M is the sum of the concentrations that was expressed as equal fractions of the EC_{50} of each component. With this index the author proposed a mixture toxicity scale(Table 1). The use of MTI has following advantages: (a) the mathematical model is simple and easy to use; (b) constant values are obtained for two reference points, no addition and concentration addition, independently of the number of compounds and the ratio between the concentrations. The MTI method has been used successfully in mixture toxicity studies by many researches(Hermens and Leeuwanch 1982, 1984; Köemann 1981b).

It was the purpose of this study: to determine toxicity of 13 heterocyclic nitrogen compounds to daphnia*(Daphnia magna Straus)*, to obtain QSARs of these chemicals based on the measured 1ogKow values and to study the toxicity of mixtures.

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Table 1. Mixture Toxicity Scale after Könemann

MTI	Classification for Toxicity of Mixtures
MTI<0	Antagonism
MTI=0	No addition(independent action)
0<MTI<1	Partial addition
MTI=1	Concentration addition(simple similar action)
MTI>1	Supra addition(potentiation of the toxic action(s) of one or more of the compounds of the mixture)

MATERIALS AND METHODS

Daphnia was reared in our laboratory, and was less than 24 hr old at the start of the experiment.

Thirteen heterocyclic nitrogen compounds(Figure 1) were synthesized by Chemistry Department of Nanjing University. The purity checked by Mass Spectroscope was greater than 99 percent, Other chemicals used in the test were of analytical reagent grade.

Dilution waters for the tests were prepared by reconstituting distilled water based on standard procedures (APHA et al 1985). The composition of the reconstituted water was: $NaHCO_3$:192mg/L, $CaSO_4.2H_20$: 120mg/L, $MgSO_4$:120mg/L, KCl:8.0mg/L, Hardness=l60-180mg$CaCO_3$/L, Alkalinity=110-120mg$CaCO_3$/L, pH=8.5.

The chemicals were dissolved in the reconstituted water with an ultrasonic device. The acute immobilization toxicity tests were conducted for 24 hr, according to APHA-AWWA-WPCF Standard Methods for the Examination of Water and Waste Water(APWH et al. 1985). The photoperiod was 16 hr daylight/8 hr darkness. The light from direct rays of sun was avoided. Exposure concentrations were selected from a logarithmic scale within the test range determined from range-finding tests. Range-finding tests consisted of a control and five chemical concentrations. We exposed 30 organisms (three replicates of 10 organisms each) to selected concentrations of each chemical. When a daphnia swam a distance less than its length in 15s, it was treated as immobile. The results were considered valid if dissolved oxygen level at the end of the test was at least equal to 25% of saturation(2.27mgL of 02 at 20), if percentage of immobilization observed for the controls was zero, and if EC50 of the reference compound(potassium dichromate) ranged between 0.9 and 2.0mg/L. Trimmed Spearman-Kärber method (Hamilton et al. 1977) was used to calculate EC_{50}. Toxicity values listed in Table 2 have been transformed to the negative logarithm of EC_{50}(pEC_{50}).

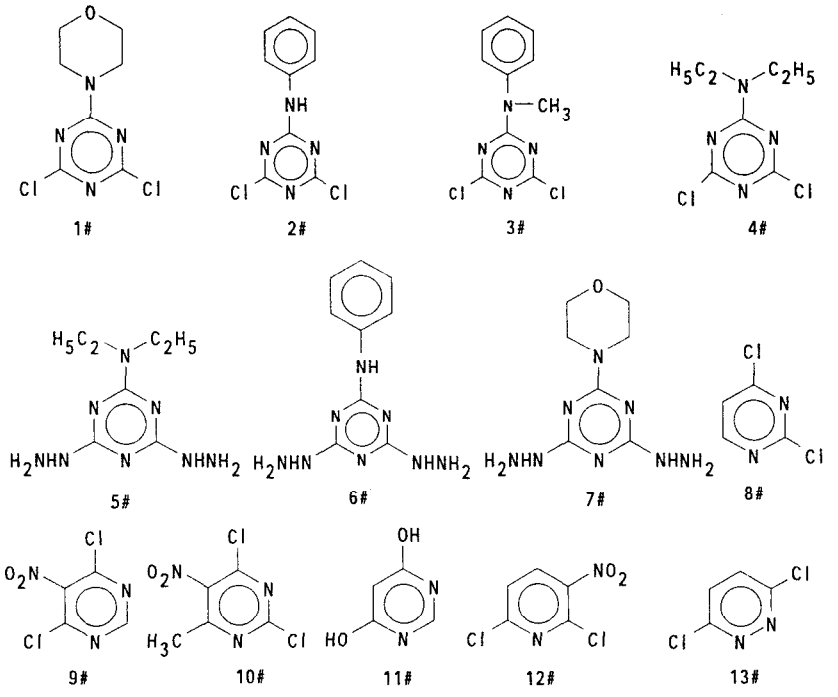

Figure 1. Structure of the Heterocyclic Nitrogen Compounds

All mixtures were prepared in equitoxic concentrations (identical fractions of EC_{50}) based on experimental EC_{50} values. The compositions of mixtures are given in Table 2. The numbers corresponded with that in Figure 1.

The octanol/water partition coefficients were determined by shake-flask method as described by Leo and Hansch(l971) for testing of chemicals at 25°C followed by centrifuging and analysis of chemical in the aqueous phase with a UV-spectrophotometer(Mode1 751 G, Made by Shanghai Analytical Apparatus Plant) against water blank. Each chemical was measured in three replicates. The measured 1ogKow values were listed in Table 2.

Statistical analyses were performed using the STATGRAPHICS software(STSC, Inc., 1987). Model adequacy was measured as square of correlation coefficient(r^2) standard error(F-ratio, and p value.

RESULTS AND DISCUSSION

Regression analysis of pEC_{50} versus 1ogKow for all the chemicals under study resulted in the following QSAR:

Table 2. Experimental Results

QSAR Studies*					Results of the Mixture Toxicity Experiment			
No.	pEC_{50}	eq.(2)	eq.(1)	logKow	No.	Chemicals in the Mixture	N	M	M T I
l#	4.83	4.42	4.39	1.25	1	2# 3# 4#	3	1.87	0.43
2#	4.74	4.98	5.08	2.32	2	1# 2# 3# 4#	4	1.60	0.66
3#	5.20	5.03	5.15	2.43	3	5# 6# 7#	3	1.05	0.96
4#	4.89	4.90	4.98	2.17	4	1# 2# 3# 4# 5# 6# 7#	7	1.35	0.85
5#	4.36	4.40	4.38	1.22	5	9# l0#	2	1.79	0.16
6#	4.32	4.33	4.28	1.07	6	8# 9# 10# ll#	4	1.43	0.74
7#	4.22	4.09	3.99	0.62	7	12# 13#	2	1.56	0.36
8#	3.97	4.37	4.34	1.16	8	9# l0# 12# 13#	4	1.82	0.57
9#	4.13	4.00	3.89	0.45	9	8# 9# l0# 11# 12# 13#	6	1.62	0.73
l0#	3.49	3.69	3.50	-0.16	10	l# 5# 7# 9# l0# 11#	6	1.29	0.86
ll#	3.89	3.82	3.66	0.10	11	1# 5# 7# 9# l0# 11# 12#	7	1.17	0.92
12#	5.63	4.79	4.85	1.96	12	1# 5# 6# 7# 9# l0# ll# 12#	8	1.14	0.94
13#	2.99	4.25	4.19	0.93	13	l# 2# 3# 4# 5# 6# 7# 8# l0# 11# 12# 13#	13	1.05	0.98

*Eq.(2) and eq.(1) represents predicted toxicity(pEC_{50}) by eq.(2) and eq.(l) respectively

$$pEC_{50}=3.60+0.636\log Kow \quad (1)$$

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

Although the correlation relationship of eq.(l) is significant(p=0.0033), it remains less satisfactory because the standard error is relatively great. Chemicals 12# and 13# were two outlier of equation(l), Deletion of the two chemicals and subsequent regression analysis yielded equation(2).

$$pEC_{50}=3.77+0.519\log Kow \quad (2)$$

$n=ll,\ r^2=0.808,\ SE=0.233,\ F=35.8,\ p=0.0002$

The significance and accuracy of equation(2) were greatly improved. As compound 12# is the only pyridine in the batch, the toxicity mechanism of it may be different from the other heterocyclic nitrogen compounds, deletion of the chemical was reasonable. In order to reveal the reasons that make compound 13# be out of the ordinary, quantum chemistry computation by MNDO Hamiltonian contained in the up-to-date version(Ver. 6.00) of the MOPAC program package was performed. The computation results showed that the net atomic charge on the nitrogen of compound 13#, which was -0.051, was much greater than the net charge of nitrogen atoms in the rings of triazines and pyrimidines, which was less than -0.236. Therefore, the derivatives of 1,3,5-triazines and pyrimidines may have charge transfer interactions or hydrogen bond interactions(Wilson and Famini 1991) with

the “target molecules” in the daphnia, and the nitrogen atoms in these chemicals provide electrons, the target molecules accept electrons. Compound 13#, the only substituted pyridazine in the batch, may has no such interactions. For these reasons, we considered that the deletion of compound 13# was reasonable too.

Equations (l)-(2) show the pEC_{50} of the compounds correlates with 1ogKow as the only parameter. The predicted toxicity by eq.(2) and es.(l) was listed in Table 2. The QSARs show the toxicity of this group of chemicals is mainly dependent on the distribution between water phase and bio-phase. However, as the substances under study have diverse complex chemical structure, similar mode of actions of these chemicals can not be concluded.

The toxicities of thirteen equitoxic mixtures were determined. The results of these experiments are given in Table 2. The joint response of the mixtures varies from partial additive to concentration additive. The results are comparable with those of Hermens and Leeuwanch(l982) whose MTI values for five mixtures of eight chemicals with diverse modes of action varied from 0.74 to 0.95 and a MTI value for a mixture of 24 chemicals with diverse mode of action was 0.74. The toxicities of mixtures of chemicals with similar mode of action are expected to be concentration additive for nonreactive organic chemicals with a potency for unspecific depression of the central nervous system. As chemicals of mixture 3 have same parent structure, they may have similar mode of actions, their joint toxicity may be additive.

Our results show, with the increase of the number of compounds in a mixture, the MTI values increased up to the limit of 1. When the number of compounds, with different modes of action, in a mixture increases, the number and diversity of joint actions between different compounds will increase. Hermens and Leeuwanch(1982) suggested that, although there will be no concentration addition based on the specific toxicity, the nonspecific toxicity(baseline toxicity) resulting from hydrophobicity will be additive. In a large mixture of different acting compounds at equitoxic concentrations, the concentrations of the individual members will be so low that their specific toxic effects will not be apparent, but the fractional nonspecific toxicity from hydrophobicity will remain and will be additive. Our results proved the hypothesis.

In conclusion, we reported the toxicity and 1ogKow values of 13 heterocyclic nitrogen compounds, and obtained two QSAR equations based on 1ogKow values in the present study. The QSAR results indicate that the toxicity of this group of chemicals is mainly dependent on the distribution between water-phase and bio-phase. Our mixture toxicity experiments show the joint toxicity of the chemicals varies from partial addition to concentration addition. When the number of

chemicals in a mixture increases, the joint toxicity tends to change from partial addition to concentration addition. The joint toxicity of mixtures of large numbers of chemicals is additive.

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