Journal of Environmental Sciences Vol. 12, No. 1, pp. 86—89, 2000

ISSN 1001—0742
CN11—2725/X

Article ID: 1001-0742(2000)01-0086-04

Quantitative structure-activity relationships for carp kidney ATPase endpoint

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Abstract: The IC_{50} values of 20 nitroaromatics were determined by the activity of ATPase of carp (*Cyprinus carpio*) kidney in vitro, and used to develop the quantitative structure activity relationship (QSAR) with 6 descriptors of $^1X^v$, $\Sigma\sigma^-$, I, ^1Ka, E_{LUMO}, log$P$. A best equation was obtained by multiple regression analysis $-\log IC_{50} = 1.306\Sigma\sigma^- + 0.657I + 0.584E_{LUMO} + 2.852 (r = 0.925)$. $\Sigma\sigma^-$ is the sum of substituent constants. I is the indicator variable. E_{LUMO} is the energy of the lowest unoccupied orbital. Results showed that the $\Sigma\sigma^-$, I and E_{LUMO} were closely correlated with toxicity of nitroaromatics. Some toxicity mechanisms by nitroaromatics are also discussed in this paper.

Key words: nitroaromatic compounds; carp kidney; ATPase; IC_{50}; QSAR
CLC number: O625.61 **Document code:** A

Introduction

Most of nitroaromatics are important environmental pollutants. The carp is the major economic fish in China and is one of the five standard test organisms recommended by OECD. Data reported indicated that the ATPase (odenosine triphosphohydrolase, EC 3.6.1.3) system could be used as a target for the toxic action of a variety of toxicants to different organisms (Boese, 1982; Davis, 1972; Kuhnert, 1976; Li, 1984; Yap, 1971). No data of IC_{50} value (50% inhibitory concentration) of ATPase activity of carp tissues in relation to nitroaromatics are available. Accordingly, we used the ATPase activity of carp kidney as endpoint to measure the inhibition of nitroaromatics in vitro. and calculated the IC_{50} of ATPase of activity carp kidney in relation to nitroaromatics. The nitroaromatics inhibited ATPase activity significantly that resulted in relation of dose-effect. QSAR models were developed with six descriptors. The mechanism of toxic effect on sublethal concentration level are discussed.

1 Materials and methods

1.1 Materials

Some good condition, active, and healthy young carps were sampled from Changchun Fish Farm for tests. They were kept in rectangular glass aquaria in laboratory at a 12 ± 3℃, pH value 7.1—7.6, DO 5.6—6.8 mg/dm^3. The average length and weight were 6.14 ± 0.10 cm and 5.08 ± 0.10g, respectively. The fish had been acclimated in laboratory condition for two weeks after washing with 0.1% (W/V) NaCl solution to avoid from possibility of infection. The death rate was lower than 1% during domestication.

1.2 Methods

The carp kidney was dissected and homogenized in an ice-cold (1—4℃) solution contained 0.25 mol/dm^3 sucrose, 1mmol/dm^3 ethylenediaminetetraacetic acid (EDTA), 0.35 mol/dm^3 mannitol ($C_6H_{14}O_6$), 1 mg/dm^3 bovine serum albumin (BSA), and 0.01 mol/dm^3 Tris-HCl (pH 7.2). The solution was then separated by centrifuging at 700g for 10 min followed by 12500g for

Foundation item: National Natural Science Foundation of China (No. 39570152)

20 min. The sediments were resuspended in an ice-cold solution containing 0.25 mol/dm^3 sucrose, 1 mmol/dm^3 EDTA, 0.35 mol/dm^3 $C_6H_{14}O_6$, and 0.01 mol/dm^3 Tris-HCl (pH 7.2). The fraction (suspension of sediment in the 12500g centrifuging) contained mitochondria and nerve ending particles. Each preparation was appropriately diluted and the samples were kept at −20℃ until the ATPase assay (Ding, 1989; Scope, 1982).

According to LC_{50} values, the sublethal concentrations were calculated in this experiment. For each compound at least seven concentration gradients were designed (including 0% and 100% inhibition concentration to ATPase activity of carp kidney and one control). Acetone (CH_3COCH_3) was used as solvent with contents of 0.05—0.1% V/V.

ATPase and protein determination was developed. IC_{50} values were determined by extrapolating the regression line. STATGRAPHICS program was used for QSAR linear regression analysis.

2 Results

2.1 Toxicity tests

Results obtained from enzyme tests indicated that the ATPase in the kidney to toxicants was more sensitive than that in the gill. A similar pattern of inhibition was also observed by Jampol (Jampol, 1970). We used the ATPase of carp kidney as endpoint in this experiment.

The observed $-\log IC_{50}$ of 20 compounds are listed in Table 1. It shows that the toxicity order of the observed compounds as follows:

DNB (except 1, 3-DNB) > DCNB > DNAn > DNT-BrNB-CNB > NT-NPh (except 3-NPh) > NAn (except 2-NAn) > NB.

2.2 QSAR studies

Based on the data reported (Deneer, 1987a, 1987b), the QSARs were developed using 6 descriptors and the IC_{50} values of the studied compounds. The values of six descriptors of compounds are listed in Table 2. ^1Ka is the molecular shape index obtained from Hall (Hall, 1989). The sum of substituent constant $\Sigma\sigma^-$, a substituent electron effect parameter, is obtained from Deneer (Deneer, 1987); $^1X^v$ is the first order valence molecular connectivity index, "I" is the indicator variable, reflecting the amount and position of the nitro-

Table 1 The actual and calculated toxicity values of the nitroaromatics to the carp kidney ATPase

No.	Name of compounds (abbreviation)	$-\log IC_{50}$, mol/dm^3		
		Act.[a]	Calc.[b]	Res.[c]
1	Nitrobenzene (NB)	3.45	3.85	−0.40
2	4-Nitrotoluene (4-NT)	3.85	3.77	0.08
3	1,2-Dinitrobenzene (1,2-DNB)	5.50	5.57	−0.07
4	1,3-Dinitrobenzene (1,3-DNB)	4.39	4.54	−0.15
5	1,4-Dinitrobenzene (1,4-DNB)	6.39	6.24	0.15
6	2,4-Dinitrotoluene (2,4-DNT)	4.17	4.49	−0.32
7	2,6-Dinitrotoluene (2,6-DNT)	4.24	4.45	−0.21
8	2-Chloronitrobenzene (2-CNB)	4.11	4.10	0.01
9	3-Chloronitrobenzene (3-CNB)	4.14	4.18	−0.04
10	4-Chloronitrobenzene (4-CNB)	4.10	4.09	0.01
11	3,4-Dichloronitrobenzene (3,4-DCNB)	4.65	4.39	0.26
12	2,5-Dichloronitrobenzene (2,5-DCNB)	4.84	4.99	−0.15
13	2,4-Dichloronitrobenzene (2,4-DCNB)	5.70	5.37	0.33
14	2-Nitroaniline (2-NAn)	4.22	4.64	−0.42
15	3-Nitroaniline (3-NAn)	3.63	3.71	−0.08
16	4-Nitroaniline (4-NAn)	3.45	3.96	−0.51
17	2,4-Dinitroaniline (2,4-DNAn)	4.54	4.62	−0.08
18	2-Nitrophenol (2-NPh)	3.88	3.43	0.45
19	3-Nitrophenol (3-NPh)	4.20	3.95	0.25
20	4-Bromonitrobenzene (4-BrNB)	4.20	4.14	0.06
21	2-Nitrotoluene (2-NT)		3.76	
22	3-Nitrotoluene (3-NT)		3.84	
23	2,3-Dinitrotoluene (2,3-DNT)		5.41	
24	2,5-Dinitrotoluene (2,5-DNT)		6.25	
25	3,4-Dinitrotoluene (3,4-DNT)		5.47	
26	3,5-Dinitrotoluene (3,5-DNT)		4.54	
27	1,3,5-Trinitrobenzene (1,3,5-TNB)		6.39	
28	4-Nitrophenol (4-NPh)		3.46	
29	2-Nitroanisole (2-NAnis)		3.65	
30	3-Nitroanisole (3-NAnis)		4.00	
31	4-Nitroanisole (4-NAnis)		3.59	
32	3-Bromonitrobenzene (3-BrNB)		4.48	
33	4-Fluoronitrobenzene (4-FNB)		3.79	

a. Act. are actual $-\log IC_{50}$ values; b: Calc. are calculated values obtained from Equation (1); c: Res. = Act. − Calc.

group, $^1X^v$ and I are obtained from Hall (Hall, 1989), I was 0.5, 1.0 or 3.0 reflecting the presence of mononitro, meta-dinitro or ortho-dinitro, para-dinitro substituents on benzene cycle (Hall, 1989). The energy of the lowest unoccupied orbital, E_{LUMO} was used because it appears in direct proportion to its electron affinity and there is an obvious relationship between E_{LUMO} and oxidation-reduction potential (Lang, 1996). log$P$ is the octanol/water partition coefficient obtained from Deneer (Deneer, 1987).

Table 2 The values of six discriptors of the nitroaromatics

No.	Name of compounds (abbreviation)	log$P$	$^1X^v$	I	^1Ka	$\Sigma\sigma^-$	E_{LUMO}
1	Nitrobenzene (NB)	1.89	2.499	0.5	6.701	0.00	1.146
2	4-Nitrotoluene (4-NT)	2.34	2.910	0.5	7.665	−0.15	1.344
3	1,2-Dinitrobenzene (1,2-DNB)	1.55	3.005	3.0	9.210	1.24	1.502
4	1,3-Dinitrobenzene (1,3-DNB)	1.52	2.999	1.0	9.210	0.71	0.170
5	1,4-Dinitrobenzene (1,4-DNB)	1.46	2.999	3.0	9.210	1.24	0.348
6	2,4-Dinitrotoluene (2,4-DNT)	2.04	3.416	1.0	10.203	0.56	0.425
7	2,6-Dinitrotoluene (2,6-DNT)	2.20	3.422	1.0	10.203	0.56	0.359
8	2-Chloronitrobenzene (2-CNB)	2.26	3.018	0.5	7.971	0.27	1.001
9	3-Chloronitrobenzene (3-CNB)	2.49	3.012	0.5	7.971	0.37	0.885
10	4-Chloronitrobenzene (4-CNB)	2.35	3.012	0.5	7.971	0.27	0.962
11	3,4-Dichloronitrobenzene (3,4-DCNB)	3.29	3.531	0.5	9.245	0.60	0.735
12	2,5-Dichloronitrobenzene (2,5-DCNB)	2.90	3.531	0.5	9.245	1.05	0.742
13	2,4-Dichloronitrobenzene (2,4-DCNB)	2.20	3.482	1.0	10.328	1.39	0.072
14	2-Nitroaniline (2-NAn)	1.85	2.705	0.5	7.650	−0.15	1.390
15	3-Nitroaniline (3-NAn)	1.37	2.699	0.5	7.650	−0.16	1.271
16	4-Nitroaniline (4-NAn)	1.39	2.699	0.5	7.650	−0.15	1.673
17	2,4-Dinitroaniline (2,4-DNAn)	1.84	3.204	1.0	10.212	0.56	0.647
18	2-Nitrophenol (2-NPh)	1.89	2.640	0.5	7.702	−0.37	1.256
19	3-Nitrophenol (3-NPh)	2.00	2.634	0.5	7.702	0.12	1.055
20	4-Bromonitrobenzene (4-BrNB)	2.73	3.408	0.5	8.167	0.23	1.129
21	2-Nitrotoluene (2-NT)	2.30	2.916	0.5	7.665	−0.15	1.333
22	3-Nitrotoluene (3-NT)	2.45	2.910	0.5	7.665	−0.07	1.292
23	2,3-Dinitrotoluene (2,3-DNT)	1.99	3.422	3.0	10.203	1.06	−1.360
24	2,5-Dinitrotoluene (2,5-DNT)	1.98	3.416	3.0	10.203	1.16	−0.148
25	3,4-Dinitrotoluene (3,4-DNT)	1.99	3.416	3.0	10.203	1.09	−1.334
26	3,5-Dinitrotoluene (3,5-DNT)	2.06	3.410	1.0	10.203	0.64	0.335
27	1,3,5-Trinitrobenzene (1,3,5-TNB)	1.18	3.498	3.0	11.753	1.42	−0.531
28	4-Nitrophenol (4-NPh)	2.04	2.634	0.5	7.702	−0.37	1.299
29	2-Nitroanisole (2-NAnis)	1.80	3.028	0.5	8.692	−0.27	1.404
30	3-Nitroanisole (3-NAnis)	2.16	3.022	0.5	8.692	0.12	1.142
31	4-Nitroanisole (4-NAnis)	2.03	3.022	0.5	8.692	−0.27	1.302
32	3-Bromonitrobenzene (3-BrNB)	2.73	3.408	0.5	8.167	0.39	1.346
33	4-Fluoronitrobenzene (4-FNB)	2.01	2.599	0.5	7.522	0.06	0.918

Through multiplicate regression analysis, a best QSAR equation contained three variables was obtained.

$$-\log IC_{50} = 1.306\Sigma\sigma^- + 0.657I + 0.584E_{LUMO} + 2.852, \quad (1)$$

$$n = 20,\ S = 0.283,\ r = 0.925,\ F = 38.53,\ Pr > F = 0.01.$$

The equation is used to estimate the toxicity of 13 compounds to ATPase of carp kidney (Table 1), most calculated toxicity values were closed to the values of LC_{50} for carp (Lang, 1996).

3 Discussion

Based on the results listed above, $\Sigma\sigma^-$ is the most important one of six discriptors of the toxicity of nitroaromatics to the carp kidney ATPase. I and E_{LUMO} are essentially correlated with electron effect or electron affinity of molecular in this QSAR.

Because of high toxicity of compounds with high $\Sigma\sigma^-$, I, E_{LUMO} values, such as DNB, DNT, we suppose that the reaction between these compounds and enzyme or macromolecules in the cells of target organs in fish may be the primary mechanism of lethality (Hall, 1986). For the compounds with lower $\Sigma\sigma^-$, I, E_{LUMO} values, such as, NPh, NAn and NT etc, may contribute little electro effects to the toxicity (Deneer, 1987).

The end point of ATPase is the protein inlaid on membrane. Active center of enzyme is ASP (aspartic acid), whose side chain has a nucleophilic effect on ATP, making it phosphorylated. Hall and Kier pointed out the nitro-group of nitroaromatics has two biochemical reactions in the cells of target organs in fish. The density of the electron cloud of the benzene cycle may be decreased by nitro-group π electrons, or the nitro-group runs away because other groups activated it. We suppose that the $-NO_2$ staying with $-CH_2$ and $-NO_2$ coexisting or respective in nitroaromatics may attract π electro cloud on benzene cycle. The positive part of benzene cycle attracts the carboxygroup of active center of enzyme. This additive reaction inhibited nucleophilic effect. ATPase activity decreases as nitroaromatics concentration added to reaction system increases. The nitroaromatics inhibit ATPase activity significantly and result in relation of dose-effect. It is proposed that the effects of toxicants on ATPase can be used as a sensitive index of sublethal endpoint in ecotoxicological risk assessment.

The data listed in Table 3 show that the correlation of IC_{50} of nitroaromatics to carp kidney ATPase and LC_{50} of these compounds to carp is better than that of fathead minnow (Hall, 1989).

Table 3 Results of linear regression

Regression equation ($y = a + bx$)					
x	y	a	b	r	n
$-\log IC_{50}$	$-\log LC_{50}$(Carp)	0.450	0.810	0.959	20
$-\log IC_{50}$	$-\log LC_{50}$(Fathead minnow)	0.598	0.786	0.919	16

The study on inhibition of ATPase activity and QSAR is able to predict ecological hazard assessment based on mechanism of action from molecular structure.

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(Received for review August 31, 1998. Accepted October 27, 1998)