Bull. Environ. Contam. Toxicol. (1997) 58:123–127

Bulletin of Environmental Contamination and Toxicology

QSAR Study of the Toxicity of Nitrobenzenes to River Bacteria and *Photobacterium phosphoreum*

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Received: 12 December 1995 / Accepted: 30 August 1996

Since nitrobenzenes constitute a class of industrial chemicals that are present in Songhua River and probably in many other industrialized countries as well, it is useful to gain insight into their potential hazard to aquatic organisms. For this reason, it was decided to determine data on the toxicity for bacteria in the Songhua River. Furthermore, the toxicity to *Ph. phosphoreum* was determined in the Microtox assay, in order to further evaluate the usefulness of this assay for hazard assessment.

Quantitative structure-activity relationships(QSARs) have been developed for aromatic nitro compound toxicity to aquatic species (Bailey et al. 1983, Deneer et al. 1989), but no data on the toxicity of nitrobenzenes to environmental bacteria were used.

In this study, the toxicity of various substituted nitrobenzenes to bacteria in Songhua River and to *Ph. phosphoreum* has been investigated, establishing quantitative structure-activity relationships with n-octanol-water partition coefficient (log P), the energy of the lowest unoccupied molecular orbital (E_{LUMO}) and the sum of substituent constant ($\Sigma\sigma^-$).

MATERIALS AND METHODS

The bacterial growth inhibition test (Alsop et al. 1980) was used to determine the 24-hr IC_{50} values of 21 nitrobenzenes to bacteria in the Songhua River. The toxicant under investigation was introduced into a mixture containing buffering agents, nutrients, growth substrates and bacterial seed (taken from the Songhua River) inocula. The mixtures containing toxicant in different concentrations were incubated for 24 hr at 22±2 °C. The turbidities were measured using a spectrophotometer (UV-190, Shimazhu, Japan) at 530 nm against a blank of an unseeded control. The absorbance values of the toxicant-amended mixtures are calculated as a percentage of the control using the simple relationship as follow. Absorbance of test bottle / Absorbance of seed control x 100% of controls. The percent of control values can be plotted against the logarithm of the toxicant concentration, and the IC_{50} (the toxicant concentration reducing growth by 50%) can be calculated from the plot.

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The tests with *Photobacterium phosphoreum* were conducted using the Microtox toxicity analyzer (DXY-2, made by the Institute of Soil Science, Academia Sinica, Nanjing, China). The concentration values causing 50% reduction of bioluminescence after 15 min of exposure(15-min EC_{50})were performed at 20°C according to the procedures described in the Instrumental Manual.

All bioassays were carried out in duplicate or triplicate for statistical purpose and the statistical analyses were performed using the STATGRAPHICS program.

The values of E_{LUMO} were calculated using the quantum chemical method CNDO/2 (Wang et al. 1981; Pople 1976). Log P and $\Sigma\sigma^-$ values were taken from Deneer et al. (1987a,b) or calculated according to Lyman et al. (1982) and Roberts (1987).

RESULTS AND DISCUSSION

The toxicological data and parameters of the tested compounds are listed in Table 1, and the QSARS established in this study are given in Table 2,3.

Deneer et al. (1989) investigated the toxicity of mono and dinitrobenzenes (including their methyl- and chloro- derivatives) towards *Daphnia magna, Chlorella pyrenoidosa* and *Photobacterium phosphoreum.* They established sets of QSARs and observed reasonable correlations with logP and σ^- for *D. magna* and *C. pyrenoidosa,* but not for *Ph. phosphoreum* (r= 0.679 and 0.255 for 15 mononitro compounds and 22 mononitro + dinitro compounds respectively). They indicated that there appears to be no obvious relationship between the susceptibilities of *Ph. phosphoreum* and other species to chemicals that cause toxic effects through modes of biological action different from general anesthesia, and concluded that the applicability of the Microtox test as a prescreening tool for the selection of compounds needs further evaluation. Roberts (1987) analyzed the data on acute toxicity of nitro-substituted benzenes to fathead minnow and obtained a QSAR based on the electronic descriptor $\Sigma\sigma^-$. It was postulated that the nitro compounds are metabolized to electrophilic C-nitroso compounds. In this work, besides mono and dinitrobenzenes (including their methyl- and halogeno - derivatives) the derivatives that contain OH, NH_2 and OCH_3 groups were also tested(considering the fact of contaminants detected in Songhua River). As for establishing the QSARs, besides logP, the sum of substituent constant $\Sigma\sigma^-$ and the energy of the lowest unoccupied molecular orbital E_{LUMO} have been selected as the parameters for reflecting the electron effect of substituents and the electrophilicity of molecules respectively.

The experimental toxicological data (Table 1) showed that both organisms exhibited similar sensitivity rule to the nitrobenzenes although the IC_{50} values for bacteria were relatively lower than the EC_{50} values for *Ph. phosphoreum* . The order of toxicity was: dinitro compounds > mononitro compounds, ortho-/para-substituted nitrobenzenes > meta- substituted nitrobenzenes except for the isomers of nitroanisole. A linear correlation for IC_{50} and EC_{50} values has been found as following:

$$\log (1/IC_{50}) = 1.130 + 0.635 \log (1/EC_{50}) \qquad [1]$$
$$n = 20 \quad r^2 = 0.806 \quad S = 0.224$$

Table 1. Toxicological data and 3 descriptors of the nitrobenzenes

No.	Nitrobenzenes	log1/EC_{50}[a]	log1/IC_{50}[a]	logP[b]	$\Sigma\sigma^-$[c]	E_{LUMO}
1	Nitrobenzene	3.26	3.19	1.89	0.00	1.146
2	1,2-Dinitrobenzene	5.86	4.97	1.55	1.24	-1.502
3	1,3-Dinitrobenzene	4.62	4.29	1.52	0.71	-0.170
4	1,4-Dinitrobenzene	5.81	5.12	1.46	1.24	-0.348
5	2,4-Dinitrotoluene	4.64	3.71	2.04	0.56	0.425
6	2,6-Dinitrotoluene	4.27	3.46	2.02	0.56	0.359
7	2-Chloronitrobenzene	3.97	3.78	2.26	0.27	1.001
8	3-Chloronitrobenzene	3.81	3.41	2.49	0.37	0.885
9	4-Chloronitrobenzene	3.94	3.76	2.35	0.27	0.962
10	3,4-Dichloronitrobenzene	4.11	3.45	3.29*	0.64	0.735
11	2,5-Dichloronitrobenzene	4.22	3.59	2.90	0.64	0.742
12	2-Nitroaniline	3.71	3.60	1.85	-0.15	1.390
13	3-Nitroaniline	3.21	3.48	1.37	-0.16	1.271
14	4-Nitroaniline	3.97	3.69	1.39	-0.15	1.673
15	2-Nitrophenol	3.53	3.51	1.89	-0.37^{Δ}	1.256
16	3-Nitrophenol	3.34	3.43	2.00	0.12^{Δ}	1.055
17	4-Nitrophenol	3.70	3.66	2.04	-0.37^{Δ}	1.299
18	2-Nitroanisole	3.50	3.15	1.80	-0.27^{Δ}	1.346
19	3-Nitroanisole	4.12	3.71	2.16	0.12^{Δ}	1.129
20	4-Nitroanisole[d]	5.32	4.31	2.03	-0.27^{Δ}	1.404
21	3-Bromonitrobenzene	4.41	3.72	2.73*	0.39^{Δ}	1.142

a. observed 15-min EC_{50} and 24-hr IC_{50} (M/L).

b. Deneer et al. (1987a), * calculated using a fragment constant method (Lyman et al. 1982).

c.Deneer et al. (1987b),Δ calculated according to Roberts (1987) and the σ^- values were taken from Xu (1987).

d.the experimental toxicity of 4-Nitroanisole was found to be obviously higher than those predicted by QSAR equations in this study, so that it was not included in the correlation analyses.

where n is the number of chemicals, r^2 is square of correlation coefficient adjusted for degrees of freedom and s is standard error. The significant relationship between IC_{50} and EC_{50} suggests that the toxic potency of tested nitrobenzenes to river bacteria can be predicted by using the Microtox test though evaluating studies about its applicability and limitation to various species are conducting extensively.

Results of simple linear regression analyses indicated a lack of dependence of toxicity of nitrobenzenes to the river bacteria and *Ph. phosphoreum* on log P (r^2 = 0.087; 0.015 respectively), but showed fair correlations with E_{LUMO} (Eqs. [2], [6]).

Table 2. QSARs for nitrobenzenes to *Ph. phosphoreum*

Eq.	$\log 1/EC_{50}(M/L)=$	n	r^2	s
[2]	$4.764 - 0.841E_{LUMO}$	20	0.730	0.376
[3]	$3.723 + 1.334\Sigma\sigma^-$	20	0.757	0.356
[4]	$4.622 + 0.053\log P - 0.847E_{LUMO}$	20	0.716	0.385
[5]	$4.364 - 0.318\log P + 1.373\Sigma\sigma^-$	20	0.799	0.324

Table 3. QSARs for nitrobenzenes to river bacteria

Eq.	$\log 1/IC_{50}(M/L)=$	n	r^2	s
[6]	$4.172 - 0.555\ E_{LUMO}$	20	0.636	0.307
[7]	$3.511 + 0.789\ \Sigma\sigma^-$	20	0.520	0.352
[8]	$4.609 - 0.224\log P - 0.526E_{LUMO}$	20	0.670	0.292
[9]	$4.425 - 0.454\log P + 0.844\Sigma\sigma^-$	20	0.722	0.268

E_{LUMO} is an electrophilicity parameter, the lower E_{LUMO} values, the stronger electrophilicity (Wang et al. 1981). Data in Table 1. showed clearly that the toxicity of dinitrobenzenes to both tested organisms is much higher than that of mononitrobenzenes and the corresponding E_{LUMO} values are much lower, the enhanced toxicity of dinitrobenzenes may be related to the intracellular reduction of NO_2 groups.

The correlation between the toxicity and the sum of the substituent electron effect $\Sigma\sigma^-$ is poor for river bacteria (Eq.[7] r^2= 0.520) but fair for *Ph. phosphoreum* (Eq.[3] r^2= 0.757). $\Sigma\sigma^-$ values reflect the ability of the substituents to withdraw electrons from the reference NO_2 group (Roberts 1987), and high positive σ constants (specifically for dinitrobenzenes) will result in a decrease of the electron density at the nitro moiety, thus facilitating the reduction to the corresponding nitroso compounds (Deneer et al. 1989). The poor correlation between $\log(1/IC_{50})$ and $\Sigma\sigma^-$ is probably due to the lower sensitivity of river bacteria to the toxicity contributed by other functional groups than nitro groups.

Multiple linear regression analyses indicated that the simple regression equations have been either slightly (from Eqs. [3], [6] to Eqs. [5], [8]) or significantly (from Eq.[7] to Eq.[9]) improved by incorporation of a log P term except for Eq. [2]. However, the negative sign of the logP coefficient indicates that the rate-determining step in toxicity of the tested nitrobenzenes is not dependent on hydrophobicity. Roberts, regarding nitrobenzene derivatives as pro-electrophiles, postulated that the rate-determining step in toxicity is alkylation of the appropriate biological nucleophiles (Dearden et al. 1995). Veith et al. (1993) concluded that the suitability of using log P to model passive transport in vivo decreases with increasing soft electrophilicity.

It may be concluded that the toxicity of tested substituted nitrobenzenes (including NO_2, OH, NH_2, OCH_3, methyl and halogeno substituents) to river bacteria and *Ph. phosphoreum* is controlled mainly by electronic factors.

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