Aquatic Toxicology, 10 (1987) 115–129
Elsevier

AQT 00237

Quantitative structure–activity relationships for the toxicity and bioconcentration factor of nitrobenzene derivatives towards the guppy (*Poecilia reticulata*)

J.W. DENEER, T.L. SINNIGE, W. SEINEN and J.L.M. HERMENS

Department of Veterinary Pharmacology, Pharmacy and Toxicology, University of Utrecht, Utrecht, The Netherlands

(Received 14 July 1986; accepted 16 January 1987)

The acute toxicity and bioconcentration factor of a series of nitrobenzene derivatives was determined for the guppy. Toxicity is found to be determined by both hydrophobicity (expressed by the octanol/water partition coefficient) and rate of reduction of the nitro group (expressed by either electrochemical halfwave reduction potential or Hammett σ values). The acute toxicity of mononitro compounds can be adequately described by hydrophobicity, and their bioconcentration factor is found to be approximately equal to the octanol/water partition coefficient. The dinitro compounds that are likely to be most easily reduced are found to have substantially lower bioconcentration factors than expected, accompanied by a marked increase in toxicity. Electrochemical reduction potentials are found to be a better descriptor of toxicity than Hammett σ^- values. Nitroanilines do not fit the QSARs established for nitrobenzene derivatives. These compounds are probably to be considered aniline derivatives.

Key words: Nitroaromatic compound; Toxicity; Bioconcentration factor; Guppy; QSAR

INTRODUCTION

Nitroaryl compounds belong to the class of bioactivated chemicals, since they are reduced in vivo to highly active intermediates like arylnitroso-compounds and arylhydroxylamines (Sternson, 1975; Uehleke, 1964). Therefore, it seems very likely that an adequate description of their toxicity should involve their tendency to be reduced. For nitroimidazoles and nitrofurans it has been shown that there is good correlation between biological activity and reduction potential (Adams et al., 1976, 1980). Biagi et al., (1983), however, found that, for the series of 5-nitroimidazoles

Correspondence to: J.W. Deneer, Department of Veterinary Pharmacology, Pharmacy and Toxicology, University of Utrecht, P.O. Box 80176, 3508 TD, Utrecht, The Netherlands

they studied, there was no obvious influence of the reduction potential upon activity.

Most studies on the aquatic toxicity and mutagenic properties of nitroaryl compounds have been directed towards nitrotoluenes, since these are important intermediates in the industrial production of various compounds, such as explosives and herbicides, and are thus most liable to enter the environment (Bailey and Spanggord, 1983; Spanggord et al., 1982). For various other nitroaryl compounds relatively few data on acute toxicity towards aquatic species are found in the literature (e.g. Holcombe et al., 1984; Heitmuller et al., 1981; LeBlanc, 1984).

Data on the bioconcentration factor (BCF) of nitrobenzene derivatives are relatively scarce. Oliver and Niimi (1985) have demonstrated that 2,3,4,5-tetrachloronitrobenzene (TCNB) and pentachloronitrobenzene (PCNB) do not accumulate to any appreciable extent in the Fathead Minnow, whereas 2,3,5,6-TCNB shows a much higher tendency for bioconcentration. Low BCF-values for 2,3,4,5-TCNB and PCNB are attributed to rapid metabolization of these compounds. For unsubstituted nitrobenzene, Veith et al. (1979) report a relatively high BCF in fathead minnows.

The object of this study was to obtain data on the acute toxicity and bioconcentration potential of various nitrobenzene derivatives in fish, and to relate these data to physico-chemical properties of the compounds studied by constructing a suitable QSAR. Since both rate of uptake and rate of metabolism would seem important, the most suitable parameters to describe acute toxicity are thought to be the hydrophobicity, of the compounds expressed by the octanol/water partition coefficient (P), and the tendency of the compounds to be reduced, which may be expressed by the electrochemical reduction potential or, possibly, Hammett σ constants.

Since it is assumed that only substances exhibiting a similar mode of toxic action should be used to formulate a reliable QSAR, precautions had to be taken to make sure that halogenated compounds that were to be included in the study did not show any tendency for alkylation. For this reason all halogenated compounds were subjected to a standard test, to establish whether they acted as an alkylating agent.

For many of the compounds studied, values of their octanol/water partition coefficient were not readily available. Therefore it was decided to determine this parameter for all substances tested. Since the traditional 'shake-flask' method is tedious and time consuming, partition coefficients were determined by measuring the capacity factor of the compounds on a 'reversed phase' HPLC column.

MATERIALS AND METHODS

Experimental animals

The fish used in the toxicity experiments were male and female guppies (*Poecilia reticulata*), reared in our laboratory. Their age varied from two to three months at the start of the experiments. For the bioconcentration tests, female guppies (5 to 8

months old) were used. The wet weight varied from 60 to 450 mg at the end of the experiment; mean fat content was 8 ± 2%. All fish were fed a commercial fishfood daily, approximately 1 h before renewal of the solutions. All experiments were carried out in standard water (SW), prepared according to Alabaster and Abram (1964), which corresponds to very soft tapwater (hardness: 25 mg/l as $CaCO_3$). All fish were acclimatized for at least 12 days prior to the experiments.

LC50 determinations

The 14 day LC50 values of the various compounds were determined following the procedure outlined by Könemann (1981), with two small changes: concentrations were increased in geometrical progression with a factor of 2, and ten guppies were exposed to each concentration. Oxygen content, pH and temperature were determined for two concentrations of each compound, on at least 4 days before and after renewal of the solutions. Oxygen content after 24 h was always above 4.5 mg/l, pH varied from 6.8 to 7.2 and the temperature was 21–23°C. Water samples were taken regularly, on at least four days during the experimental period, both before and after renewal of the solutions, and analyzed after hexane extraction, using a Tracor 550 gaschromatograph equipped with both an electron capture and a flame ionization detector. Except for ortho- and para-dinitrobenzene, concentrations found after 24 h corresponded to at least 80% of the amount added. For the two aforementioned compounds, solutions turned yellow after the fish had been exposed to them for some hours. Upon analysis after 24 h of exposure, up to 40% of the compound added was seen to be converted into the corresponding nitroaniline. This effect only occurred when fish were present in the solution. LC50 experiments for these two compounds were repeated under the same conditions, but using 5 l glass jars; 4 l of each solution were renewed daily. In these experiments, concentrations found after 24 h of exposure corresponded to at least 90% of the amount added. Chemicals were used as purchased. Their purity is given in Table II. LC50 values were calculated by logit transformation, and are based on the amount of compound added.

Bioconcentration factor

The required amount of each compound was dissolved in one l SW, giving stock solutions corresponding to LC50 concentrations. Immediately before use, these stock solutions were diluted 1:5 in SW; the resulting solutions had concentrations equal to 1/5 LC50. The experiments were carried out in glass jars of 1.5 l, containing one l of solution and 9 female guppies. New solutions were prepared from the stock solutions every day. Two water samples of 2 ml each were taken each day from every jar before and after renewal of the solutions, and extracted with 2–5 ml toluene. Extracts were kept in the dark at approx. 4°C, and stored for a maximum of 6 days. After 1 and 2 days of exposure two guppies were taken for analysis; at

the end of day three all remaining fish were taken. Fish were killed by immersion in liquid nitrogen, and immediately underwent the extraction and clean-up procedure.

In some preliminary experiments it was established that the uptake of the chemicals proceeded very fast, thus enabling the use of this short-term assay.

All fish were analyzed separately, using a slightly modified version of the clean-up procedure as outlined by Edgerton et al. (1985). After immersion in liquid nitrogen, each fish was dried on a tissue and weighed, after which it was ground with four times its own weight of dried sodium sulphate (Merck, p.a.). Extractions with 20 ml of hexane (Baker Resi Analyzed) were carried out in a Soxhlet-apparatus for 3 h. Ten ml of the extract were evaporated to dryness under nitrogen to determine the fat content; the other half was evaporated to approx. 0.5 ml, and deposited on a clean-up column consisting of 1 g silica gel (Merck, 70–230 mesh) and 0.25 g dried sodium sulphate. The column was prerinsed with 5 ml hexane. The nitroaryl compounds were eluted with 15 ml of a hexane/toluene mixture (2:3 v/v). The resulting solution was evaporated to 1 ml and, if necessary, diluted with hexane. Reported values of bioconcentration factors are corrected for extraction efficiencies which were determined twice for each compound and were always above 80%, except for para-chloronitrobenzene (approx. 60%).

All analyses were carried out using either a Tracor 550 gaschromatograph using a CP-Sil 5 CB capillary column (10 m, 0.22 mm i.d.) or a Pye Unicam 304 GC, using a CP-Wax 57 CB column (25 m, 0.32 mm i.d.). Both chromatographs were equipped with an electron capture detector, and a Shimadzu CR1A integrator.

Test for alkylating power

All halogen-containing compounds were subjected to the 4-nitrobenzylpyridine (NBP) test as outlined by Hermens et al. (1985). The concentration of NBP and test compound were 0.20 M and 7.5 mM resp. The mixture was refluxed for maximally 6 h.

For the spectrophotometric determinations, a Pye Unicam SP 1800 UV/VIS spectrometer was used at 500–580 nm and 0.25 nm bandwidth.

Determination of HPLC capacity factors

All capacity factors were determined at four compositions of the mobile phase and extrapolated to 100% water, yielding k_0, following the recommendations by Hammers et al. (1982). The HPLC-apparatus used consisted of a Pye Unicam 4010 double piston pump operated at 1 ml/min flow and a Pye Unicam 4020 UV-detector, operated at 254 nm. All solvents used were filtered over a 0.45 μm filter, and degassed prior to use. Determinations were carried out at room temperature on a 'reversed phase' C-18 column (Merck Lichrosorb, particle size 10 μm, length 10 cm, 4.6 mm i.d.).

RESULTS AND DISCUSSION

Alkylating power

All halogenated compounds tested were found to be non-reactive in the NBP-test, after refluxing for 6 h.

HPLC capacity factors

Experimental capacity factors for various eluent compositions, including calculated values of k_0, and some literature values of log P (Hansch and Leo, 1979) are given in Table I. The correlation coefficient between composition (% methanol) and log k was always larger than 0.9958.

Since formamide showed different retention times for mobile phases of varying polarity, this compound could not be used to determine t_0. Instead, methanol was used, which showed a much more constant retention time, thus considerably improving the determination of t_0. Linear regression of log k_0 and experimental values of log P resulted in Equation 1, which was used to calculate the values of log P given in Table I.

$$\log P = (1.051 \pm 0.037) \log k_0 + 0.10 \quad (N=11,\ r=0.995\ s=0.04), \tag{1}$$

where N is the number of observations, r is the correlation coefficient, and s is the standard error of estimate. Coefficients are given as (value ± standard error).

Toxicity data and bioconcentration factors

In general fish showed loss of balance, lethargy and, at lower concentrations, an increase in appetite. At relatively high concentrations various fish showed signs of severe cyanosis, apparent from a blue hue of their skin. This effect was especially marked with various dinitro compounds.

Experimental values of the LC50 and bioconcentration factors for the compounds tested are given in Table II. The LC50 values given for the dinitrobenzenes were obtained using the 5-l aquaria and are, because of higher availability, slightly lower than the corresponding values obtained when using the 1.5 l standard jars.

The LC50 values reported here are somewhat lower than literature data for 96 h LC50 of Fathead Minnows (Holcombe et al., 1984; Bailey and Spanggord, 1983), but are in general in good agreement with values found in the literature.

Graphical representation of LC50 data versus log P reveals a marked influence of the hydrophobicity on the toxicity of mononitro compounds (Fig. 1). The plotted regression line was calculated for all chemicals, excluding the dinitro compounds, the nitro-anilines and 4-chloronitrobenzene (Equation 2).

TABLE I

Logarithm of capacity factors and calculated values of log P for various nitroaryl compounds.

Compound	Mobile phase (CH_3OH/H_2O v/v%)					log P		
	80/20	70/30	60/40	50/50	40/60	0/100[a]	calc[b]	lit[c]
Nitrobenzene	−0.3691	−0.0964	0.1504	0.4125	–	1.709	1.89	1.87[d]
2-chloronitrobenzene	−0.2515	−0.0153	0.2963	0.6164	–	2.056	2.26	2.24[d]
3-chloronitrobenzene	−0.1285	0.1356	0.4580	0.7734	–	2.278	2.49	2.43[d]
4-chloronitrobenzene	−0.1972	0.0586	0.3717	0.6792	–	2.141	2.35	2.40[d]
2,3-dichloronitrobenzene	−0.0734	0.2267	0.6038	0.9994	–	2.776	3.01	
2,4-dichloronitrobenzene	−0.0889	0.2107	0.5754	0.9486	–	2.672	2.90	
2,5-dichloronitrobenzene	−0.0998	0.2007	0.5679	0.9406	–	2.667	2.90	
3,5-dichloronitrobenzene	0.0690	0.3949	0.7687	1.1243	–	2.890	3.13	
2-nitrotoluene	−0.2039	0.0433	0.3503	0.6586	–	2.094	2.30	2.30[d]
3-nitrotoluene	−0.1598	0.0936	0.4101	0.7218	–	2.191	2.40	2.42[d]
4-nitrotoluene	−0.1859	0.0629	0.736	0.6842	–	2.132	2.34	2.39[d]
4-chloro-2-nitrotoluene	−0.0230	0.2913	0.6701	1.0410	–	2.816	3.05	
2-chloro-6-nitrotoluene	−0.0203	0.2953	0.6776	1.0569	–	2.851	3.09	
2,3-dimethylnitrobenzene	−0.1078	0.1825	0.5442	0.9098	–	2.602	2.83	
3,4-dimethylnitrobenzene	−0.0573	0.2387	0.6041	0.9685	–	2.676	2.91	
1,2-dinitrobenzene	−0.4309	−0.2417	−0.0076	0.2553	–	1.384	1.55	1.58[d]
1,3-dinitrobenzene	−0.3567	−0.1696	0.0586	0.2849	–	1.354	1.52	1.49[d]
1,4-dinitrobenzene	−0.4355	−0.2245	−0.0097	0.2103	–	1.284	1.45	1.47[d]
2,4-dinitrotoluene	−0.2720	−0.0482	0.2392	0.5246	–	1.851	2.04	1.98[d]
2,6-dinitrotoluene	−0.2659	−0.0685	0.2246	0.5236	–	1.833	2.02	
2-nitroaniline	–	−0.2944	−0.0432	0.2029	0.4815	1.502	1.67	1.81
3-nitroaniline	–	−0.3830	−0.1908	0.0194	0.2634	1.109	1.26	1.37
4-nitroaniline	–	−0.4400	−0.2572	−0.0575	0.1911	1.1010	1.16	1.39

[a] Calculated by linear extrapolation of experimental values of log k at various solvent compositions.

[b] Calculated from Equation 1.

[c] Data taken from Hansch and Leo (1979); if several reliable values were available, the mean value was used.

[d] Value used to calculate Equation 1.

TABLE II

14 Day LC50 and bioconcentration factors for the guppy of various nitroaryl compounds.

Compound	Purity (%)	log LC50[a]	log BCF[b]	log P[c]	log (BCF × LC50)	$LC50/LC50_{MT}$[d]
Nitrobenzene	>99	2.70	1.47 ± 0.12 (15)	1.89	4.17	0.30
2-chloronitrobenzene	>99	2.28	2.29 ± 0.05 (5)	2.26	4.57	0.24
3-chloronitrobenzene	>98	1.99	2.42 ± 0.10 (6)	2.49	4.41	0.19
4-chloronitrobenzene	>98	1.58	2.46 ± 0.11 (6)	2.35	4.04	0.06
2,3-dichloronitrobenzene	>98	1.34	3.01 ± 0.04 (7)	3.01	4.35	0.12
2,4-dichloronitrobenzene	>98	1.54	3.02 ± 0.05 (17)	2.90	4.56	0.16
2,5-dichloronitrobenzene	>98	1.41	2.92 ± 0.04 (7)	2.90	4.33	0.12
3,5-dichloronitrobenzene	>98	1.47	3.01 ± 0.05 (8)	3.13	4.48	0.21
2-nitrotoluene	>99	2.38	2.28 ± 0.06 (6)	2.30	4.66	0.33
3-nitrotoluene	>98	2.34	2.31 ± 0.04 (5)	2.40	4.65	0.36
4-nitrotoluene	>98	2.43	2.37 ± 0.05 (6)	2.34	4.80	0.40
4-chloro-2-nitrotoluene	>97	1.56	3.02 ± 0.09 (6)	3.05	4.58	0.22
2-chloro-6-nitrotoluene	>99	1.48	3.09 ± 0.06 (6)	3.09	4.57	0.20
2,3-dimethylnitrobenzene	>99	1.61	2.86 ± 0.06 (6)	2.83	4.47	0.16
3,4-dimethylnitrobenzene	>99	1.79	2.84 ± 0.05 (6)	2.91	4.63	0.28
1,2-dinitrobenzene	>98	0.85	1.02 ± 0.09 (5)	1.55	1.87	0.002
1,3-dinitrobenzene	>99	1.36	1.87 ± 0.04 (7)	1.52	3.23	0.005
1,4-dinitrobenzene	>97	0.37	<0.70 (8)	1.45	<1.07	0.0006
2,4-dinitrotoluene	98	1.84	2.31 ± 0.03 (15)	2.04	4.15	0.06
2,6-dinitrotoluene	98	1.99	2.44 ± 0.04 (13)	2.02	4.43	0.08
2-nitroaniline	>99	1.85	–	1.67	–	0.03
3-nitroaniline	>98	2.57	–	1.26	–	0.06
4-nitroaniline	>99	2.59	–	1.16	–	0.05

[a] LC50 expressed in μmol/l.

[b] BCF on the basis of fat weight, given as: value ± standard deviation (number of determinations).

[c] Taken from Table I.

[d] $LC50_{MT}$ denotes 'minimum toxicity', calculated from $-\log LC50_{MT} = 0.87 \log P - 4.87$ (Könemann, 1981).

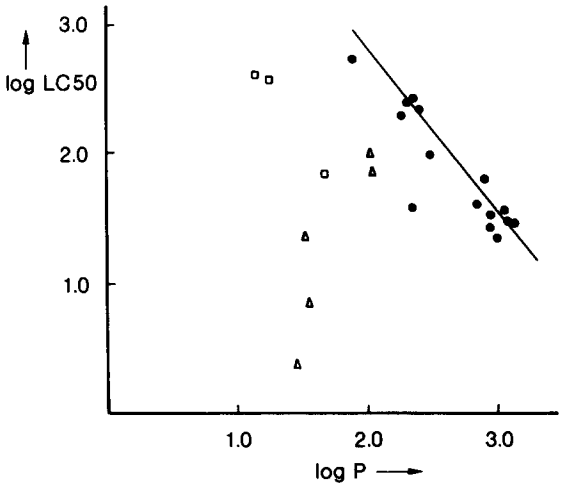

Fig. 1. The influence of log P on the toxicity of nitroaryl compounds. Plotted line calculated from Equation 2; ●, mono-nitro compound; Δ, dinitro compound; □, nitroaniline.

$$-\log \mathrm{LC50} = (1.13 \pm 0.10) \log P - 4.92 \quad (2)$$
$$(N = 14 \; r = 0.959 \; s = 0.14)$$

Deviations from the regression line by all compounds used to calculate Equation 2 are probably due to experimental error. 4-Chloronitrobenzene is about 5 times as toxic as would be expected on the basis of Equation 2. Since its LC50 was determined in duplicate, twice yielding the same value, it is improbable that the deviation is the result of experimental error. The reason for this increased toxicity is not yet clear. It is suspected, however, that although the compound was negative in the NBP-test, it does possess a slight tendency to alkylate various tissue components. This would also explain the low extraction efficiency of the compounds in the bioconcentration experiments (60%).

The nitro-anilines clearly do not fit Equation 2. Since these substances are only very weak bases, it seems unlikely that ionization plays any role under the circumstances at which the toxicity tests were conducted. Their toxicity can be much better described by the QSAR for alkyl- and chloro-anilines as published by Hermens et al. (1984), given in Equation 3.

$$-\log \mathrm{LC50} = 0.88 \log P - 3.83 \quad (3)$$
$$(N = 11 \; r = 0.959 \; s = 0.24)$$

There is no apparent relationship between toxicity and partition coefficient for the dinitro compounds (see Fig. 1). The marked increase in toxicity upon introduc-

tion of a second nitro group into the aromatic nucleus is probably the result of relatively fast reduction of these compounds to the corresponding arylhydroxylamines, which can be explained by considering their reduction potentials.

Halfwave reduction potentials ($E_{1/2}$), taken from Pearson (1948), together with some additional LC50 values taken from Bailey and Spanggord (1983), are given in Table III. Unfortunately, these halfwave potentials cannot be converted to standard reduction potentials because of the mechanism of the electrode reaction used to measure them. As Pearson (1948), however, has demonstrated in his paper, $E_{1/2}$ directly reflects the velocity of the reaction between the compounds in question and hydrogen atoms formed at the mercury electrode. The halfwave potentials may therefore be used as a kinetic parameter to describe the reduction process. Various other authors (e.g. Maki and Geske, 1961; Bencheikh-Sayarh et al., 1983) give values of $E_{1/2}$ for some nitroaryl compounds, sometimes differing considerably from the data given by Pearson; the discrepancies mostly stem from the use of different reaction media, different pH etc. On the basis of these data it may be concluded, however, that in neutral aqueous media chloronitrobenzenes do not differ to any great extent from nitrobenzene and nitrotoluenes as far as the rate of reduction reactions is concerned. The chloronitrobenzenes, dimethylnitrobenzenes and the chloronitrotoluenes are, because of the lack of suitable data, assumed to have the same halfwave potentials as nitrobenzene. The error introduced by this assumption will be seen to be quite small.

Introduction of the halfwave reduction potentials into a QSAR leads to Equation 4a, in which 4-chloronitrobenzene, 2,4-dinitrotoluene, 2,6-dinitrotoluene and the nitroanilines have not been taken into account, since they deviate considerably from the line calculated, as can be seen in Table III.

$$-\log \mathrm{LC50} = (0.96 \pm 0.11) \log P + (8.81 \pm 0.63) E_{1/2} + 0.68 \quad (4a)$$
$$(N=20\ r=0.964\ s=0.18)$$

$$\log P = (4.5 \pm 0.8) E_{1/2} + 0.05 \quad (4b)$$
$$(N=20\ r=0.815\ s=0.37)$$

Because of the high correlation between log P and $E_{1/2}$ (Equation 4b), the coefficients in Equation 4a are probably not very precise.

It is not clear why the two dinitrotoluenes are much less toxic than could be expected on the basis of Equation 4a. The reason might be that the arylhydroxylamines formed upon reduction of these compounds are much less stable than the corresponding compounds formed from other isomers, thus shortening their time of existence in the body appreciably. Data given by Pearson on the potential of the second polarographic wave, which corresponds to the reduction of arylhydroxylamines to the corresponding amines, seem to be in accordance with this hypothesis.

Considering that the error in the assumed halfwave potential of dimethylnitro-

TABLE III

Comparison of observed log LC50 with predictions based on Equations 4a and 5a.

Compound	log LC50 observed	$-E_{1/2}$[a] Volt	$\Sigma\sigma^{-}$[b]	log P[c]	log LC50 calc. Eq. 4a	residual	log LC50 calc. Eq. 5a	residual
Nitrobenzene	2.70	0.58	0.00	1.89	2.61	0.09	2.22	0.48
2-chloronitrobenzene	2.28	0.58*	0.27	2.26	2.26	0.02	1.84	0.44
3-chloronitrobenzene	1.99	0.58*	0.37	2.49	2.04	−0.05	1.68	0.31
4-chloronitrobenzene	1.58	0.58*	0.27	2.35	2.17	0.59	1.85	−0.27
2,3-dichloronitrobenzene	1.34	0.58*	0.64	3.01	1.54	−0.20	1.26	0.08
2,4-dichloronitrobenzene	1.54	0.58*	0.54	2.90	1.64	−0.10	1.40	0.14
2,5-dichloronitrobenzene	1.41	0.58*	0.64	2.90	1.64	−0.23	1.28	0.13
3,5-dichloronitrobenzene	1.47	0.58*	0.74	3.13	1.42	0.05	1.12	0.35
2-nitrotoluene	2.38	0.62	−0.15	2.30	2.57	−0.19	2.31	0.07
3-nitrotoluene	2.34	0.59	−0.07	2.40	2.21	0.13	2.20	0.14
4-nitrotoluene	2.43	0.58	−0.15	2.34	2.18	0.25	2.30	0.13
4-chloro-2-nitrotoluene	1.56	0.58*	0.22	3.05	1.50	0.06	1.73	−0.17
2-chloro-6-nitrotoluene	1.48	0.58*	0.22	3.09	1.46	0.02	1.72	−0.24
2,3-dimethylnitrobenzene	1.61	0.58*	−0.22	2.83	1.71	−0.10	2.28	−0.67
3,4-dimethylnitrobenzene	1.79	0.58*	−0.22	2.91	1.63	0.16	2.26	−0.47
1,2-dinitrobenzene	0.85	0.33	1.24	1.55	0.74	0.11	0.87	−0.02
1,3-dinitrobenzene	1.36	0.39	0.71	1.52	1.30	0.06	1.48	−0.12
1,4-dinitrobenzene	0.37	0.31	1.24	1.45	0.66	−0.29	0.89	−0.52
2,4-dinitrotoluene	1.84	0.40	0.56	2.04	0.89	0.89	1.59	0.25
2,6-dinitrotoluene	1.99	0.46	0.56	2.02	1.43	0.56	1.60	0.39
2-nitroaniline[d]	1.85	0.58*	−0.15[e]	1.67	2.83	−0.98	2.50	−0.65
3-nitroaniline[d]	2.57	0.58*	−0.16[e]	1.26	3.22	−0.65	2.61	−0.04
4-nitroaniline[d]	2.59	0.58*	−0.15[e]	1.16	3.32	−0.73	2.63	−0.04
2,3-dinitrotoluene	1.00[f]	0.40	1.06	1.99*	0.93	0.07	0.99	−0.01
3,4-dinitrotoluene	0.92[f]	0.36	1.09	1.99*	0.58	0.34	0.95	0.03
1,3,5-trinitrobenzene	0.71[f]	0.31	1.42	1.18*	0.92	−0.21	0.74	0.03

[a] Values taken from Pearson (1948) or estimated as outlined in the text; estimated values are denoted by *.
[b] Values taken from Hansch and Leo (1979); σ^{-} for ortho-position taken equal to value for para-position.
[c] Taken from Table I; values denoted by * are calculated according to Rekker (1977).
[d] log LC50 calculated from Equation 4: 2.36, 2.72 and 2.81 for ortho-, meta- and para-isomer, respectively.
[e] σ^{-} of amino-group.

benzenes, chloronitrobenzenes and chloronitrotoluenes may be approximately 20 mV, the error in the calculated values of log LC50 for these compounds would amount up to 0.17 log units, which, in view of experimental error, is quite acceptable.

When using Hammett sigma values as a parameter to describe the toxicity data one has to apply σ^- values, since the nitro-group is a strong electron acceptor (Exner, 1978). Correlation of log LC50 with log P and $\Sigma\sigma^-$ leads to Equation 5a. Parachloronitrobenzene and the nitroanilines were excluded from the data used to calculate Equation 5a. The correlation between log P and $\Sigma\sigma^-$ is given in Equation 5b.

$$-\log \mathrm{LC50} = (0.24 \pm 0.14) \log P + (1.16 \pm 0.17)\, \Sigma\sigma^- - 2.73 \quad (N=22\ r=0.860\ s=0.33) \tag{5a}$$

$$\log P = (-0.644 \pm 0.22)\, \Sigma\sigma^- + 2.64 \quad (N=22\ \ r=0.550\ \ s=0.51) \tag{5b}$$

Since the coefficient of log P appears not to be significantly different from 0, a QSAR using only the Hammett constants was calculated (Equation 6). However, elimination of log P from the QSAR did not result in a better description of the toxicity data.

$$-\log \mathrm{LC50} = (1.00 \pm 0.15)\, \Sigma\sigma^- - 2.10 \quad (N=22\ \ r=0.838\ \ s=0.34) \tag{6}$$

The use of Hammett sigma constants leads to a significantly ($P<0.01$) larger standard error of estimate compared to the use of reduction potentials, which leads us to believe that the former parameter is not as suitable for the description of the acute toxicity data of nitrobenzene derivatives as are halfwave reduction potentials.

The nitroanilines, epecially the ortho-isomer, do not fit Equations 4a and 5a very well. It seems likely that these compounds act like anilines.

Bioconcentration data

Experimental values of the bioconcentration factor are given in Table II. The bioconcentration factor of 1,4-dinitrobenzene could not be determined because of too-low concentrations in fish tissue.

Three out of 207 fish died during the experiments. Concentrations in these fish did not significantly differ from concentrations found in surviving fish. Data from dead fish are, however, not used to calculate BCF.

In some preliminary experiments it was established that the type of short-term assay used to determine BCF did not lead to erroneous results. Sixteen female guppies were exposed to 1/5 LC50 of 2,4-dichloro nitrobenzene, taking two fish and

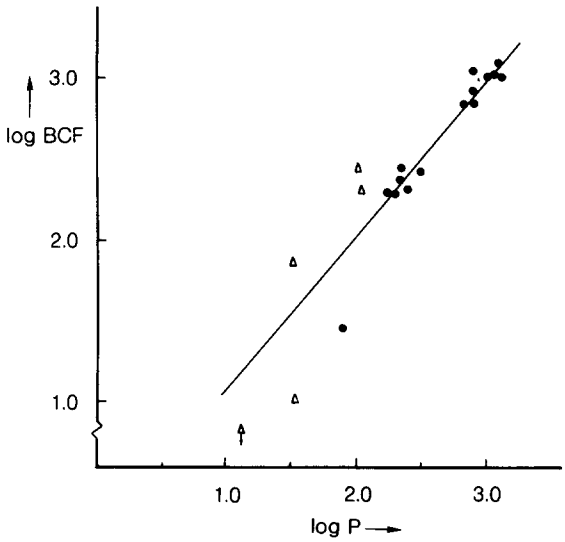

Fig. 2. Bioconcentration factor versus log P. Plotted line calculated from Equation 7; ●, mono-nitro compound; △, dinitro compound.

two water samples after 2, 4, 6, 8, 24, 48 and 72 h. Solutions were renewed after 24 and 48 h. The concentrations found in fish and water showed that the steady state value for C(fish)/C(water) was reached after approximately 6 h. This result was confirmed in the actual BCF determinations since there was never an apparent difference between BCFs determined on subsequent days.

In an additional experiment, the BCF of 2,4-dichloronitrobenzene was determined at 1/50 LC50. The value found (log BCF = 3.04 ± 0.06; N = 9) was not different from the value determined at 1/5 LC50 (log BCF = 2.98 ± 0.02; N = 8).

Fig. 2 represents the correlation between log P and log BCF. The plotted line (Equation 7) was calculated excluding all dinitro compounds and nitrobenzene. Considering the sometimes relatively large standard deviations in the BCF data, the calculated regression equation gives a surprisingly good estimation of actual values.

$$\log \text{BCF} = (0.96 \pm 0.06) \log P + 0.09 \quad (N = 14 \quad r = 0.975 \quad s = 0.08) \tag{7}$$

Inclusion of meta-dinitrobenzene and the two dinitrotoluenes leads to Equation 8, which gives a somewhat poorer description of the BCF data.

$$\log \text{BCF} = (0.77 \pm 0.06) \log P + 0.65 \quad (N = 17 \quad r = 0.954 \quad s = 0.12) \tag{8}$$

The relatively low value of BCF for nitrobenzene might be due to experimental

difficulties in the determination of nitrobenzene in fish, because of the relatively high volatility of this compound.

All dinitro compounds are seen to deviate from the regression line given by Equation 7. Ortho- and para-dinitrobenzene have a BCF substantially lower than expected on the basis of Equation 7, which most probably is the result of metabolization of these compounds. Meta-dinitrobenzene, 2,4-dinitrotoluene and 2,6-dinitrotoluene, somewhat surprisingly, have BCFs which are approximately 1.5–2 times higher than expected on the basis of Equation 7. Since the BCFs for the dinitrotoluenes were found to be very reproducible in duplicate experiments, it seems unlikely that these discrepancies are the result of experimental error.

Inspection of the product of LC50 and BCF (Table II) reveals a remarkable constancy for many of the compounds studied. Gross deviations only occur for the dinitro benzenes and *p*-chloronitrobenzene. Excluding these compounds as well as nitrobenzene, the mean value of log (BCF $\times$ LC50) is found to be 4.53 $\pm$ 0.34 (95% confidence level). The value for 2,6-dinitrotoluene (4.43) is interesting, since it does not deviate from the mean value established for the mononitro compounds, indicating that this compound is reduced only very slightly, due to its relatively low reduction potential. This is in good agreement with the observations of Turner et al. (1985) that, in humans exposed to technical dinitrotoluene (which consists of 76% 2,4-DNT and 19% 2,6-DNT) no reduced metabolites of 2,6-DNT were found, although 2,4-DNT was reduced to various amino-nitro compounds. It must be stressed, however, that large differences exist between various species as to their ability to reduce nitro compounds; e.g. Fischer 344 rats are very well able to reduce 2,6-dinitrotoluene (Rickert et al., 1984).

From the foregoing discussion, the following conclusions may be drawn:

The acute toxicity towards fish of nitroaryl compounds is found to be dependent upon both hydrophobicity and the tendency of the compounds to be transformed, through reduction of the nitro moiety.

The mono-nitro compounds studied are seen to be reduced only very slightly, since their toxicity and bioconcentration can be described satisfactorily with log P as the only parameter. The toxicity of poly-nitro compounds is, however, heavily dependent upon their reduction potential; many of these compounds are probably reduced very easily in vivo. This is further substantiated by the fact that the bioconcentration factor of substances possessing relatively high halfwave reduction potentials is appreciably lower than would be expected on the basis of their octanol/water partition coefficient.

The toxicity of the nitroanilines studied is best described by a QSAR for chloroanilines published by Hermens et al. (1984). Therefore it seems likely that these compounds should be considered as aniline derivatives.

Hammett σ^- values are not as good descriptors for the estimation of toxicity as are the halfwave potentials, probably because steric factors are not accounted for by the former parameter.

Bioconcentration factors of mono-nitro compounds can be very well described using log *P* as the only parameter. As expected, the most toxic compounds (ortho- and para-dinitrobenzene), possess markedly lower bioconcentration factors than would be expected from their log *P*. All tested dinitro compounds with nitro-groups in meta-position relative to each other, bioconcentrate to approximately twice the values expected on the basis of their log *P*.

In spite of the reasonably good correlations obtained in this study, one should be very cautious when attempting to predict the toxicity of nitroaryl compounds on the basis of the parameters presently used, since the presence of a nitro moiety in the molecule does not necessarily mean that the compound exerts its toxicity mainly through the formation of arylnitroso-compounds and arylhydroxylamines. Unfortunately this is a general shortcoming of QSAR studies; if it is not clear to which class of compounds a specific chemical belongs, one is not able to reliably predict its toxicity from a QSAR.

ACKNOWLEDGEMENTS

These investigations were supported by the Netherlands Foundation for Technical Research (STW), future Technical Science Branch/Division of the Netherlands Organization for the Advancement of Pure Research (ZWO), as well as by the Dutch Ministry of Transport and Public Works.

The authors would like to thank Prof. Dr. H. van Genderen, Dr. H. Könemann and Drs. C. van Leeuwen for their stimulating discussions and suggestions.

REFERENCES

Adams, G.E., E.D. Clarke, R.S. Jacobs, I.J. Stratford, R.G. Wallace, P. Wardman and M.E. Watts, 1976. Mammalian cell toxicity of nitro compounds: dependence upon reduction potential. Biochem. Biophys. Res. Commun. 72, 824–829.

Adams, G.E., I.J. Stratford, R.G. Wallace, P. Wardman and M.E. Watts, 1980. Toxicity of nitro compounds toward hypoxic mammalian cells in vitro: dependence on reduction potential. J.N.C.I. 64, 555–560.

Alabaster, J.S. and F.S.H. Abrams, 1964. Development and use of a direct method of evaluating toxicity to fish, in: Adv. Water Poll. Res., Proc. 2nd Int. Conf. Tokyo, Vol. I, Pergamon Press, Oxford.

Bailey, H.C. and R.J. Spanggord, 1983. The relationship between the toxicity and structure of nitroaromatic chemicals. In: Aquatic Toxicology and Hazard Assessment: Sixth Symposium, ASTM Philadelphia, edited by W.E. Bishop, R.D. Cardwell and B.B. Heidolph, STP 802, pp. 98–107.

Bencheikh-Sayarh, S., P. Pouillen, A.-M. Martre and P. Martinet, 1983. Réduction électrochimique de composes organiques en présence de tensio-actifs I. Etude polarographique de nitrobenzenes monosubstitués en milieu acide. Electrochim. Acta 28, 627–634.

Biagi, G.L., A.M. Barbaro, M.C. Guerra, G. Cantelli Forti, G. Aicardi and P.A. Borea, 1983. Quantitative relationship between structure and mutagenic activity in a series of 5-nitroimidazoles. Teratog. Carcinog. Mutagen. 3, 429–438.

Edgerton, T.R., W. Scott and R.E. Linder, 1985. Methodology for the determination of dinitroaniline herbicides in tissue and excreta. J. Anal. Toxicol. 9, 15–19.

Exner, O., 1978. In: Correlation analysis in chemistry; recent advances, edited by N.B. Chapman and J. Shorter, Plenum Press, New York.

Hammers, W.E., G.J. Meurs and C.L. De Ligny, 1982. Correlations between liquid chromatographic capacity ratio data on Lichrosorb RP-18 and partition coefficients in the octanol-water system. J. Chromatogr. 247, 1-13.

Hansch, C. and A. Leo, 1979. Substituent constants for correlation analysis in chemistry and biology, John Wiley & Sons, New York.

Heitmuller, P.T., T.A. Hollister and P.R. Parish, 1981. Acute toxicity of 54 industrial chemicals to Sheepshead Minnow *(Cyprinodeon variegatus)*. Bull. Environ. Contam. Toxicol. 27, 596-604.

Hermens, J., F. Busser, P. Leeuwangh and A. Musch, 1985. Quantitative correlation studies between the acute lethal toxicity of 15 organic halides to the guppy and chemical reactivity towards 4-nitrobenzylpyridine. Toxicol. Environ. Chem. 9, 219-236.

Hermens, J., P. Leeuwangh and A. Musch, 1984. Quantitative structure-activity relationships and mixture toxicity studies of chloro- and alkylanilines at an acute lethal toxicity level to the guppy (*Poecilia reticulata*). Ecotoxicol. Environ. Saf. 8, 388-394.

Holcombe, G.W., G.L. Phipps, M.L. Knuth and T. Felhaber, 1984. The acute toxicity of selected substituted phenols, benzenes and benzoic acid esters to Fathead Minnows, *Pimephales promelas*. Environ. Pollut. (Series A) 35, 367-381.

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

LeBlanc, G.A. 1984. Interspecies relationships in acute toxicity of chemicals to aquatic organisms. Environm. Toxicol. Chem. 3, 47-60.

Maki, A.H. and D.H. Geske, 1961. Electron spin resonance and polarographic investigation of substituted nitrobenzene negative ions. J. Am. Chem. Soc. 83, 1852-1860.

Oliver, B.G. and A.J. Niimi, 1985. Bioconcentration factors of some halogenated organics for rainbow trout: Limitations in their use for prediction of environmental residues. Environ. Sci. Technol. 19, 842-849.

Pearson, J., 1948. The reduction of nitro-compounds at the dropping mercury cathode. Part I: The nitrobenzenes and nitrotoluenes. Trans. Faraday Soc. 44, 683-697.

Rekker, R.F., 1977. The hydrophobic fragmental constant. Elsevier, Amsterdam/New York.

Rickert, D.E., B.E. Butterworth and J.A. Popp, 1984. Dinitrotoluene: Acute toxicity, oncogenicity, genotoxicity and metabolism. CRC Crit. Rev. Toxicol. 13, 217-234.

Spanggord, R.J., K.E. Mortelmans, A.F. Griffin and V.F. Simmon, 1982. Mutagenicity in *Salmonella typhimurium* and structure-activity relationships of wastewater components emanating from the manufacture of trinitrotoluene. Environ. Mutagen. 4, 163-179.

Sternson, L.A., 1975. Detection of arylhydroxylamines as intermediates in the metabolic reduction of nitro-compounds. Experientia 31, 268-270.

Turner Jr., M.J., R.J. Levine, D.D. Nystrom, Y.S. Crume and D.E. Rickert, 1985. Identification and quantification of urinary metabolites of dinitrotoluenes in occupationally exposed humans. Toxicol. Appl. Pharmacol. 80, 166-174.

Uehleke, H., 1964. Nitrosobenzol im Blute von Katzen nach Verabreichung von Nitrobenzol. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 247, 412-418.

Veith, G.D., D.L. DeFoe and B.V. Bergstedt, 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Res. Board Can. 36, 1040-1048.