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Calculated values of the octanol–water partition coefficient and aqueous solubility for aminoazobenzene dyes and related structures

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Abstract

Results from calculations of the logarithm of the octanol–water partition coefficient, log *P*, and the aqueous solubility, *S*, for a wide range of arylamine dyes and related structures are presented. Since many arylamine dyes have functional groups that easily form ions, the logarithm of the apparent partition coefficient for dissociative systems, log *D*, has also been calculated as a function of pH for some of these compounds. Correlations of the observed biological behavior of various dyes with log *P* and *S* are discussed.

Keywords: Octanol–water partition coefficient; Log *P*; Aqueous solubility; Aminoazobenzene dyes; Structure–activity relationships

1. Introduction

Recent advances in combinatorial chemistry [1] have enabled large numbers of new compounds to be synthesized rapidly in parallel, which has increased the demand for reliable methods to assess the potential toxicity of emerging drugs, pesticides, dyes, surfactants, etc. Rigorous animal-based testing of all new and existing chemicals is not economically practical [2,3]. One alternative approach that has proved to be useful in screening broad classes of chemicals, e.g. aromatic and heterocyclic amines [4], for a specific toxicological problem, e.g. mutagenicity, involves the development of quantitative structure–activity relationships (QSARs). QSARs are mathematical models that attempt to correlate the biological activity of a compound with some of its structural or property descriptors.

The octanol–water partition coefficient, *P*, is one physical property of a compound that has often been linked to its biological behavior [5]. This coefficient is a property of the two-phase system in which water and 1-octanol are in equilibrium at a fixed temperature (typically 25 °C) and the substance is distributed between the water-rich and octanol-rich phases. *P* is defined as the ratio of the equilibrium concentration of the substance in the octanol-rich phase to that in the water-rich phase, in the limit of zero concentration. It is a measure of the relative lipophilic/hydrophobic behavior of a chemical and tends to be large for compounds with extended non-polar structures, e.g. long chain or multi-ring hydrocarbons, and small for compounds with highly polar groups. *P* has been measured for

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a large number of chemicals and has been found to vary over more than 12 orders of magnitude [6]. Consequently, it is the logarithm of P, log P, that is commonly reported in the literature. Studies have shown that log P is an indicator of a chemical's ability to permeate membranes and interact with biological receptors [7,8]. As such, it has become a valuable parameter in developing QSARs for the pharmaceutical, environmental, biochemical and toxicological sciences [7–10].

Although log P is widely used for correlating the biological effects of organic substances, relatively few experimental values of this property are currently available in the literature for azobenzene-based dyes [11–13]. The purpose of the present paper is to report calculated values of log P for a variety of monosubstituted 4-aminoazobenzene (AAB), *N*-methyl-4-aminoazobenzene (MAB) and *N*,*N*-dimethyl-4-aminoazobenzene (DAB) derivatives, as well as, for some of their proposed metabolites. We also report calculated log P values for a selection of the arylamine dyes listed in the color index (CI) [14]. Many azo-based dyes, however, have functional groups that can easily form ions. In these cases the partitioning of the dye between the water and octanol phases depends on the extent of ionization of the dye and the partition constants of the various microspecies present in the mixture. In such cases, the logarithm of the apparent partition coefficient for dissociative systems, log D, as a function of pH gives a more appropriate description of this composite equilibria [15]. We report calculated log D versus pH curves for several AAB, MAB and DAB derivatives. Since the aqueous solubility, S, of an azo compound is often an important consideration for its use in many textile applications, we provide quantitative predictions of S for a wide range of azo dyes. The potential use of log P and S for QSAR development involving the mutagenicity/carcinogenicity of azo compounds is also discussed.

2. Computational section

A variety of methods have been developed to calculate log P [10, 16–23]. These methods generally assume that log P is an additive-constitutive molecular property [4]. Despite this assumption, experience has shown that values of log P can be reliably predicted, particularly for a family of closely related compounds [24]. Unfortunately, many of these methods have not yet been fully developed and they predict identical values of log P for *all* the positional isomers of a compound [4,25], even though experimental measurements of log P have clearly shown that there can be substantial differences [4].

For this study, we have employed the log D suite from Advanced Chemical Development, Inc. (ACD) [15] to calculate log P, log D, and the aqueous solubility of a variety of azobenzene-based structures. This suite calculates log P using a unique additive-constitutive algorithm which employs a database of experimental log P values for over 3600 structures with 500 different functional groups. It incorporates contributions to log P from separate atoms, structural fragments, and intramolecular interactions between structural fragments. These latter pair-wise group contributions generally depend on the separation between the fragments, which can lead to different values of log P for the positional isomers of a compound. For azobenzene derivatives and dyes it is important to differentiate the properties of their positional isomers because there are some well-known examples where different positional isomers display radically different biological activities, e.g. 3-OMe–AAB is a potent hepatocarcinogen in the rat [26], whereas 2-OMe–AAB is a noncarcinogen under similar conditions; neither of these dyes, however, is mutagenic per se in the *Salmonella typhimurium* strains TA98 or TA100 [26].

In general, log P can only be calculated accurately for uncharged species. However, if a compound contains one or more ionogenic groups, e.g. NH_2, OH, etc., it may exist as a mixture of ionic forms (each of which has its own octanol–water partition coefficient) that is strongly pH dependent. This is illustrated for 2′-OH–AAB in Fig. 1. In such cases the logarithm of the partition coefficient for dissociative systems, log D, gives a better description of the complex octanol-water partitioning equilibria. Log D is defined as

$$\log\ D = \log\left(\frac{\Sigma a_i^{\text{octanol}}}{\Sigma a_i^{\text{H}_2\text{O}}}\right),$$

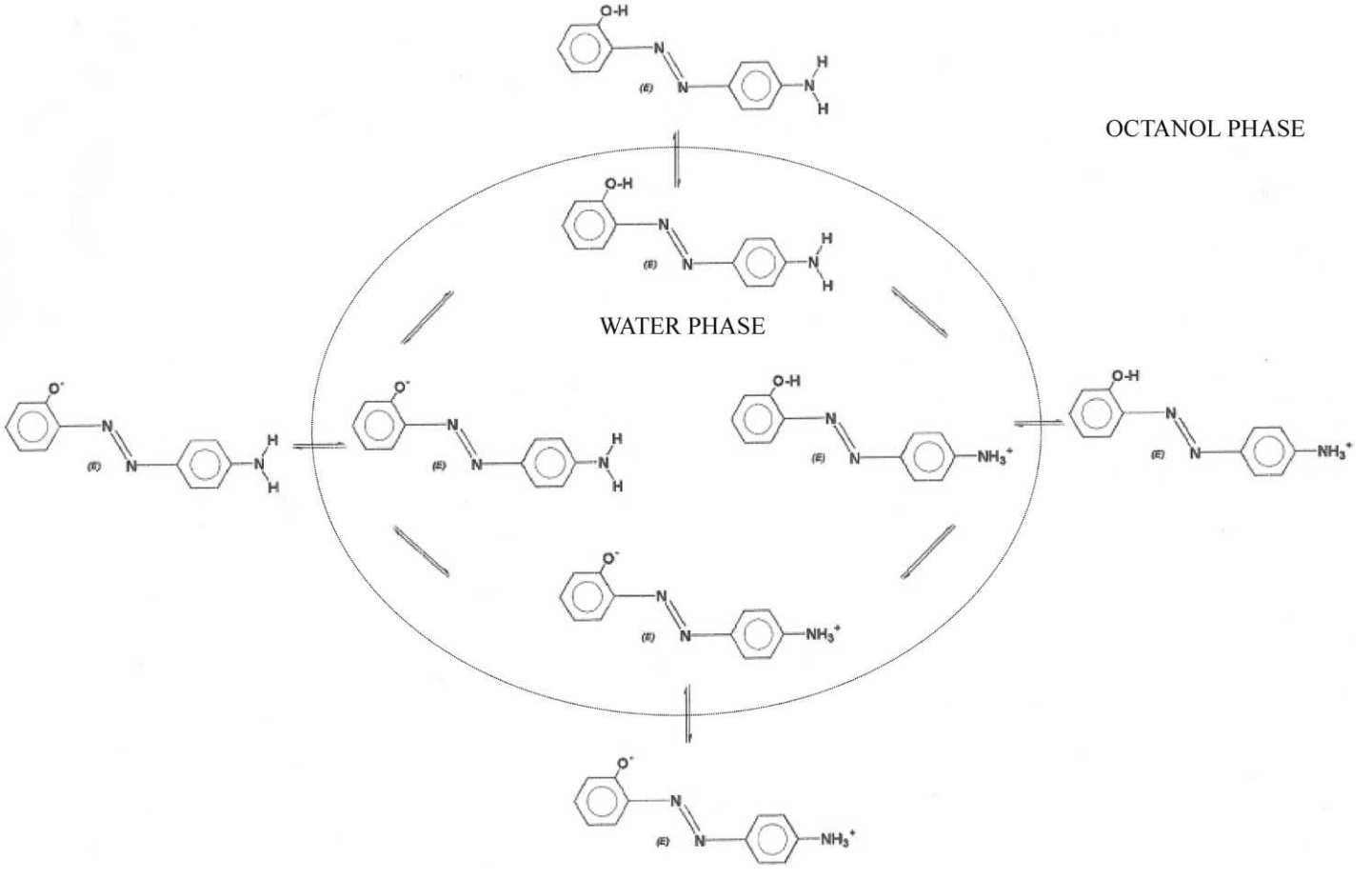


Fig. 1. Illustration of the complex equilibria involved with compounds containing ionogenic groups.

where a_i^{octanol} is the concentration of the ith microspecies in the octanol phase, and a_i H_2O is the concentration of the ith microspecies in the aqueous phase *at a given pH*.

It should be noted that the ACD software allows the user to test compounds for alternative tautomeric forms e.g. *ortho*-hydroxy azobenzene dyes (2′-OH–AAB) and log *D* can then be calculated for these structures as well. Since only vague descriptions of the aqueous solubility of most azo dyes are usually available in the literature, we have employed the ACD/aqueous solubility suite [15] to calculate *S* (g/l) at 25 °C and at various values of the pH for some of these dyes. In general, the melting point of a compound is required to accurately predict its aqueous solubility from the ACD software. Unfortunately, melting points of many azo dyes are not available in the literature, which leads to less reliable estimates of their aqueous solubility.

3. Results and discussion

The numbering system that we are using for the AAB, MAB and DAB derivatives is shown in Fig. 2. Calculations have shown that substitution at the 2- and 6-positions or at the 3- and 5-positions (as well as for the 2′- and 6′-positions and for the 3′- and 5′-positions) yield distinct conformers that have different electronic properties [25, 27, 28]. Nevertheless, such distinctions are not usually made in the experimental literature. The ACD/log *D* suite gives identical values of log *P* and log *D* for some of the positional isomers, e.g. 3-X–AAB and 5-X–AAB, but it can give different values for the positional isomers 2-X–AAB and 3-X–AAB, as well as for 2′-, 3′-, and 4′-X–AAB.

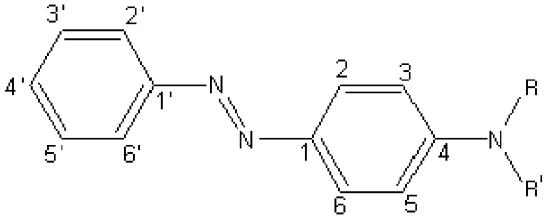


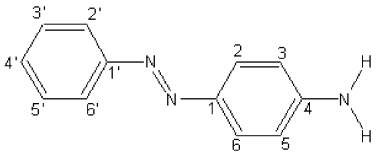
Fig. 2. Numbering system for AAB (R = R′ = H), MAB (R = H, R′ = CH_3) and DAB (R = R′ = CH_3).

In Table 1, we list calculated log *P* values for the 2(6)-, 3(5)-, 2′(6′)-, 3′(5′)-, and 4′-positional isomers of a variety of monosubstituted AAB compounds. The corresponding results for DAB are given in Table 2 and the values for the analogous MAB derivatives are about midway between those for AAB and DAB, e.g. log *P* for 2-Cl–AAB, 2-Cl–MAB, and 2-Cl–DAB are 3.95, 4.76, and 5.33 respectively. The estimated uncertainty in each log *P* value that is reported from the ACD/log *P* software [15] is also listed in these tables. We note that the experimental value of log *P* for DAB is 4.58 [13]; its calculated value, 4.43 ± 0.33, is in good agreement with the experimental result. As can be seen from Tables 1 and 2, there are instances where the values of log *P* among the positional isomers of a compound differ by more than one log unit for a given substituent, e.g. the values of log *P* for 3-NO_2–AAB and 3′-NO_2–AAB are 4.37 and 3.04 respectively. On the other hand, the predicted values of log *P* are the same within the uncertainty of the calculations for methyl substitution at each of the possible positions in AAB. Experimental evidence from other classes of compounds do suggest that changing the position of a methyl group has relatively little effect on the value of log *P*, e.g. the experimental values of log *P* for 2-, 3-, and 4-methylaniline are, 1.32, 1.40, and 1.39 respectively, while those for 2-, 3-, 4-, 6-, and 7-methylquinoline are 2.59, 2.53, 2.61, 2.57, and 2.47 respectively [4, 24].

The range of log *P* values for the monosubstituted AAB compounds in Table 1 (or for the monosubstituted DAB compounds in Table 2) is rather large, indicating that the octanol–water partition coefficient varies over some 4 orders of magnitude. This finding shows clearly that there are substantial differences in the hydrophobicity of these compounds, despite their common backbone. It may be noted that methyl substitution on the amino group increases lipophilicity, e.g. the values of log *P* for AAB, MAB, and DAB are 3.13, 3.79, and 4.43 respectively.

Log *P* has become an increasingly important descriptor in QSAR development [29–31]. For example, Debnath et al. [4] have shown that the mutagenic activity of some aromatic and heteroaromatic amines can be correlated to their hydro-

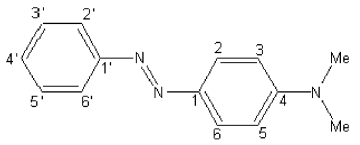
Table 1
Values of log *P* calculated using the ACD/log *P* suite [15] for the positional isomers of monosubstituted AAB



Substituent/position	2	3	2′	3′	4′
–H	3.13±0.32	3.13±0.32	3.13±0.32	3.13±0.32	3.13±0.32
$-CH_3$	3.59±0.32	3.59±0.32	3.59±0.32	3.59±0.32	3.59±0.32
$-CF_3$	4.98±0.42	5.12±0.43	4.10±0.40	4.50±0.41	4.10±0.39
–C≡N	4.07±0.42	3.44±0.41	3.37±0.41	2.74±0.38	3.07±0.39
–COOH	3.78±0.40	3.46±0.39	3.61±0.38	2.87±0.35	3.18±0.35
–CHO	3.67±0.42	3.56±0.42	3.36±0.41	2.61±0.38	2.92±0.38
–F	3.52±0.43	3.58±0.43	3.13±0.42	3.30±0.42	3.42±0.42
–Cl	3.95±0.39	4.22±0.39	3.67±0.39	3.84±0.39	3.97±0.39
–Br	4.24±0.43	4.51±0.43	3.85±0.42	4.02±0.42	4.14±0.42
–I	4.88±0.44	4.61±0.43	4.11±0.42	4.28±0.42	4.40±0.42
–OH	2.45±0.38	2.97±0.39	2.31±0.38	2.73±0.38	2.55±0.38
$-OCH_3$	3.87±0.40	3.48±0.39	3.88±0.40	3.24±0.39	2.95±0.39
$-OCH_2CH_3$	4.40±0.40	4.02±0.39	4.42±0.40	3.77±0.39	3.48±0.39
$-OCH_2CH_2CH_3$	4.93±0.40	4.55±0.39	4.95±0.40	4.31±0.39	4.01±0.39
$-OCH_2CH_2CH_2CH_3$	5.46±0.40	5.08±0.39	5.48±0.40	4.81±0.39	4.54±0.39
$-OCH_2CH_2OH$	2.89±0.41	2.51±0.40	2.91±0.41	2.27±0.40	1.97±0.39
$-NH_2$	1.30±0.39	2.58±0.39	1.77±0.38	2.19±0.39	2.44±0.39
$-N(CH_2CH_3)_2$	5.14±0.41	4.56±0.39	5.14±0.40	4.50±0.39	4.80±0.39
$-N(CH_2CH_2OH)_2$	2.56±0.72	1.98±0.71	2.56±0.72	1.92±0.71	2.22±0.71
$-NO_2$	4.20±0.41	4.37±0.41	3.67±0.37	3.04±0.35	3.51±0.36
$-SO_3H$	1.14±0.78	2.68±0.40	1.38±0.78	1.78±0.39	1.84±0.39

phobicity/lipophilicity using log *P*; AAB based dyes, however, were not included in their study. In Table 3A [32–42] we list the mutagenic activity (rev/μmol) for 3-OMe, 3-OEt, 3-OPr-, and 3-OBu–AAB observed in *Salmonella* mutagenicity tests (Ames test) with the TA98 bacterial strain as reported by Freeman et al. [32]; these results were obtained in the presence of a mammalian microsomal activation system (+S9). Also included in this table are the values of log *P* calculated using the ACD software [15]. As noted by Freeman et al. [32], the observed number of rev/μmol *decreases* as the length of the alkoxy side chain increases, although the value for 3-OEt–AAB appears to be exceptionally low. The corresponding values of log *P increase*, showing that the lipophilicity increases as the length of this chain increases. Since Freeman et al. [32] also reported the melting points of these compounds, we used the ACD software to estimate their aqueous solubility at neutral pH, see Table 3A. (Since the ACD software uses both the melting point and molecular weight of a compound to estimate its aqueous solubility, we have also listed these parameters in Table 3.) For this series of alkoxy compounds, as log *P* increases, the value of *S* decreases but, as we shall see, these parameters do not always act in opposition. The solubilities of these alkoxy dyes are all relatively small, but *decrease* as the length of the side chain increases. Thus, for this small series of related dyes,

Table 2
Values of log *P* calculated using the ACD/log *P* suite for the positional isomers of various monosubstituted DAB compounds



Substituent/position	2	3	2′	3′	4′
–H (DAB)[a]	4.43±0.33	4.43±0.33	4.43±0.33	4.43±0.33	4.43±0.33
$-CH_3$	4.89±0.34	4.89±0.34	4.89±0.34	4.89±0.34	4.89±0.34
$-CF_3$	6.15±0.43	6.52±0.44	5.40±0.41	5.80±0.42	5.40±0.40
–C≡N	5.05±0.42	4.25±0.41	4.68±0.42	4.05±0.39	4.37±0.40
–COOH	5.01±0.41	3.98±0.39	4.91±0.39	4.17±0.36	4.48±0.36
–CHO	4.83±0.42	3.72±0.41	4.66±0.42	3.91±0.39	4.22±0.39
–F	4.73±0.44	4.91±0.44	4.43±0.43	4.60±0.43	4.72±0.43
–Cl	5.33±0.39	5.50±0.39	4.97±0.39	5.14±0.39	5.27±0.39
–Br	5.51±0.44	5.68±0.44	5.15±0.43	5.32±0.43	5.44±0.43
–I	5.80±0.44	5.38±0.44	5.41±0.43	5.58±0.43	5.70±0.43
–OH	3.58±0.39	3.82±0.39	3.61±0.39	4.03±0.39	3.85±0.39
$-OCH_3$	5.34±0.41	4.44±0.40	5.19±0.41	4.54±0.39	4.25±0.39
$-OCH_2CH_3$	5.87±0.41	4.98±0.40	5.72±0.41	5.08±0.39	4.78±0.39
$-OCH_2CH_2CH_3$	6.40±0.41	5.51±0.40	6.25±0.41	5.61±0.39	5.31±0.39
$-OCH_2CH_2CH_2CH_3$	6.93±0.41	6.04±0.40	6.78±0.41	6.14±0.39	5.84±0.39
$-OCH_2CH_2OH$	4.37±0.41	3.47±0.41	4.21±0.41	3.57±0.40	3.27±0.40
$-NH_2$	3.07±0.39	3.55±0.39	3.07±0.39	3.49±0.39	3.74±0.39
$-N(CH_2CH_3)_2$	6.54±0.41	5.35±0.40	6.44±0.41	5.80±0.40	6.10±0.40
$-N(CH_2CH_2OH)_2$	3.96±0.72	2.77±0.72	3.86±0.72	3.22±0.72	3.52±0.72
$-NO_2$	4.54±0.39	5.65±0.41	4.97±0.39	4.34±0.36	4.81±0.37
$-SO_3H$	3.12±0.79	3.08±0.39	2.68±0.78	3.08±0.39	3.14±0.39

[a] The experimental value for the log *P* of DAB is 4.58 [13].

a decrease in *S*, or an increase in log *P*, is correlated with a lower level of mutagenic behavior in the Ames test with the TA98 bacterial strain.

Unfortunately, high-quality mutagenic data for complete series of related AAB, MAB, and DAB compounds is extremely scarce. In Table 3B–E [32–42] we list observed mutagenicity data for AAB, 4-nitroazobenzene (NAB), MAB, DAB, and their 2-, 3-, and 4′-OMe derivatives; when no quantitative data could be found, we included qualitative data (mild, severe, etc.), if it was available. Calculated values of log *P* for these compounds and their aqueous solubility at neutral pH are also given in the tables. These methoxy-derivatives are important because their positional isomers often show radically different carcinogenic and/or mutagenic behavior. It is generally believed that methoxy substitution at the 2-position in AAB, NAB, MAB, or DAB gives compounds that are non-mutagenic or weakly mutagenic in TA98 (+S9) and are non-carcinogenic; methoxy substitution at the 3-position gives compounds that show significant mutagenic activity in TA98 (+S9) and are often potent carcinogens. The available data in Table 3 is in accord with this notion, although quantitative data for MAB and DAB are lacking. In each of these series of compounds, it is quite striking that the calculated value of log *P* is consistently the *largest* (and the aqueous solubility is the *lowest*) for methoxy substitution at the 2-position, where the resulting compound has the lowest mutagenic activity. It is also worth noting that 3-OMe–NAB is significantly less mutagenic than 3-OMe–AAB and is

Table 3
Observed mutagenicity (rev/μmol) in the TA98 *Salmonella* bacterial strain, calculated log *P* [15], calculated aqueous solubility [15], experimental melting point (°C), and molecular weight, of **A.** 3-OR–AAB (R = Me, Et, Pr, Bu), **B.** *n*-OMe–AAB (*n* = 2, 3, 4′), **C.** *n*-OMe–NAB, and **D.** *n*-OMe–MAB, **E.** *n*-OMe–DAB

Compound	TA98 rev/μmol (+S9) ([Ref.])	ACD/log *P* (neutral form)	ACD/Aqueous solubility (g/l) pH = 7	Melting point (°C) ([Ref.])	Molecular weight (g/mol)
A.					
AAB	204 [32]	3.13	0.041	124–125 [32]	197.236
3-OMe–AAB	77065 [32]	3.48	0.021	110–111 [32]	227.262
3-OEt–AAB	13802 [32]	4.02	0.0063	107–109 [32]	241.289
3-OPr–AAB	18919 [32]	4.55	0.0022	97–98 [32]	255.315
3-OBu–AAB	4983 [32]	5.08	0.0012	63–65 [32]	269.342
B.					
AAB	204 [32]	3.13	0.041	124–125 [32]	197.236
2-OMe–AAB	10 [33]	3.87	0.0037	157–159 [34]	227.262
3-OMe–AAB	77065 [32]	3.48	0.021	110–111 [34]	227.262
4′-OMe–AAB	2300 [33]	2.95	0.027	155–159 [34]	227.262
C.					
NAB	400 [35]	4.20	0.0029	132–134 [36]	227.219
2-OMe–NAB	20 [35]	5.26	0.00054	90–91 [36]	257.245
3-OMe–NAB	2250 [35]	4.18	0.0025	131–132 [36]	257.245
4′-OMe–NAB	40 [35]	4.02	0.0022	158–159 [36]	257.245
D.					
MAB	183 [37]	3.79	0.018	87.5–88[a] [37]	211.263
2-OMe–MAB	–	4.34	0.0028	114–115 [34]	241.289
3-OMe–MAB	–	3.88	0.016	72–74 [34]	241.289
4′-OMe–MAB	–	3.61	0.020	93–95 [40]	241.289
E.					
DAB	140 [42]	4.43	0.0024	117–118 [41]	225.289
2-OMe–DAB	–[c]	5.34	0.00035	104–105 [34]	255.315
3-OMe–DAB	–[d]	4.44	(0.0030)[b]	–	255.315
2′-OMe–DAB	–[e]	5.19	0.00065	90–91 [34]	255.315
3′-OMe–DAB	–[f]	4.54	0.0027	87–88 [34]	255.315
4′-OMe–DAB	–[e]	4.25	0.0017	146–147 [40]	255.315

[a] Other values of the melting point have been reported as 86° [38] and 89° [39].
[b] Calculated without melting point data which is not available in the literature.
[c] Noncarcinogen [42].
[d] Carcinogen, but less so than 3-OMe–AAB and 3-OMe–MAB.
[e] Mild carcinogen [42]
[f] Severe carcinogen [42].

nearly a factor of 10 less soluble (the value of log *P* is also higher); a similar comparison holds between 4′-OMe–NAB and 4′-OMe–AAB, see Table 3.

Other factors (electronic, steric, etc.), however, must also have an effect on the mutagenic behavior of these compounds [4,24,27,28]. For example, it is well known that 4′-OMe–AAB is mutagenic in TA98 in the presence of S9 [33], but to a much lesser degree than 3-OMe–AAB, see Table 3B. The value of log *P* for 4′-OMe–AAB, 2.95, however, is smaller than the value of log *P* for 3-OMe–AAB, 3.48, (4-OMe–AAB is also more soluble in water than 3-OMe–AAB) the reverse of what one might have expected based on our observations above. To determine the extent to which altering the pH changes the relative octanol–water equilibria for 2-OMe–, 3-OMe–, and 4′-OMe–AAB, we have plotted log *D* versus pH for these compounds in Fig. 3; the corresponding aqueous solubility versus pH curves are plotted in Fig. 4. For this collection of compounds, log *D* is

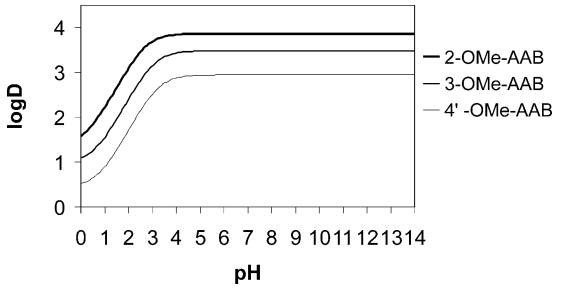


Fig. 3. Plot of log *D* versus pH for 2-OMe–, 3-OMe–, and 4′-OMe–AAB.

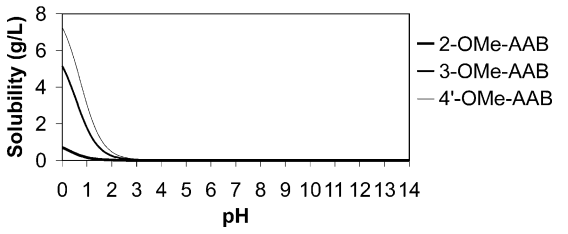


Fig. 4. Plot of aqueous solubility, *S* (g/l), versus pH for 2-OMe–, 3-OMe–, and 4′-OMe–AAB.

consistently smallest for 4′-OMe–AAB and highest for 2-OMe–AAB at all values of the pH. It is interesting to note that under extremely acidic conditions, the solubility of the non-carcinogen 2-OMe–AAB is substantially lower than that of either 3- or 4′-OMe–AAB, see Fig. 4. This finding may be of importance in situations where these dyes are administered orally in animal testing. Thus, the observed difference in mutagenic behavior of 2-OMe–, 3-OMe– and 4′-OMe–AAB cannot be explained entirely on the basis of log *P*, *S*, or the variation of these parameters with pH. The mutagenic behavior of 3-OMe–AAB appears to be abnormally high based on its values of log *P* and *S*. This extreme activity may be a result of interactions that involve the methoxy group in the ultimate covalent adduct formed from the nitrenium ion, 3-OMe–AAB–NH^+, and DNA, especially at guanine residues [43,44]. (A methoxy group at the 3-position is relatively close to the point where the nitrenium ion bonds to the DNA.)

The only reliable mutagenicity information we could find for methoxy substitution at the 2′- and 3′-positions was for DAB, see Table 3E. The dye 2′-OMe–DAB is considered a mild mutagen and a mild carcinogen, whereas 3′-OMe–DAB is a severe carcinogen. Again, the less mutagenic compound, 2′-OMe–DAB, has the larger value for log *P*, 5.19 (only slightly below that of 2-OMe–DAB, 5.34) and the lower value for *S*. Clearly, however, other effects must be considered since 4′-OMe–DAB is also a mild mutagen [42], but its calculated value of log *P*, 4.25, is lower than that of either 2′- or 3′-OMe–DAB. More quantitative mutagenic data on all the methoxy derivatives of AAB, MAB, and DAB (hopefully from a single laboratory to ensure the data is consistent) would be very helpful in sorting out the role of log *P* in predicting possible mutagenic behavior of member in this class of dyes.

Various substitutions at the 3′-position in AAB, MAB, and DAB have also resulted in compounds that show mutagenic activity. In Table 4A [38,40–42, 45] we list the observed mutagenicity (rev/μmol) for DAB and its 3′-CH_2OH, 3′-CHO, and 3′-COOH derivatives. These substitutions result in increased activity in TA98 (+S9) compared to DAB. (The activity of AAB, MAB, and DAB are *all* increased by –CH_2OH substitution at the 3′-position, see Table 4B, to a greater extent than by CH_3 substitution at this position, see Table 4C.) The least mutagenic of the compounds in Table 4A, DAB, is again found to have the lowest value of *S* and the largest value of log *P*. The correlation between mutagenic activity and solubility (or log *P*), however, is not direct: 3′-CH_2OH–DAB is some three times more potent as a mutagen than 3′-COOH–DAB, but it is a factor of ten less soluble at neutral pH. (It should be noted, however, that 3′-COOH–DAB is found to be more soluble than all of the compounds we considered in Table 3; this may be an indication of some kind of bilinear dependence of mutagenicity on solubility [4,24].)

In Table 5 [37,46,47] we list the mutagenicity data for MAB, 3′-Me–MAB, and 4′-Me–MAB and some of their possible metabolites in the bacterial strain TA98; calculated values of log *P* and *S* (g/l) are also given. We chose this series of compounds because there is a relatively complete collection of experimental data available in the literature. Unfortunately, the mutagenic behavior of MAB, 3′-Me–MAB, and 4′-Me–MAB are

Table 4
Observed mutagenicity (rev/μmol) in the TA98 *Salmonella* bacterial strain, calculated log *P* [15], calculated aqueous solubility, melting point (°C), and molecular weight, of **A.** 3′-R–DAB (R = H, CH_2OH, CHO, COOH), **B.** 3′-CH_2OH–R (R = AAB, MAB, DAB), and **C.** 3′-Me–R (R = AAB, MAB, DAB)

Compound	TA98 rev/μmol (+S9) ([Ref.])	ACD/log *P* (neutral form)	ACD/aqueous solubility (g/l) pH = 7	Melting point (°C) ([Ref.])	Molecular weight (g/mol)
A.					
DAB	140 [42]	4.43	0.0024	117–118 [41]	225.289
3′-CH_2OH–DAB	601 [38]	3.25	0.023	122–123 [45]	255.315
3′-CHO–DAB	383 [38]	3.91	0.0087	97–99 [45]	253.299
3′-COOH–DAB	201 [38]	4.17	0.30	212–213 [45]	269.299
B.					
3′-CH_2OH–AAB	596 [38]	1.94	0.59	107–109 [45]	227.262
3′-CH_2OH–MAB	503 [38]	2.60	0.10	119–121 [45]	241.289
3′-CH_2OH–DAB	601 [38]	3.25	0.023	122–123 [45]	255.315
C.					
3′-Me–AAB	240 [38]	3.59	0.027	89–91 [41]	211.263
3′-Me–MAB	445 [38]	4.25	0.0042	109–109.5 [41]	225.289
3′-Me–DAB	356 [38]	4.89	0.00031	168–170 [40]	239.316

Table 5
Observed mutagenicity (rev/μmol) in the TA98 *Salmonella* bacterial strain, calculated log *P*, aqueous solubility, melting point (°C), and molecular weight of MAB, 3′-Me–MAB, 4′-Me–MAB, and some of their possible metabolites

Compound	TA98 rev/μmol (+S9) ([Ref.])	ACD/log *P* (neutral form)	ACD/aqueous solubility (g/l) pH = 7	Melting point (°C) ([Ref.])	Molecular weight (g/mol)
A.					
MAB	183 [37]	3.79	0.018	87.5–88 [37]	211.263
3′-Me–MAB	233 [46]	4.25	0.0042	109–109.5 [46]	225.289
4′-Me–MAB	283 [46]	4.25	0.0045	105–105.5 [46]	225.289
B.					
N–OH–MAB	650 [47]	2.74	0.032	171–174[b] [46]	227.262
N–OH–3′-Me–MAB	1000 [46]	3.20	(0.040)[c]	–	241.289
N–OH–4′-Me–MAB	1132 [46]	3.20	(0.040)[c]	–	241.289
C.					
N–OAc–MAB	26,000[a] [46]	2.94	0.098	74–76 [47]	269.999
N–OAc–3′-Me–MAB	28,000[a] [46]	3.40	0.027	85.5–86.5 [46]	283.325
N–OAc–4′-Me–MAB	55,000[a] [46]	3.40	0.021	98–99 [46]	283.325
D.					
N–OBz—MAB	32,000[a] [46]	4.69	0.0011	89–91 [46]	331.368
N–OBz–3′-Me–MAB	52,000[a] [46]	5.15	0.00032	96–97 [46]	345.395
N–OBz–4′-Me–MAB	38,095[a] [46]	5.15	0.00034	95–96 [46]	345.395

[a] These results were recorded in the *absence* of a mammalian microsomal activation system (−S9).
[b] A much lower value for the melting point of N–OH–MAB, 45°, was given in reference [48].
[c] Calculated without melting point data. This is expected to reduce the reliability of the calculated aqueous solubility.

rather similar and, as in AAB, methyl substitution at 3′- or 4′-position on MAB gives the same value for log *P*, see Table 5A. Methyl substitution on the phenyl ring in MAB (as in AAB or DAB, see Tables 1 and 2) increases log *P* relative to MAB and reduces its (already low) aqueous solubility.

The first step in the metabolic activation of MAB and its derivatives is generally believed to be *N*-hydroxylation [49]. The resulting N–OH compounds have lower values of log *P* (the reduction is about 1 log unit) and greater solubility, see Table 5B. The increase in the solubility of N–OH–

Table 6
Values of log *P* and *S* (g/l) calculated using the ACD/log *P* suite [15] for selected arylamine dyes from the color index [14]

CI number	Class	ACD/log *P* (neutral form)	ACD/aqueous solubility pH = 7 S (g/l)[a]	Melting point (°C)	Molecular weight (g/mol)
11000	CI Solvent Yellow (Aniline Yellow, AAB)	3.13±0.32	8.9×10^{-2} [3.9×10^{-2}]	127.5	197.236
11005	CI Disperse Orange 3				
	CI Solvent Orange 9	3.51±0.36	2.2×10^{-2} [2.7×10^{-3}]	210–212	242.234
11005:1	CI Disperse Orange 3:1	3.97±0.36	7.4×10^{-3}		256.260
11015	Disperse Dye	2.98±0.46	1.5×10^{-2}		330.256
11020	CI Solvent Yellow 2 (DAB)	4.43±0.33	4.9×10^{-3} [2.5×10^{-3}]	115	225.289
11021	CI Solvent Yellow 56	5.49±0.33	4.2×10^{-4}		253.342
11025	CI Disperse Black 3	3.74±0.39	1.5×10^{-2} [2.6×10^{-3}]	186–187	240.304
11030	Disperse Dye	3.88±0.36	5.8×10^{-3}		282.340
11035	CI Disperse Black 7	4.56±0.40	1.8×10^{-3}		274.749
11040	CI Disperse Red 41	5.87±0.42	9.6×10^{-5}		300.313
11060	Disperse Dye	6.35±0.42	1.9×10^{-5}		343.337
11070	Disperse Dye	6.93±0.42	7.9×10^{-6}		328.366
11077	CI Disperse Blue 165	4.96±0.54	9.7×10^{-5}		405.410
11078	CI Disperse Blue 183	5.68±0.35	9.7×10^{-6}		459.297
11079	CI Disperse Red 210	4.27±0.48	2.7×10^{-4}		422.930
11100	CI Disperse Orange 5	5.34±0.61	8.7×10^{-5}		367.186
11110	CI Disperse Red 1	4.58±0.59	9.0×10^{-4} [4.1×10^{-4}]	160	314.339
11113	CI Disperse Red 8	5.06±0.62	1.7×10^{-4}		359.337
11114	CI Disperse Red 72	4.14±0.50	4.6×10^{-4}		406.395
11115	CI Disperse Red 13	5.22±0.60	1.5×10^{-4}		348.784
11116	CI Disperse Red 73	4.71±0.47	4.1×10^{-4}		348.359
11117	CI Disperse Red 90	4.10±0.50	4.6×10^{-4}		406.395
11118	CI Disperse Red 2	3.77±0.60	2.6×10^{-3}		344.365
11119	CI Disperse Orange 30	5.39±0.46	2.0×10^{-5}		450.275
11120	CI Disperse Violet 12	6.12±0.62	1.4×10^{-5}		387.390
11121	CI Disperse Orange 52	5.27±0.66	5.4×10^{-5}		404.914
11125	Solvent Dye	6.28±0.60	1.2×10^{-5}		376.837
11129	CI Solvent 58	2.91±0.71	3.6×10^{-2}		285.341
11130	CI Disperse Red 19	3.29±0.72	8.3×10^{-3}		330.339
11140	CI Disperse Red 82	4.76±0.51	8.0×10^{-5}		439.422
11145	CI Disperse Orange 138	4.02±0.60	2.4×10^{-3}		320.731
11150	CI Disperse Red 7	4.19±0.75	8.3×10^{-4}		364.783
11152	CI Disperse Brown 1	5.48±0.77	2.2×10^{-5}		433.673
11152:1	CI Disperse Brown 1:1	5.36±0.81	1.3×10^{-5}		478.124
11160	CI Solvent Yellow 3	4.05±0.33	1.0×10^{-2} [7.5×10^{-3}]	100	225.289
11180	Disperse Dye	3.85±0.59	4.6×10^{-3}		300.313
11190	CI Disperse Red 32	5.80±0.61	2.8×10^{-5}		383.229
11191	CI Disperse Red 98				
	CI Solvent Red 116	5.56±0.65	2.9×10^{-5}		407.262
11192	CI Disperse Red 109	5.18±0.74	7.5×10^{-5}		395.904
11195	CI Disperse Violet 13	6.58±0.62	4.6×10^{-6}		401.417
11200	CI Disperse Violet 24	6.91±0.68	6.4×10^{-7}		480.313
11205	Disperse Dye	6.92±0.70	Insoluble		510.339
11210	CI Disperse Red 17	3.75±0.72	2.7×10^{-3}		344.365
11215	CI Disperse Red 5				
	CI Solvent Red 117	4.39±0.75	4.5×10^{-4}		378.810
11218	CI Disperse Violet 33	5.22±0.51	2.6×10^{-5}		453.448

(*continued on next page*)

Table 6 (*continued*)

CI number	Class	ACD/log *P* (neutral form)	ACD/aqueous solubility pH = 7 S (g/l)[a]	Melting point (°C)	Molecular weight (g/mol)
11220	CI Disperse Black	3.42±0.76	5.2×10^{-3}		344.408
11225	CI Disperse Red 16	3.65±0.76	5.3×10^{-3}		360.365
11227	CI Disperse Orange 25	4.68±0.41	6.5×10^{-4}		323.349
11228	CI Disperse Red 65	5.77±0.42	3.6×10^{-5}		371.821
11230	Disperse Dye	3.85±0.60	2.0×10^{-3}		353.375
11232	CI Disperse Red 97	3.87±0.63	1.1×10^{-3}		384.346
11239	CI Disperse Orange 62	7.41±0.46	Insoluble		512.344
11240	CI Disperse Orange 7	4.43±0.37	2.4×10^{-3}		270.287
11245	Disperse Dye	3.15±0.41	2.8×10^{-2}		272.303
11250	CI Disperse Red 31	4.33±0.41	2.3×10^{-3}		286.286
11255	CI Disperse Black 2	3.25±0.40	2.9×10^{-2}		256.303
11260	CI Disperse Blue 11	4.69±0.44	8.9×10^{-4}		302.286
11275	Oxidation Dye	0.60±0.40	7.31		227.265
11282	CI Disperse Red 63	1.68±0.40	5.9×10^{-1}		257.248
11285	CI Solvent Brown 1	2.53±0.39	1.1×10^{-1}		262.309
11290	CI Mordant Brown 12	1.09±0.42	9.26×10^{2}		318.245
11300	CI Mordant Brown 48	2.41±0.44	4.28×10^{1}		352.690
11310	Disperse Dye	3.48±0.46	9.2×10^{-3}		302.246
11330	CI Solvent Brown 2	2.99±0.39	3.5×10^{-2} [2.1×10^{-2}]	148	276.336
11335	CI Mordant Brown 4	1.55±0.42	3.05×10^{2}		332.272
11337	CI Disperse Blue 337	4.30±0.61	7.6×10^{-5}		493.515
11343	CI Disperse Blue 90	4.21±0.71	9.2×10^{-5}		493.268
11345	CI Disperse Blue 79	5.30±0.60	8.6×10^{-7}		639.410
11350	CI Solvent Yellow 4	4.36±0.32	4.0×10^{-3} [2.2×10^{-3}]	123	247.295
11360	CI Solvent Brown 3	5.59±0.33	1.7×10^{-4}		297.353
11365	CI Disperse Black 1	3.67±0.39	1.2×10^{-2} [4.4×10^{-3}]	159–160	262.309
11370	CI Disperse Blue 85	5.93±0.64	7.6×10^{-6}		445.813
11380	CI Solvent Yellow 5	3.69±0.31	1.4×10^{-2} [1.3×10^{-2}]	104	247.295
11385	CI Solvent Red 5	4.07±0.35	3.4×10^{-3} [9×10^{-4}]	185.5	292.292
11390	CI Solvent Yellow 6	4.15±0.31	4.8×10^{-3} [2.9×10^{-3}]	126	261.321
11395	CI Mordant Green 24	3.49±0.41	5.20		353.289
11405	CI Disperse Blue 338	7.19±0.59	Insoluble		493.515
11406	CI Disperse Blue 335	7.70±0.60	Insoluble		347.402
11420	Disperse Dye	5.06±0.49	5.5×10^{-5}		427.252
11430	CI Disperse Blue 38	4.44±1.08	3.0×10^{-4}		398.800
13020	CI Acid Red 2	4.91±0.39	1.82		269.299
13056	CI Disperse Orange 10	2.77±0.58	1.17×10^{-2}		348.421
13058	CI Pigment Red 100	3.39±0.72	1.16×10^{1}		329.351
13379	CI Disperse Red 6	2.81±0.58	1.1×10^{-2}		370.427
13430	Pigment	2.42±1.06	2.23×10^{3}		371.368
13710	CI Acid Black 180	4.99±0.57	8.4×10^{-5}		429.881

[a] Values in [ ] were calculated using the melting point in the next column.

MAB relative to that of MAB, however, is less than one might have expected for *N*-hydroxylation. It should be noted that the reported melting point for N–OH–MAB (171–174 °C) [46] is significantly higher than that of MAB (87.5–88 °C) [37], and that a relatively high melting point for a compound generally reduces its aqueous solubility. A similar differential in melting points has been observed for AAB (124–125 °C) and N–OH–AAB (195–197 °C) [41]. In this case the predicted solubility of N–OH–AAB, 0.013 g/l, is actually *less* than that of AAB, 0.014 g/l. (The value of log *P*

for N–OH–AAB, 2.98, is also *less* than that of AAB, 3.13.) On the other hand, the melting point of N–Ac–AAB (144–146 °C) is slightly higher than that of its *N*-hydroxy derivative, N–Ac–N–OH–AAB (136–138 °C), and the predicted solubility of N–Ac–N–OH–AAB (0.12 g/l) is more than seven times greater than that of N–Ac–AAB (0.016g/l). Although we could not find melting points in the literature for N–OH–3′-Me or N–OH–4′-Me–MAB, it seems likely that their solubilities are greater than those of 3′-Me- and 4′-Me–MAB, see Table 5B. The compounds N–OH–MAB, N–OH–3′-Me–MAB, and N–OH–4′-Me–MAB all show approximately a four fold increase in their mutagenic activity in TA98 (+S9) compared to their corresponding parent compounds.

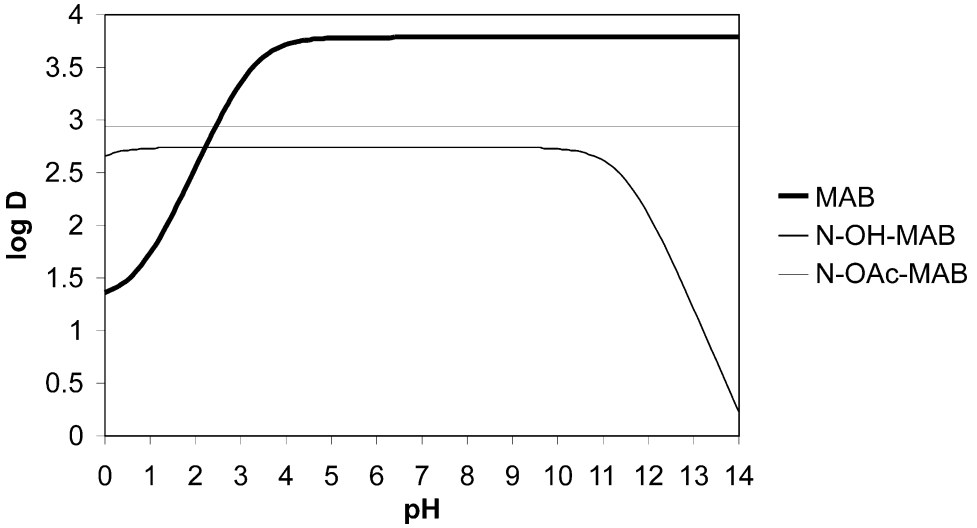


Fig. 5. Plot of log *D* versus pH for MAB, N–OH–MAB, and N–OAc–MAB.

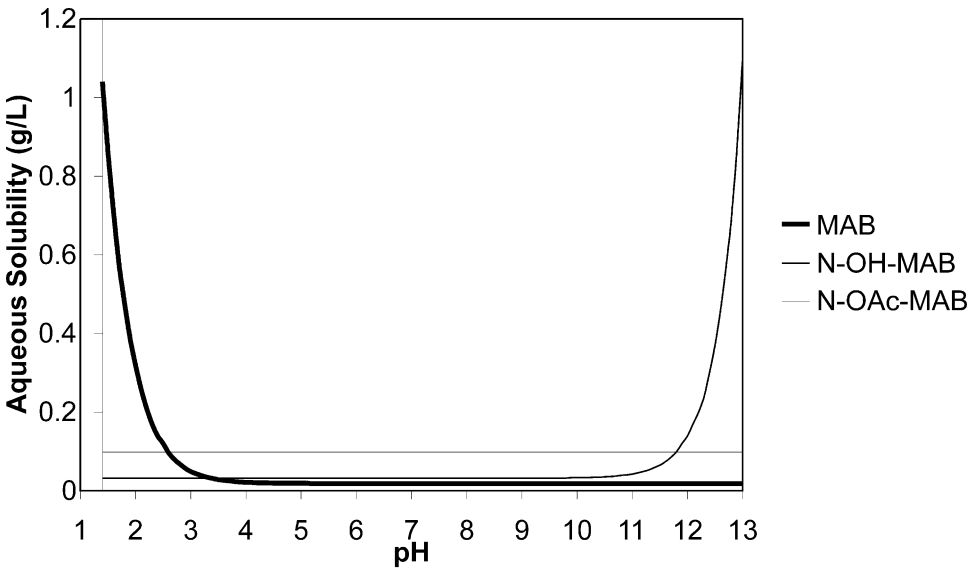


Fig. 6. Plot of aqueous solubility, *S* (g/l), versus pH for MAB, N–OH–MAB, and N–OAc–MAB.

The second step in the activation of MAB is most likely the formation of reactive N–O esters such as N–OAc–MAB [50]. The esters N–OAc–MAB, N–OAc–3′-Me–MAB, and N–OAc–4′-Me–MAB are all highly mutagenic in TA98 in the *absence* of S9, see Table 5C. (The corresponding results for N–OBz–MAB are given in Table 5D.) The calculated values of log *P* for these N–OAc compounds are about 0.2 log units *greater* than the corresponding *N*-hydroxy derivatives. The melting points of these esters, however, are substantially lower than those of the *N*-hydroxides, and their aqueous solubilities are about three times *greater* at neutral pH. For comparison, we note that the melting point for N–Ac–N–OAc–AAB (64–65 °C) is much lower than that of N–Ac–N–OH–AAB (136–138 °C) and the predicted solubility of the N–OAc derivative (0.22 g/l) is nearly double that of the corresponding N–OH derivative (0.12 g/l). In Fig. 5 we plot log *D* versus pH for MAB, N–OH–MAB, and N–OAc–MAB; the corresponding plots of *S* versus pH are given in Fig. 6. The importance of pH on the relative values of log *P* is very evident from Fig. 5. MAB is clearly less lipophilic than either its N–OH or N–OAc derivative at very low values of the pH, but more lipophilic at all other values of the pH. This behavior of log *D* is reflected in a greater aqueous solubility for MAB at low pH; at higher values of the pH, N–OH–MAB is more soluble, see Fig. 6.

In Table 6 we have compiled a list of calculated log *P* values for 86 arylamine dyes given in the CI [14]. We have excluded from the table those dyes which are listed as sodium salts; log *D* appears to be essential in understanding the complex octanol–water partitioning equilibria in these instances. We also list the calculated aqueous solubilities of the dyes at neutral pH. Unfortunately, in many instances we were unable to find melting points for these dyes, and this reduces the reliability of their predicted solubility. To be as consistent as possible, we calculated values of *S* for all the dyes without utilizing the melting point. In those instances where a melting point for the dye was available, we recalculated the aqueous solubility using the melting point and this value is also listed in the Table. Small changes in pH (near neutral) for dyes with relatively low solubility do not usually alter the predicted aqueous solubility of the dye significantly, e.g. for CI Disperse Orange 3 the predicted solubility is 0.0027 g/l at all values of pH from 5 to 9; the solubility of some dyes, e.g. CI Mordant Dye 24, are more sensitive to changes in the pH: 0.76 g/l at pH = 5 to 5.53 g/l at pH = 9.

Most of the compounds in Table 6 are solvent and/or disperse dyes, although a few are mordant or acid dyes. Log *P* varies over a broad range of values (0.60–7.70) for the arylamine dyes in Table 6: from 2.98 to 6.93, 2.91 to 6.28, and 1.09 to 3.49 for disperse, solvent, and mordant dyes, respectively. (The value of log *P* is 0.60 for the one oxidation dye in the table, CI number 11275; the values of log *P* for the two acid dyes are 4.91 and 4.99, CI numbers 13020 and 13710 respectively.) Aqueous solubilities for the solvent and disperse dyes in the table are generally quite low, although

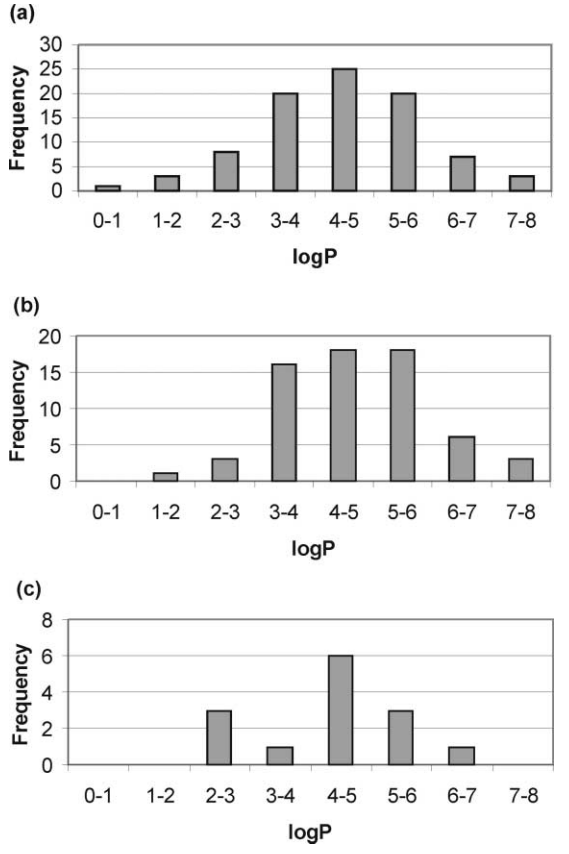


Fig. 7. Distribution of log *P* values for (a) all, (b) the disperse, and (c) the solvent arylamine dyes in Table 6.

they range over several orders of magnitude. As expected, the mordant and acid dyes are far more soluble.

The distribution of log *P* for all of the 86 arylamine dyes listed in Table 6 are shown in Fig. 7(a). The values of log *P* for nearly 75% of these dyes are in the range from 3 to 6 and 35% are above 5. For comparison, we note that a study of the successful drugs developed by the pharmaceutical industry have shown that log *P* for these molecules was invariably less than 5 [51]. Thus, many dyes in common use are more lipophilic than typical drugs. The distribution of log *P* for the 65 disperse dyes from Table 6 is shown in Fig. 7(b). Approximately 80% of these dyes have log *P* values in the range from 3 to 6 and over 41% are above 5. There are 14 solvent dyes in Table 6 and the distribution of their log *P* values are shown in Fig. 7(c). More than 70% of these dyes have a value of log *P* above 4.

4. Conclusions

Log *P* has proved to be a valuable descriptor in understanding the biological behavior of various classes of compounds [4,24]. It is often used in conjunction with other molecular descriptors, e.g. the energy of the highest occupied molecular orbital, HOMO, or lowest unoccupied molecular orbital, LUMO, refractive index, etc., in developing predictive QSARs. Log *P* has an important advantage as a molecular descriptor—it can be calculated quickly. Currently, the expected accuracy is about ± 0.5 log units. The accuracy of the calculations, however, is improving and additional conformational information, including stereochemistry, is being incorporated into the algorithms [15]. Progress is also being made in estimating the aqueous solubility of a compound, particularly when its melting point is known [15].

It now appears that log *P* and *S* may be valuable descriptors in understanding the biological behavior of dyes. This finding may eventually enable us to devise safer dyes. We have shown that for the series of 2-, 3-, and 4′-methoxy substituted AAB, MAB, DAB, and NAB dyes, the compound with the highest log *P*, or the lowest aqueous solubility, has the lowest mutagenic activity in that series; similar results are found for DAB and its 3′-CH_2OH, 3′-CHO, and 3′-COOH derivatives.

To some extent this correlation even works when comparing two different methoxy series. For example, 3-OMe–AAB is far more mutagenic than 3-OMe–NAB; log *P* is greater and *S* is lower for the NAB derivative. Similar results hold for 4′-OMe–AAB and 4′-OMe–NAB. On the other hand, simply increasing log *P* or decreasing aqueous solubility does not automatically decrease the mutagenicity of the dye. Consider 3-OEt- and 3-OPr–AAB, where log *P* is greater and the aqueous solubility is lower for 3-Opr–AAB, but the reported mutagenic activity of 3-OPr–AAB is greater than that of 3-OEt–AAB. Furthermore, related compounds can have similar log *P* and *S* values, but significantly different mutagenic activity, e.g. NAB (log $P = 4.20$, $S = 0.0029$ g/l: 400 rev/μmol) and 3-OMe–NAB (log $P = 4.18$, $S = 0.0025$ g/l: 2250 rev/μmol). More high-quality, quantitative mutagenicity data covering a broader range of azo dyes are clearly needed to establish a baseline of the importance of log *P* and/or *S* in predicting the mutagenic behavior of these dyes. Once such a baseline is established, it will be easier to identify compounds whose mutagenic activity is due to other factors, e.g. steric, electronic, etc. It already appears evident from this limited study that only a fraction of the exceptionally high activity of 3-OMe–AAB can be linked to its values of log *P* or aqueous solubility.

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