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A QSAR Investigation of the Role of Hydrophobicity in Regulating Mutagenicity in the Ames Test: 1. Mutagenicity of Aromatic and Heteroaromatic Amines in *Salmonella typhimurium* TA98 and TA100

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Quantitative structure-activity relationships (QSAR) have been derived for the mutagenic activity of 88 aromatic and heteroaromatic amines acting on *Salmonella typhimurium* TA98 + S9 and 67 amines acting on TA100 + S9. Mutagenic activity is linearly dependent on hydrophobicity, the energy of the highest occupied molecular orbital, and the energy of the lowest unoccupied molecular orbital of the amine. The dependence of mutagenic activity on hydrophobicity and electronic effects is nearly identical for TA98 and TA100. Mutagenic activity in TA98 is also found to depend on the size of the aromatic ring system. Different QSARs are derived for the mutagenic activity of hydrophilic amines (log $P < 1$) acting on either TA98 or TA100. The mechanism of amine activation and reaction with DNA is considered in light of these findings.

Key words: structure-activity relationships

INTRODUCTION

Understanding and predicting the chronic toxic effects of chemicals, especially carcinogenicity, has become one of the major problems facing chemists involved with the development of industrial chemicals (pesticides, drugs, etc.), as well as scientists studying the toxicology of natural products. While the toxic effects of many of the chemicals now entering the public marketplace are being routinely examined, many thousands of new and untested compounds are being synthesized annually in thousands of laboratories and chemists are becoming increasingly concerned about the potential toxicity of the new chemicals they are being exposed to.

The classic approach to toxicology (i.e., the in-depth study of the mechanism of action of a few chemicals), is inadequate for the Herculean task of discovering potentially toxic chemicals among the vast number of new chemicals entering the environment. An alternate approach, which might be called predictive toxicology, is slowly beginning to take shape. In our view, the goal of predictive toxicology is the development of models for predicting various kinds of toxicity starting solely with a knowledge of a given chemical's structure. It is towards this goal that we have been developing quantitative structure-activity relationships (QSAR) for nonspecific toxicity [Hansch et al., 1989] and gene toxicity.

The development of a model for predicting toxicity necessitates a test system capable of providing reproducible and quantitative estimates of toxic activity. Inexpensive mutagenicity assays, such as the Ames test [Maron and Ames, 1983], though imperfect in their predictions, still afford the best means for predicting potential carcinogenic activity. In any case, compounds found to be mutagenic in these assays are likely to modify human DNA as well and are not desirable for human use.

Our preliminary results, which have examined the mutagenicity of triazenes [Shusterman et al., 1989] and nitroarenes [Compadre et al., 1990; Debnath et al., 1991] in the Ames test using *Salmonella typhimurium*, have encouraged us to develop QSARs for other families of chemical mutagens. In this article we report on two new QSARs which describe the behavior of aromatic and heteroaromatic amines in two test systems: *S. typhimurium* TA98 and TA100. In the following paper [Debnath et al., 1992], we

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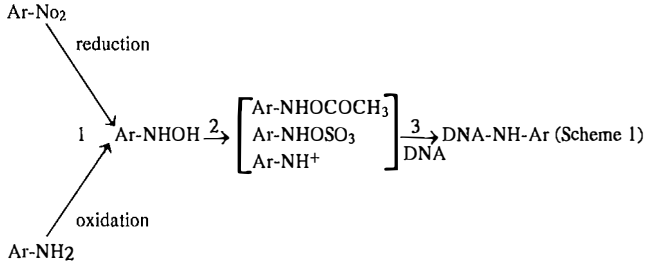
present a new, expanded QSAR for the behavior of nitroarenes in the TA100 system, and examine how these QSARs depend on the structure of the mutagen (nitroarene vs. aminoarene) and the test system (TA98 vs. TA100).

The precise mechanisms of activation of aminoarene and nitroarene compounds to mutagenic species are not known; however, they are believed to share many common features (Scheme 1). It is generally accepted that both types of



compounds are converted to a common hydroxylamine intermediate (step 1), which is then converted to an electrophilic nitrogen species (step 2) which reacts with DNA (step 3). We do not mean to imply that the hydroxylamines all progress through the same intermediate; any or all of the three pathways may be operative. Any difference between the two mechanisms should lie entirely in the initial activation step (i.e., the formation of a hydroxylamine). The amino compounds are mutagenic only in the presence of the S9 microsomal preparation and are believed, therefore, to be oxidized to the hydroxylamine by cytochrome P-450 residing in S9. It is the unstable hydroxylamine, then, that must diffuse to and penetrate the bacterial membrane. The nitro compounds, on the other hand, do not require S9 activation, and it appears that they are reduced to the hydroxylamine by cytosolic reductases. Thus, it is the nitroarene which penetrates the cell, and the metabolic intermediates that are generated have a shorter random walk to their sites of action. The differing demands of oxidation versus reduction, and the different sites of activation, suggest that different structural factors will determine the mutagenic activity of these two classes of compounds.

Equation 1 [Debnath et al., 1991] has been derived to correlate the mutagenicity of aromatic and heteroaromatic nitro compounds in *S. typhimurium* TA98 (parent structures are given in Fig. 1). Examination of the QSAR shows how such equations can not only provide a means for predicting mutagenicity, but can also reveal aspects of the activation mechanism. In this expression, log TA98

$$\log \text{TA98} = 0.65 \log P - 2.90 \log (\beta 10^{\log P} + 1) - 1.38\, \epsilon_{LUMO} + 1.88\, I_L - 2.89\, I_a - 4.15$$
$$n = 188,\ r = 0.900,\ s = 0.886,\ \log P_0 = 4.93,\ \log \beta = -5.48 \quad (1)$$

refers to the mutation rate in log (revertants/nmol), P is the octanol/water partition coefficient, and ϵ_{LUMO} is the energy of the molecule's *l*owest *u*noccupied *m*olecular *o*rbital as calculated by the AM1 method [Dewar et al., 1985]. The statistics describing Eq. 1 are: n, the number of data points upon which the QSAR is based; r, the correlation coefficient; s, the standard deviation from the regression; and β, which is a disposable parameter estimated by an iterative procedure [Kubinyi, 1979]. In Eq. 1 the indicator variable I_L is assigned the value of one for all instances where three or more fused rings are present in the aromatic portion of the mutagen (e.g., anthracene, phenanthrene, etc.) and zero when less than three fused rings are present (e.g., naphthalene, quinoline, benzene, etc.). The coefficient with I_L indicates that the large compounds are about 75 times more potent, other factors being equal, than those with one or two fused rings. The variable I_a takes the value of one for the five nitroacenthrylene compounds in the data set, bringing out their surprisingly low potency.

Equation 1 covers a very wide range of structures, including over 40 heterocycles. Furthermore, Eq. 1 reveals structural factors affecting mutagenic activity which might not have been discovered merely by inspection of Scheme 1. The most important parameter turns out to be log P, on which activity is bilinearily dependent. Mutagenicity increases as 0.65 log P until log P = 4.93 (log P_0) and then decreases as −2.25 log P (0.65–2.90). The dominant role of hydrophobicity in determining relative mutagenicity has also been demonstrated for mutagenic triazenes [Shusterman et al., 1989]. The negative coefficient with ϵ_{LUMO} shows that the more easily a nitro compound is reduced (i.e., the lower its ϵ_{LUMO}, the more mutagenic it will be).

Encouraged by the success of our previous studies, as well as by the attempts of others to construct QSARs for mutagenic amines [Loew et al., 1979; DeFlora et al., 1985; Klopman et al., 1985; Frierson et al., 1986], we have compiled mutagenicity data in TA98 and TA100 and physico-chemical parameters for a broad spectrum of aromatic amines. In addition, we report new mutagenicity data for several amines, along with partition coefficients, and the results of AM1 electronic structure calculations on all of the amines. The resulting QSARs, while consistent with our previous work, contain several novel and unexpected features.

MATERIALS AND METHODS

Chemicals

3-aminoquinoline, 5-aminoquinoline, 6-aminoquinoline, 8-aminoquinoline, 2-aminonaphthalene, and 4-cyclohexylaniline were purchased from Aldrich Chemical Company, Milwaukee, WI. All compounds were purified (>99%) using HPLC (Spectra-Physics). Preparative scale separations were performed using Whatman Partisil 10 (normal phase; 25 cm × 22 mm; hexane/isopropanol; isocratic) and

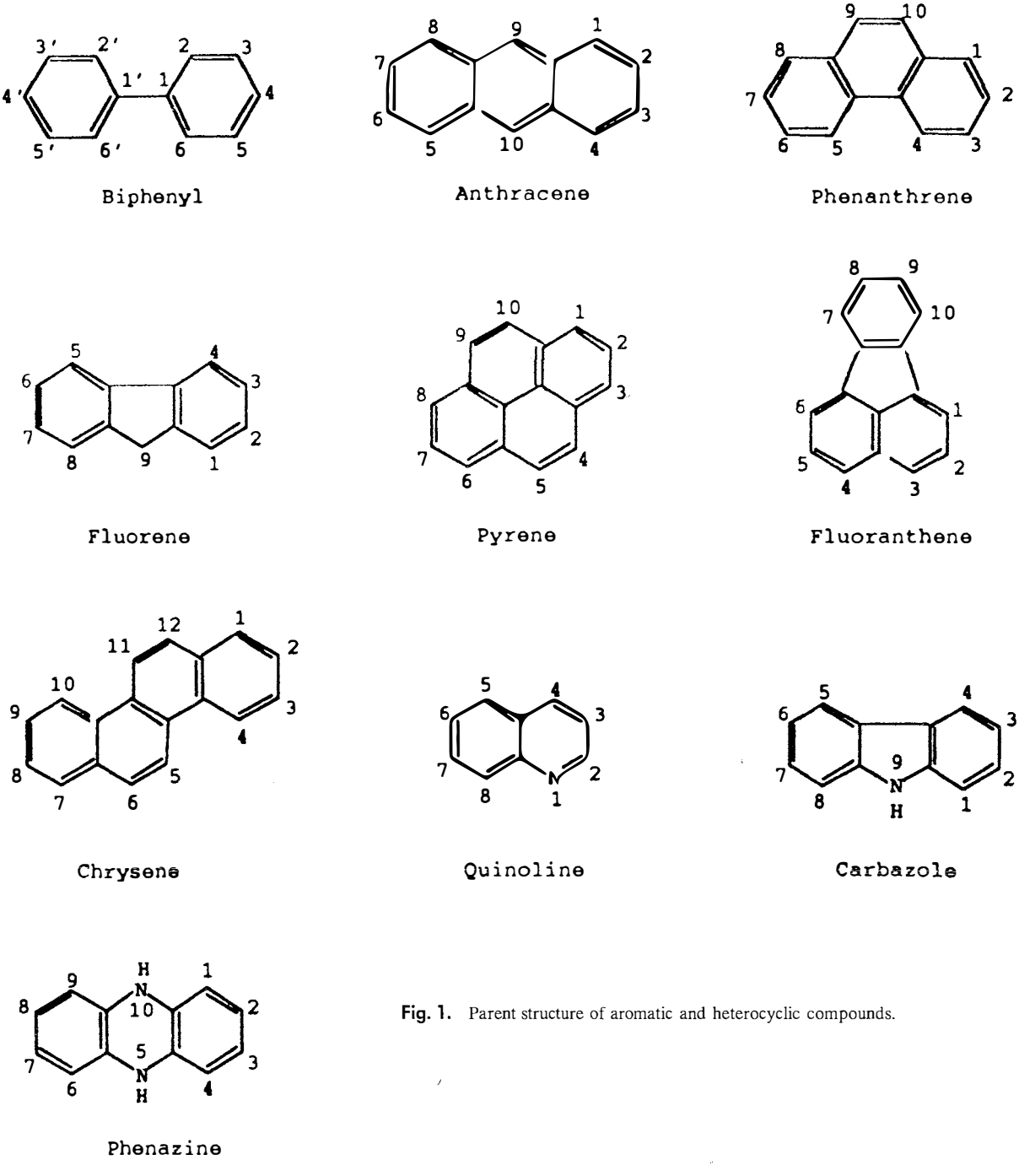


Fig. 1. Parent structure of aromatic and heterocyclic compounds.

Whatman Partisil 10 ODS-3 (C_{18} reverse phase; 25 cm × 22 mm; methanol/water; isocratic) columns.

Electronic Descriptors

The electronic descriptors, ϵ_{LUMO} and ϵ_{HOMO}, were calculated using the semi-empirical AM1 method (MOPAC 4.10, Quantum Chemistry Program Exchange No. 455, VAX version) developed by Dewar and his colleagues [1985]. The molecular structure of each amino compound was optimized with respect to total energy using algorithms built into the MOPAC program.

Octanol/Water Partition Coefficient (P)

The octanol/water partition coefficients of 67 aromatic and heteroaromatic amines were measured in our laboratory or obtained from the literature (Table I). Partition coeffi-

TABLE I. Comparison of Experimental and Calculated log P Values

No.	Name of Compound	log P (expt.)	Clog P[a]	log P-Clog P
1.	4-bromoaniline	2.26	2.08	0.18
2.	4-chloro-1, 2-phenylenediamine	1.28	0.86	0.46
3.	2-methoxy-5-methylaniline (p-cresidine)	1.74	1.52	0.22
4.	4-methoxy-2-methylaniline (m-cresidine)	1.23	1.52	−0.29
5.	3, 4-diaminotoluene	0.66	0.19	0.47
6.	2, 4-diffuoroaniline	1.54	1.66	−0.12
7.	1, 3-phenylenediamine	−0.33	−0.31	−0.02
8.	2, 4-dimethylaniline (2, 4-xylidine)	1.68	1.91	−0.23
9.	2, 5-dimethylaniline (2, 5-xylidine)	1.83	1.91	−0.08
10.	2-amino-4-chlorophenol	1.81	1.81	0.00
11.	2-amino-4-methylphenol	1.16	1.15	0.01
12.	3-chloroaniline	1.88	1.93	−0.05
13.	2-chloroaniline	1.90	1.93	−0.03
14.	4-chloroaniline	1.88	1.93	−0.05
15.	Sulfanilamide[b]	−0.62	−0.63	0.01
16.	2-aminobiphenyl	2.84	2.80	0.04
17.	4-aminobiphenyl	2.86	2.80	0.06
18.	2-aminophenol[b]	0.62	0.65	−0.03
19.	3-amino-$\alpha\alpha\alpha$-trifluorotoluene	2.29	2.33	−0.04
20.	2-methoxyaniline[b]	1.18	1.02	0.16
21.	4-cyclohexylaniline	3.65	3.54	0.11
22.	2-aminonaphthalene	2.28	2.09	0.19
23.	1-aminofluorene	3.18	2.70	0.48
24.	2-aminofluorene	3.14	2.70	0.44
25.	1-aminoanthracene	3.69	3.26	0.43
26.	9-aminophenanthrene	3.56	3.26	0.30
27.	1-aminopyrene	4.31	3.72	0.59
28.	2-aminofluoranthene	4.20	3.72	0.48
29.	1-aminonaphthalene	2.25	2.09	0.16
30.	3-aminoquinoline	1.63	1.73	−0.10
31.	5-aminoquinoline	1.16	1.28	−0.12
32.	6-aminoquinoline	1.28	1.28	0.00
33.	8-aminoquinoline	1.79	1.28	0.51
34.	Benzidine	1.34	1.58	−0.24
35.	4, 4′-methylenedianiline	1.59	1.61	−0.02
36.	4-aminophenylsulfide	2.18	2.03	0.15
37.	4-aminophenylether	1.36	2.06	−0.70
38.	4-phenoxyaniline	2.96	3.20	−0.24
39.	1, 2-phenylenediamine	0.15	−0.31	0.46
40.	4-ethoxyaniline (p-phenetedine)	1.24	1.55	−0.31
41.	2, 4-diaminotoluene	0.14	0.19	−0.05
42.	3, 3′-dimethylbenzidine	2.34	2.57	−0.23
43.	4-fluoroaniline	1.15	1.36	−0.21
44.	2-bromo-7-aminofluorene	3.92	3.62	0.30
45.	3, 3′-dichlorobenzidine	3.51	3.64	−0.13
46.	2-nitro-1, 4-phenylenediamine	0.53	0.87	−0.34
47.	4-nitro-1, 2-phenylenediamine	0.88	0.40	0.48
48.	4-chloro-2-nitroaniline	2.72	2.64	0.08
49.	4-chloro-1, 3-phenylenediamine	0.85	0.86	−0.01
50.	2-amino-7-nitrofluorene	3.06	2.57	0.49
51.	2-methylaniline[b]	1.32	1.41	−0.09
52.	3-methylaniline[b]	1.40	1.41	−0.01
53.	4-methylaniline[b]	1.39	1.41	−0.02
54.	2-ethylaniline[b]	1.74	1.94	−0.20
55.	4-ethylaniline[b]	1.96	1.94	0.02
56.	2-bromoaniline[b]	2.11	2.08	0.03
57.	2-fluoroaniline[b]	1.26	1.36	−0.10
58.	2-lodoaniline[b]	2.32	2.34	−0.02
59.	4-lodoaniline[b]	2.34	2.34	0.00

Continued

TABLE I. Comparison of Experimental and Calculated log P Values (Continued)

No.	Name of Compound	log P (expt.)	Clog P[a]	log P-Clog P
60.	2, 4-dichloroaniline[b]	2.91	2.80	0.11
61.	2, 4, 6-trichloroaniline[b]	3.69	3.58	0.11
62.	3-aminophenol[b]	0.17	0.25	−0.08
63.	4-aminophenol[b]	0.04	0.25	−0.21
64.	Aniline[b]	0.90	0.92	−0.02
65.	3-methoxyaniline[b]	0.93	1.02	−0.09
66.	4-methoxyaniline[b]	0.95	1.02	−0.07
67.	2, 2′-diaminobiphenyl[b]	1.34	1.58	−0.24

[a] Calculated using the CLOGP program, version 3.54.
[b] These compounds are inactive and were not included in developing the regression equations.

cients (Clog P) were also calculated for these compounds using the CLOGP program, release 3.54 [Leo, 1988] (Table I). Equation 2 demonstrates the good agreement between the two sets of coefficients. The CLOGP program, which assumes that log P is an additive constitutive molecular property [Hansch and Leo, 1979] was used to calculate log P values for the remaining amino compounds for which experimental coefficients were unavailable. It should be noted that our present version of CLOGP program calculates log P only for unionized compounds. Consequently our present data set was limited to compounds that would be neutral under the conditions used for the mutagenicity assay.

$$\log P = 1.04\ (\pm 0.06)\ \text{Clog P} - 0.04\ (\pm 0.12)$$
$$n = 67,\ r = 0.975,\ s = 0.248 \qquad (2)$$

Mutagenicity Assay

Mutagenic activities of several amino compounds in *S. typhimurium* TA98 + S9 microsomal preparation and *S. typhimurium* TA100 + S9 microsomal preparation were measured by Microbiological Associates of Rockville, MD using previously described methods [Debnath et al., 1991]. The mutagenic activities of the remaining amino compounds were obtained from the literature (see Tables IIa–c and IIIa–c). Wherever more than one activity value has been reported for a particular compound, the average of all of the reported values was used since this appears to give better results [Compadre et al., 1990].

RESULTS

Physico-chemical parameters of various aromatic and heteroaromatic amines are listed in Table IIa, along with their mutagenic activities in *S. typhimurium* TA98 + S9. The relationship between these parameters and activity is given by Eq. 3. Mutagenic activity in log (rev/nmol) is represented by log TA98, and the AM1 molecular orbital energies are given in eV (HOMO and LUMO stand for *highest occupied* and *lowest unoccupied* molecular orbital, respectively).

$$\log \text{TA98} = 1.08\ (\pm 0.26)\ \log P + 1.28\ (\pm 0.64)\ \epsilon_{HOMO} - 0.73\ (\pm 0.41)\ \epsilon_{LUMO} + 1.46\ (\pm 0.56)\ I_L + 7.20\ (\pm 5.4)$$
$$n = 88,\ r = 0.898,\ s = 0.860,\ F_{1,83} = 12.6 \qquad (3)$$

$$\log \text{TA98} = 1.69\ (\pm 0.35)\ \log P - 4.23\ (\pm 0.86)$$
$$n = 88,\ r = 0.723,\ s = 1.329,\ F_{1,86} = 94.1 \qquad (3a)$$

$$\log \text{TA98} = 3.09\ (\pm 0.55)\ I_L - 1.31\ (\pm 0.32)$$
$$n = 88,\ r = 0.771,\ s = 1.224,\ F_{1,86} = 126 \qquad (3b)$$

$$\log \text{TA98} = 1.09\ (\pm 0.27)\ \log P + 2.23\ (\pm 0.47)\ I_L - 3.57\ (\pm 0.62)$$
$$n = 88,\ r = 0.875,\ s = 0.938,\ F_{1,85} = 61.6 \qquad (3c)$$

$$\log \text{TA98} = 1.15\ (\pm 0.27)\ \log P + 1.98\ (\pm 0.51)\ I_L + 0.60\ (\pm 0.55)\ \epsilon_{HOMO} + 1.40\ (\pm 4.6)$$
$$n = 88,\ r = 0.882,\ s = 0.918,\ F_{1,84} = 4.7 \qquad (3d)$$

Equations 3a–d show the stepwise development of Eq. 3. Mutagenic activity correlates most strongly with log P and I_L. However, application of the F test to each equation shows that all of the terms in Eq. 3 are statistically significant ($F_{1,60\ \alpha.05} = 4.00$, $F_{1,60\ \alpha.001} = 11.97$).

It is interesting to note that the coefficient with log P is approximately one in Eqs. 3, 3c, and 3d. This phenomenon appears to be fairly general for mutagenesis [Debnath et al., submitted for publication]. The bilinear relationship between activity and log P found previously in Eq. 1 is not duplicated in Eq. 3. This difference is due to the generally greater hydrophilicity of the amino compounds studied here compared with the nitro compounds used in the derivation of Eq. 1. The electronic terms in Eq. 3, ϵ_{HOMO} and ϵ_{LUMO}, though statistically significant, account for only 4% of the variance in activity.

The indicator variable, I_L, has the same meaning in Eq.

TABLE IIa. Mutagenicity (*S. typhimurium* TA98 With Metabolic Activation) of Aromatic and Heteroaromatic Amines and Their Physico-chemical Parameters Used to Derive Equation 3

No.	Compounds	log. Rev./nmol Obs.	log. Rev./nmol Pred.	Dev.	log P	ϵ_{LUMO}	ϵ_{HOMO}	I_L	Reference
1.	2-bromo-7-aminofluorene	2.62	2.62	0.00	3.92[a]	−0.405	−8.236	1	Vance et al. [1987]
2.	2-methoxy-5-methylaniline (p-cresidine)	−2.05	−2.06	0.01	1.74[a]	0.419	−8.449	0	Zeiger et al. [1988]
3.	5-aminoquinoline	−2.00	−2.02	0.02	1.16[a]	−0.395	−8.395	0	MBA*
4.	4-ethoxyaniline (p-phenetidine)	−2.30	−2.32	0.02	1.24[a]	0.513	−8.182	0	Zeiger et al. [1988]
5.	1-aminonaphthalene	−0.60	−0.63	0.03	2.25[a]	−0.195	−8.108	0	Connor et al. [1983]
6.	4-aminofluorene	1.13	1.10	0.03	2.70	−0.157	−8.256	1	Later et al. [1984]
7.	2-aminoanthracene	2.62	2.65	−0.03	3.26	−0.771	−7.869	1	Later et al. [1984]
8.	7-aminofluoranthene	2.88	2.83	0.05	3.72	−0.895	−8.186	1	Later et al. [1984]
9.	8-aminoquinoline	−1.14	−1.08	−0.06	1.79[a]	−0.294	−8.134	0	MBA*
10.	1, 7-diaminophenazine	0.75	0.82	−0.07	1.64	−1.045	−8.089	1	Watanabe et al. [1989]
11.	2-aminonaphthalene	−0.67	−0.74	0.07	2.28[a]	−0.198	−8.227	0	MBA*
12.	4-aminopyrene	3.16	3.25	−0.09	3.72	−0.850	−7.837	1	Later et al. [1984]
13.	3-amino-3′-nitrobiphenyl	−0.55	−0.44	−0.11	2.68	−1.034	−8.801	0	Nohara et al. [1985]
14.	2, 4, 5-trimethylaniline	−1.32	−1.19	−0.13	2.41	0.581	−8.244	0	Mortelmans et al. [1986]
15.	3-aminofluorene	0.89	1.02	−0.13	2.70	−0.182	−8.333	1	Later et al. [1984]
16.	3, 3′-dichlorobenzidine	0.81	0.67	0.14	3.51[a]	−0.142	−8.125	0	Messerly et al. [1987]
17.	2, 4-dimethylaniline (2, 4-xylidine)	−2.22	−2.05	−0.17	1.68[a]	0.605	−8.288	0	Zeiger et al. [1988]
18.	2, 7-diaminofluorene	0.48	0.31	0.17	1.47	0.000	−7.753	1	Vance et al. [1987]
19.	3-aminofluoranthene	3.31	3.49	−0.18	4.20[a]	−0.818	−8.033	1	Later et al. [1984]
20.	2-aminofluorene	1.93	1.73	0.20	3.14[a]	−0.108	−8.106	1	Later et al. [1984]
21.	2-amino-4′-nitrobiphenyl	−0.62	−0.42	−0.20	2.68	−1.148	−8.852	0	Nohara et al. [1985]
22.	4-aminobiphenyl	−0.14	−0.34	0.20	2.86[a]	0.048	−8.263	0	Haworth et al. [1983]
23.	3-methoxy-4-methylaniline (o-cresidine)	−1.96	−2.19	0.23	1.52	0.583	−8.274	0	Zeiger et al. [1988]
24.	2-aminocarbazole	0.60	0.84	−0.24	2.30	0.025	−8.023	1	LaVoie et al. [1981]
25.	2-amino-5-nitrophenol	−2.52	−2.26	−0.26	1.36	−0.923	−9.052	0	Zeiger et al. [1987]
26.	2, 2′-diaminobiphenyl	−1.52	−1.79	0.27	1.58	0.267	−8.193	0	Nohara et al. [1985]
27.	2-hydroxy-7-aminofluorene	0.41	0.69	−0.28	2.03	−0.127	−7.998	1	Vance et al. [1987]
28.	1-aminophenanthrene	2.38	2.01	0.37	3.26	−0.357	−8.132	1	Later et al. [1984]
29.	2, 5-dimethylaniline (2, 5-xylidine)	−2.40	−2.02	−0.38	1.83[a]	0.581	−8.404	0	Zeiger et al. [1988]
30.	4-amino-2′-nitrobiphenyl	−0.92	−0.53	−0.39	2.68	−0.554	−8.597	0	Nohara et al. [1985]
31.	2-amino-4-methylphenol	−2.10	−2.50	0.40	1.16[a]	0.478	−8.274	0	Zeiger et al. [1988]
32.	2-aminophenazine	0.55	0.97	−0.42	2.18	−1.165	−8.495	1	Watanabe et al. [1989]
33.	4-aminophenylsulfide	0.31	−0.13	0.44	2.18[a]	0.047	−7.528	0	LaVoie et al. [1979]
34.	2, 4-dinitroaniline	−2.00	−2.46	0.46	1.84	−1.491	−9.931	0	Haworth et al. [1983]
35.	2, 4-diaminoisopropylbenzene	−3.00	−2.53	−0.47	1.12	0.722	−8.121	0	Shahin et al. [1980]
36.	2, 4-difluoroaniline	−2.70	−2.22	−0.48	1.54[a]	−0.085	−8.695	0	Zeiger et al. [1988]
37.	4, 4′-methylenedianiline	−1.60	−2.11	0.51	1.59[a]	0.576	−8.274	0	LaVoie et al. [1979]
38.	3, 3′-dimethylbenzidine	0.01	−0.50	0.51	2.34[a]	0.185	−7.873	0	Haworth et al. [1983]
39.	2-aminofluoranthene	3.23	2.71	0.52	3.72	−0.887	−8.273	1	Later et al. [1984]
40.	2-amino-3′-nitrobiphenyl	−0.89	−0.37	−0.52	2.68	−1.029	−8.746	0	Nohara et al. [1985]
41.	1-aminofluoranthene	3.35	2.81	0.54	3.72	−0.816	−8.159	1	Later et al. [1984]
42.	4, 4′-ethylenebis(aniline)	−2.15	−1.60	−0.55	2.13	0.589	−8.318	0	Messerly et al. [1987]
43.	4-chloroaniline	−2.52	−1.96	−0.56	1.88[a]	0.284	−8.568	0	Motelmans et al. [1986]
44.	2-aminophenanthrene	2.46	1.89	0.57	3.26	−0.365	−8.233	1	Later et al. [1984]
45.	4-fluoroaniline	−3.32	−2.73	−0.59	1.15[a]	0.275	−8.560	0	Zimmer et al. [1980]
46.	9-aminophenanthrene	2.98	2.38	0.60	3.56[a]	−0.364	−8.099	1	Later et al. [1984]
47.	3, 3′-diaminobiphenyl	−1.30	−1.92	0.62	1.58	0.056	−8.414	0	Nohara et al. [1985]
48.	2-aminopyrene	3.50	2.86	0.64	3.72	−0.865	−8.144	1	Later et al. [1984]
49.	2, 6-dichloro-1, 4-phenylenediamine	−0.69	−1.33	0.64	1.79	−0.029	−8.180	0	Mortelmans et al. [1986]
50.	2-amino-7-acetamidofluorene	1.18	0.53	0.65	1.72	−0.143	−7.876	1	Vance et al. [1987]
51.	2, 8-diaminophenazine	1.12	0.46	0.66	1.64	−1.039	−8.369	1	Watanabe et al. [1989]
52.	6-aminoquinoline	−2.67	−1.96	−0.71	1.28[a]	−0.383	−8.443	0	MBA*
53.	4-methoxy-2-methylaniline (m-Cresidine)	−3.00	−2.27	−0.73	1.23[a]	0.474	−8.152	0	Zeiger et al. [1988]

Continued

TABLE IIa. Mutagenicity (*S. typhimurium* TA98 With Metabolic Activation) of Aromatic and Heteroaromatic Amines and Their Physico-chemical Parameters Used to Derive Equation 3 (Continued)

No.	Compounds	log. Rev./nmol Obs.	Pred.	Dev.	log P	ϵ_{LUMO}	ϵ_{HOMO}	I_L	Reference
54.	3-amino-2′-nitrobiphenyl	−1.30	−0.55	−0.75	2.68	−0.648	−8.665	0	Nohara et al. [1985]
55.	2, 4′-diaminobiphenyl	−0.92	−1.69	0.77	1.58	0.215	−8.144	0	Nohara et al. [1985]
56.	1, 6-diaminophenazine	0.20	0.99	−0.79	1.64	−1.058	−7.967	1	Watanabe et al. [1989]
57.	4-aminophenyldisulfide	−1.03	−0.20	−0.83	1.99	−1.33	−8.209	0	LaVoie et al. [1979]
58.	2-bromo-4, 6-dinitroaniline	−0.54	−1.41	0.87	2.78	−1.691	−10.018	0	Zeiger et al. [1987]
59.	2, 4-diamino-n-butylbenzene	−2.70	−1.82	−0.88	1.77	0.702	−8.130	0	Shahin et al. [1980]
60.	4-aminophenylether	−1.14	−2.03	0.89	1.36[a]	0.317	−8.167	0	LaVoie et al. [1979]
61.	2-aminobiphenyl	−1.49	−0.59	−0.90	2.84[a]	0.107	−8.407	0	Haworth et al. [1983]
62.	1, 9-diaminophenazine	0.04	0.94	−0.90	1.64	−1.040	−7.997	1	Watanabe et al. [1989]
63.	1-aminofluorene	0.43	1.38	−0.95	3.18[a]	−0.191	−8.467	1	Later et al. [1984]
64.	8-aminofluoranthene	3.80	2.83	0.97	3.72	−0.853	−8.166	1	Later et al. [1984]
65.	2-chloroaniline	−3.00	−2.01	−0.99	1.90[a]	0.268	−8.632	0	Zeiger et al. [1987]
66.	3-amino-aaa-trifluorotoluene	−0.80	−1.79	0.99	2.29[a]	−0.104	−9.032	0	Haworth et al. [1983]
67.	2-amino-1-nitronaphthalene	−1.17	−0.17	−1.00	2.95	−0.998	−8.800	0	Connor et al. [1983]
68.	3-amino-4′-nitrobiphenyl	0.69	−0.43	1.12	2.68	−1.178	−8.879	0	Nohara et al. [1985]
69.	4-bromoaniline	−2.70	−1.57	−1.13	2.26[a]	0.212	−8.620	0	Zeiger et al. [1988]
70.	2-amino-4-chlorophenol	−3.00	−1.87	−1.13	1.81[a]	0.154	−8.508	0	Zeiger et al. [1988]
71.	3, 3′-dimethoxybenzidine	0.15	−1.01	1.16	1.81	−0.005	−7.927	0	Haworth et al. [1983]
72.	4-cyclohexylaniline	−1.24	−0.06	−1.18	3.65[a]	0.620	−8.380	0	MBA*
73.	4-phenoxyaniline	0.38	−0.82	1.20	2.96[a]	0.255	−8.598	0	LaVoie et al. [1979]
74.	4, 4′-methylenebis (o-ethylaniline)	−0.99	0.22	−1.21	3.66	0.605	−8.181	0	Rao et al. [1982]
75.	2-amino-7-nitrofluorene	3.00	1.79	1.21	3.06[a]	−1.164	−8.595	1	Rosenkranz and Mermelstein [1985]
76.	Benzidine	−0.39	−1.63	1.24	1.34[a]	0.147	−7.931	0	LaVoie et al. [1979]
77.	1-amino-4-nitronaphthalene	−1.77	−0.48	−1.29	2.48	−1.035	−8.663	0	Connor et al. [1983]
78.	4-amino-3′-nitrobiphenyl	1.02	−0.27	1.29	2.68	−0.959	−8.630	0	Nohara et al. [1985]
79.	4-amino-4′-nitrobiphenyl	1.04	−0.27	1.31	2.68	−1.102	−8.707	0	Nohara et al. [1985]
80.	1-aminophenazine	−0.01	1.31	−1.32	2.18	−1.164	−8.230	1	Watanabe et al. [1989]
81.	4, 4′-methylenebis (o-fluoroaniline)	0.23	−1.09	1.32	2.50	0.186	−8.467	0	Rao et al. [1982]
82.	4-chloro-2-nitroaniline	−2.22	−0.80	−1.42	2.72[a]	−1.019	−9.110	0	Haworth et al. [1983]
83.	3-aminoquinoline	−3.14	−1.71	−1.43	1.63[a]	−0.376	−8.534	0	MBA*
84.	3-aminocarbazole	−0.48	1.12	−1.60	2.30	−0.107	−7.877	1	LaVoie et al. [1981]
85.	4-chloro-1, 2-phenylenediamine	−0.49	−2.27	1.78	1.28[a]	0.241	−8.325	0	Zeiger et al. [1988]
86.	3-aminophenanthrene	3.77	1.91	1.86	3.26	−0.203	−8.122	1	Later et al. [1984]
87.	3, 4′-diaminobiphenyl	0.20	−1.67	1.87	1.58	0.103	−8.187	0	Nohara et al. [1985]
88.	1-aminoanthracene	1.18	3.20	−2.02	3.69[a]	−0.804	−7.820	1	Later et al. [1984]
89.	1-aminocarbazole[b]	−1.04	0.93	−1.97	2.30	−0.115	−8.035	1	LaVoie et al. [1981]
90.	9-aminoanthracene[b]	0.87	2.95	−2.08	3.26	−0.712	−7.600	1	Later et al. [1984]
91.	4-aminocarbazole	−1.42	0.92	−2.34	2.30	−0.029	−7.993	1	LaVoie et al. [1981]
92.	6-aminochrysene[b]	1.83	4.30	−2.47	4.98	−0.592	−7.926	1	Connor et al. [1983]
93.	1-aminopyrene[b]	1.43	4.10	−2.67	4.31[a]	−0.700	−7.580	1	Later et al. [1984]
94.	4, 4′-methylenebis (o-isopropyl-aniline)[b]	−1.77	1.10	−2.87	4.46	0.610	−8.165	0	Rao et al. [1982]
95.	2, 7-diaminophenazine[b]	3.97	0.81	3.16	1.64	−1.069	−8.110	1	Watanabe et al [1989]

[a] Experimental log P.
[b] These data points were not included for deriving Eq. 3.
*These compounds were assayed by Microbiological Associates, Rockville, MD (see Methods).

3 as it did in Eq. 1; it is one for compounds containing three or more fused aromatic rings and is zero for all other compounds. The coefficients with I_L in Eqs. 1 and 3 are similar, suggesting a common mechanistic origin.

The correlation matrix for the parameters used in deriving Eq. 3 is:

	log P	ϵ_{LUMO}	ϵ_{HOMO}	I_L
log P	1	−0.36	0.03	0.46
ϵ_{LUMO}		1	0.37	−0.37
ϵ_{HOMO}			1	0.40
I_L				1

TABLE IIb. Mutagenicity (*S. typhimurium* TA98 + S9 Microsomal Preparation) of Aromatic Amines and the Physico-Chemical Parameters Used to Derive Equation 4

No.	Compound	log Rev./nmol Obs.	log Rev./nmol Pred.	Dev.	log P	ϵ_{HOMO}	ϵ_{LUMO}	Reference
1.	1,2-phenylenediamine	−0.75	−0.74	−0.01	0.15[a]	−8.186	0.578	Zeiger et al. [1988]
2.	1, 3-phenylenediamine	−0.46	−0.44	−0.02	−0.33[a]	−8.226	0.740	Zeiger et al. [1988]
3.	3, 3′-diaminobenzidine	−0.04	−0.10	0.06	−0.88	−7.738	0.184	Messerly et al. [1987]
4.	2, 4-diaminoethylbenzene	−0.87	−1.09	0.22	0.72	−8.133	0.705	Shahin et al. [1980]
5.	3-amino-6-methylphenol	−1.40	−1.11	−0.29	0.75	−8.334	0.513	Zeiger et al. [1988]
6.	3, 4-diaminotoluene	−1.42	−1.06	−0.37	0.66[a]	−8.086	0.567	Zeiger et al. [1988]
7.	4-chloro-1, 3-phenylenediamine	−0.77	−1.17	0.40	0.85[a]	−8.297	0.428	Haworth et al. [1983]
8.	2, 4-diaminotoluene[b]	−1.29	−0.73	−0.56	0.14[a]	−8.117	0.697	Haworth et al. [1983]
9.	2-nitro-1, 4-phenylenediamine[b]	−0.05	−0.98	0.93	0.53[a]	−8.215	−0.727	Zeiger et al. [1988]
10.	4-nitro-1, 3-phenylenediamine[b]	−2.40	−1.19	−1.21	0.87	−8.921	−0.529	Zeiger et al. [1988]
11.	4-nitro-1, 2-phenylenediamine[b]	0.35	−1.19	1.54	0.88[a]	−8.953	−0.857	Mortelmans et al. [1986]

[a] Experimental log P.
[b] This compound was not used in the derivation of Eq. 4.

TABLE IIc. Physico-Chemical Parameters of Inactive Aromatic Amines and Predicted Mutagenicity in log Rev./nmole (*S. typhimurium* TA98 + S9 Microsomal Preparation) Using Equation 3

No.	Compound	Pred.	log P	ϵ_{HOMO}	ϵ_{LUMO}	I_L	Reference
1.	2-methyl-4-chloroaniline	−1.41	2.43	−8.633	0.224	0	Haworth et al. [1983]
2.	2-chloro-4-methylaniline	−1.35	2.43	−8.559	0.274	0	Haworth et al. [1983]
3.	4-methoxyaniline	−2.66	0.95[a]	−8.212	0.492	0	Haworth et al. [1983]
4.	3-methoxyaniline	−3.16	0.93[a]	−8.527	0.597	0	Haworth et al. [1983]
5.	Aniline	−3.21	0.90[a]	−8.530	0.615	0	Haworth et al. [1983]
6.	3-chloroaniline	−2.16	1.88[a]	−8.739	0.246	0	Zeiger et al. [1987]
7.	3-ethoxyaniline	−2.48	1.55	−8.504	0.621	0	Zeiger et al. [1988]
8.	2-ethoxyaniline	−2.21	1.55	−8.344	0.525	0	Zeiger et al. [1988]
9.	4-aminophenol	−3.65	0.04[a]	−8.269	0.408	0	Zeiger et al. [1988]
10.	3-aminophenol	−3.91	0.17[a]	−8.523	0.504	0	Zeiger et al. [1988]
11.	4, 4′-methylenebis (2, 6-diisopropylaniline)	4.26	7.31	−8.059	0.666	0	Rao et al. [1982]
12.	4, 4′-methylenebis (2, 6-diethylaniline)	2.50	5.72	−8.111	0.643	0	Rao et al. [1982]
13.	4, 4′-methylenebis (2-methyl-6-t-butylaniline)	3.13	6.26	−8.058	0.673	0	Rao et al. [1982]
14.	4, 4′-methylenebis (2-methyl-6-isopropylaniline)	2.24	5.46	−8.097	0.641	0	Rao et al. [1982]
15.	4, 4′-methylenebis (2-methyl-6-ethylaniline)	1.36	4.66	−8.113	0.636	0	Rao et al. [1982]
16.	4, 4′-methylenebis (2, 6-Dimethylaniline)	0.22	3.60	−8.120	0.624	0	Rao et al. [1982]
17.	3-aminobiphenyl	−0.67	2.80	−8.498	−0.003	0	Nohara et al. [1985]
18.	2, 3-diaminobiphenyl	−1.86	1.58	−8.305	0.167	0	Nohara et al. [1985]
19.	2-methoxyaniline	−2.59	1.18[a]	−8.331	0.525	0	Haworth et al. [1983]
20.	2-aminophenol	−3.17	0.62[a]	−8.356	0.448	0	Haworth et al. [1983]
21.	4-aminophenanthrene	1.55	3.26	−8.524	−0.411	1	Later et al. [1984]

[a] Experimental log P.

The two most important parameters in the QSARs, log P and I_L, are weakly correlated with each other. This correlation shows up during the stepwise regression as a change in the coefficient of either log P or I_L whenever the other parameter is added to the QSAR (i.e., the change can be seen by comparing either Eq. 3a or 3b with Eq. 3c). I_L is also weakly correlated with the two MO energies.

The most hydrophilic aromatic amines (log P < 1) could not be treated using Eq. 3. The mutagenic activities and physico-chemical properties of these amines are listed in Table IIb, and a QSAR correlating their behavior with log P is given by Eq. 4. While the correlation coefficient for Eq. 4 is less than that of Eq. 3, the standard deviation of Eq. 4 is also smaller. Unfortunately, the small number of compounds of this type makes construction of a more significant QSAR impossible. Nevertheless, the negative coefficient with log P in Eq. 4 suggests that these amines may act by a different mechanism than do their more hydrophobic counterparts. One possible explanation is that these more hydrophilic amines are activated in a hydrophilic cell compartment by an oxidase whose characteristics differ significantly from the isozymes of the cytochrome P-450 family.

$$\log TA98 = -0.62\ (\pm 0.47)\ \log P - 0.65\ (\pm 0.31)$$
$$n = 7,\ r = 0.837,\ s = 0.294,\ F_{1,5} = 11.7 \quad (4)$$

Another unusual feature of this class of compounds is that all but two compounds (3-amino-4-methylphenol and 3,3′-diaminobenzidine) are phenylenediamines. While it is tempting to postulate a novel mechanism of action specific for diamines, it must be noted that Eq. 3 adequately predicts the behavior of several, more hydrophobic (log P > 1), diamines (including three phenylenediamines), (i.e., 1,9-diaminophenazine, 2,4-diamino-n-butylbenzene, 1,6-diaminophenazine, 2,4′-diaminobiphenyl, 2,8-diaminophenazine, 2,6-dichloro-1,4-phenylenediamine, 3,3′-diaminobiphenyl, 2,4-diaminoisopropylbenzene, 2,2′-diaminobiphenyl, 2,7-diaminoflourene, and 1,7-diaminophenazine). Furthermore, any mechanism designed to address the behavior of the highly hydrophilic diamines must also take into account the four phenylenediamines whose activities could not be fit using Eq. 4: 2,4-diaminotoluene, 4-nitro-1,2-phenylenediamine, 2-nitro-1,4-phenylenediamine, and 4-nitro-1,3-phenylenediamine.

One other group of aminoarenes requiring special consideration is the family of amines that also contain a nitro substituent. Compounds of this type can be found in Tables IIa and IIb and have been treated here as substituted amines. That is, we have assumed that activation of these compounds occurs solely by S9-mediated oxidation of the amino group to the hydroxylamine rather than by reduction of the nitro group to the hydroxylamine. This assumption is supported by the data in Table IIa, which indicates that the more hydrophobic nitroamines are, in fact, adequately fit by Eq. 3. However, since reduction of the nitro group does not require S9, reduction may still occur even when S9 is present. If nitro reduction is still occurring under these conditions, then any agreement between measured mutagenic activities and the activities predicted by Eq. 3 is fortuitous. The mutagenicities of various nitroamines have also been successfully correlated with the mutagenicities of "normal" nitroarenes [Debnath et al., 1991]. However, since the mutagenicity assay employed for nitroarenes does not include S9, it is likely that the primary mechanism for inducing mutations involves reduction of the nitro group.

The mutagenic activities of various aromatic and heteroaromatic amines in *S. typhimurium* TA100 + S9, along with several physico-chemical parameters for each amine, are listed in Table IIIa. The relationship between these parameters and activity is given by Eq. 5. Mutagenic activity in log (rev/nmol) is represented by log TA100, and Eqs. 5a and 5b show the stepwise development of Eq. 5.

$$\log TA100 = 0.92\ (\pm 0.23)\ \log P + 1.17\ (\pm 0.83)\ \epsilon_{HOMO} - 1.18\ (\pm 0.44)\ \epsilon_{LUMO} + 7.35\ (\pm 6.9)$$
$$n = 67,\ r = 0.877,\ s = 0.708 \quad (5)$$

$$\log TA100 = 1.30\ (\pm 0.26)\ \log P - 3.16\ (\pm 0.69)$$
$$n = 67,\ r = 0.777,\ s = 0.914,\ F_{1,65} = 99.23 \quad (5a)$$

$$\log TA100 = 1.20\ \log P\ (\pm 0.24) + 1.70\ (\pm 0.95)\ \epsilon_{HOMO} + 11.07\ (\pm 8)$$
$$n = 67,\ r = 0.818,\ s = 0.842,\ F_{1,64} = 12.42 \quad (5b)$$

The correlation matrix for the variables of Eq. 5 is:

	log P	ϵ_{LUMO}	ϵ_{HOMO}
log P	1	−0.50	0.22
ϵ_{LUMO}		1	−0.31
ϵ_{HOMO}			1

The hydrophobicity of each amine is by far the most important determinant of its relative mutagenicity; variation in log P accounts for 60% of the variance in log TA100. The two electronic terms account for only 17% of the variance in log TA100. The coefficients with log P, ϵ_{HOMO}, and ϵ_{LUMO} in Eq. 5 are similar to those found in Eq. 3. One important difference between Eq. 3 and Eq. 5 is the absence of the I_L term from the latter equation. The structural changes affecting the indicator variable do not appear to influence mutagenic activity in TA100.

There is also a second category of amines whose behavior can not be correlated by Eq. 5. These amines are all much more hydrophilic (log P < 1) than the amines used to derive Eq. 5. Activity data and physico-chemical parameters for these hydrophilic amines are listed in Table IIIb. The relationship between activity and log P for these amines is given by Eq. 6. One hydrophilic amine, 2,4-diaminoethylbenzene, does not conform to Eq. 6. Equation 6 is quite similar to Eq. 4, the QSAR for the mutagenicity of hydrophilic amines in TA98 + S9.

$$\log TA100 = -0.54\ (\pm 0.32)\ \log P - 1.62\ (\pm 0.18)$$
$$n = 6,\ r = 0.921,\ s = 0.156,\ F_{1,4} = 22.5 \quad (6)$$

DISCUSSION

Equations 3 and 5 both identify hydrophobicity as the single most important structural factor affecting the relative mutagenicity of aminoarenes. Other structural factors, such as steric and electronic factors, are of secondary importance, at least for this set of compounds. Since these conclusions are based on the mathematical structure of the two QSARs, it is important to carefully assess the reliability of these equations. The following discussion addresses the reliability and the limitations of these QSARs from several perspectives. One particularly useful method for verifying Eqs. 3 and 5 is to compare them with analogous QSARs that have been developed for nitroarenes acting in the same systems. This method of "lateral verification" of QSARs is based on the similar mutagenic activation mechanisms that are be-

TABLE IIIa. Mutagenicity (*S. typhimurium* TA100 With Metabolic Activation) of Aromatic and Heteroaromatic Amines and Their Physico-Chemical Parameters Used to Derive Equation 5

No.	Compounds	log Rev./nmol Obs.	log Rev./nmol Pred.	Dev.	log P	ϵ_{LUMO}	ϵ_{HOMO}	Reference
1.	2, 3-dimethylaniline (2, 3-xylidine)	−1.36	−1.37	0.01	1.91	0.581	−8.403	Zimmer et al [1980]
2.	2, 5-dimethylaniline (2, 5-xylidine)	−1.43	−1.44	0.01	1.83[a]	0.581	−8.404	Zeiger et al. [1988]
3.	4-chloro-1, 2-phenylenediamine	−1.44	−1.46	0.02	1.28[a]	0.241	−8.325	Zeiger et al. [1988]
4.	4-aminophenylsulfide	0.48	0.53	−0.05	2.18[a]	0.047	−7.528	LaVoie et al. [1979]
5.	4-aminopyrene	2.69	2.63	0.06	3.72	−0.850	−7.837	Later et al. [1984]
6.	2-amino-4-methylphenol	−1.68	−1.78	0.10	1.16[a]	0.478	−8.274	Zeiger et al. [1988]
7.	1-aminofluoranthene	2.34	2.21	0.13	3.72	−0.816	−8.159	Later et al. [1984]
8.	2-aminofluorene	0.78	0.91	−0.13	3.14[a]	−0.108	−8.106	Later et al. [1984]
9.	Benzidine	−0.66	−0.83	0.17	1.34[a]	0.147	−7.931	LaVoie et al. [1979]
10.	4-methyl-2-bromoaniline	−0.64	−0.45	−0.19	2.58	0.228	−8.498	Zimmer et al. [1980]
11.	8-aminoquinoline	−0.34	−0.14	−0.20	1.79[a]	−0.294	−8.134	MBA*
12.	3, 4-dimethylaniline (3, 4-xylidine)	−1.08	−1.29	0.21	1.91	0.602	−8.318	Zimmer et al. [1980]
13.	3-aminofluorene	0.10	0.33	−0.23	2.70	−0.182	−8.333	Later et al. [1984]
14.	4-methyl-2-chloroaniline	−0.40	−0.64	0.24	2.43	0.293	−8.477	Zimmer et al. [1980]
15.	4-aminofluorene	0.64	0.39	0.25	2.70	−0.157	−8.256	Later et al. [1984]
16.	4-chloroaniline	−1.51	−1.24	−0.27	1.88[a]	0.284	−8.568	Mortelmans et al. [1986]
17.	8-aminofluoranthene	1.98	2.25	−0.27	3.72	−0.853	−8.166	Later et al. [1984]
18.	2-ethyl-4-chloroaniline	0.08	−0.20	0.27	2.96	0.305	−8.502	Zimmer et al. [1980]
19.	2-aminopyrene	2.58	2.29	0.29	3.72	−0.865	−8.144	Later et al. [1984]
20.	2-aminonaphthalene	0.39	0.09	0.30	2.28[a]	−0.198	−8.227	MBA*
21.	4-cyclohexylaniline	−0.14	0.21	−0.35	3.65[a]	0.620	−8.380	MBA*
22.	6-aminoquinoline	−1.22	−0.86	−0.36	1.28[a]	−0.383	−8.443	MBA*
23.	2-amino-1-methylnaphthalene	0.84	0.48	0.36	2.59	−0.203	−8.142	El-Bayoumy et al. [1981]
24.	4-amino-3-methylbiphenyl	1.12	0.73	0.39	3.30	0.069	−8.208	El-Bayoumy et al. [1981]
25.	4, 4′-ethylenebis (aniline)	−1.51	−1.08	−0.43	2.13	0.589	−8.318	Messerly et al. [1987]
26.	2-methoxy-5-methylaniline (p-cresidine)	−1.85	−1.39	−0.46	1.74[a]	0.419	−8.449	Zeigler et al. [1988]
27.	2, 4, 5-trimethylaniline	−0.26	−0.72	0.46	2.41	0.581	−8.244	Mortelmans et al. [1986]
28.	2, 4-diamino-n-butylbenzene	−0.84	−1.32	0.48	1.77	0.702	−8.130	Shahin et al. [1980]
29.	7-aminofluoranthene	2.76	2.28	0.48	3.72	−0.895	−8.186	Later et al. [1984]
30.	1-aminocarbazole	−0.25	0.24	−0.49	2.30	−0.115	−8.035	LaVoie et al. [1981]
31.	1-aminophenanthrene	1.79	1.29	0.50	3.26	−0.357	−8.132	Later et al. [1984]
32.	3-amino-4-methylbiphenyl	0.09	0.60	−0.51	3.30	−0.007	−8.398	El-Bayoumy et al. [1981]
33.	3-aminocarbazole	−0.11	0.41	−0.52	2.30	−0.107	−7.877	LaVoie et al. [1981]
34.	4-methoxy-2-methylaniline (m-cresidine)	−2.10	−1.57	−0.53	1.23	0.474	−8.152	Zeiger et al. [1988]
35.	2-aminobiphenyl	−0.51	0.04	−0.55	2.84[a]	0.107	−8.407	Haworth et al. [1983]
36.	3-aminofluoranthene	2.25	2.81	−0.56	4.20[a]	−0.818	−8.033	Later et al. [1984]
37.	4-aminobiphenyl	0.85	0.29	0.56	2.86[a]	0.048	−8.263	Haworth et al. [1983]
38.	3, 3′-dichlorobenzidine	0.66	1.27	−0.61	3.51[a]	−0.142	−8.125	Messerly et al. [1987]
39.	2, 6-dichloro-1, 4-phenylenediamine	−1.12	−0.50	−0.62	1.79	−0.029	−8.180	Mortelmans et al. [1986]
40.	3, 3′-dimethoxybenzidine	−0.85	−0.22	−0.63	1.81[a]	−0.005	−7.927	Haworth et al. [1983]
41.	4-aminophenyldisulfide	0.54	1.18	−0.64	1.99	−1.334	−8.209	LaVoie et al. [1979]
42.	2-aminocarbazole	−0.56	0.08	−0.64	2.30	0.025	−8.023	LaVoie et al. [1981]
43.	4-aminocarbazole	−0.47	0.18	−0.65	2.30	−0.029	−7.993	LaVoie et al. [1981]
44.	1-aminofluorene	−0.04	0.63	−0.67	3.18[a]	−0.191	−8.467	Later et al. [1984]
45.	2-aminoanthracene	2.76	2.08	0.68	3.26	−0.771	−7.869	Later et al. [1984]
46.	2-amino-3-methylnaphthalene	1.09	0.39	0.70	2.59	−0.199	−8.214	El-Bayoumy et al. [1981]
47.	2-aminofluoranthene	2.87	2.16	0.70	3.72	−0.887	−8.273	Later et al. [1984]
48.	3-aminoquinoline	0.07	−0.65	0.72	1.63[a]	−0.376	−8.534	MBA*
49.	4, 4′-methylenebis (o-fluoraniline)	−1.16	−0.44	−0.72	2.50	0.186	−8.467	Rao et al. [1982]
50.	3-methoxy-4-methylaniline (0-cresidine)	−0.81	−1.58	0.77	1.52	0.583	−8.274	Zeiger et al. [1988]
51.	2-chloroaniline	−2.05	−1.28	−0.77	1.90[a]	0.268	−8.632	Zeiger et al. [1987]
52.	4-phenoxyaniline	0.63	−0.25	0.88	2.96[a]	0.255	−8.598	LaVoie et al. [1979]
53.	2-amino-4-chlorophenol	−2.00	−1.08	−0.92	1.81	0.154	−8.508	Zeiger et al. [1988]
54.	1-amino-2-methylnaphthalene	−0.37	0.57	−0.94	2.59	−0.176	−8.034	El-Bayoumy et al. [1981]
55.	6-aminochrysene	2.41	3.38	−0.97	4.98	−0.592	−7.926	Connor et al. [1983]
56.	2-methyl-4-bromoaniline	0.46	−0.52	0.98	2.58	0.226	−8.562	Zimmer et al. [1980]
57.	4-aminophenanthrene	−0.11	0.89	−1.00	3.26	−0.411	−8.524	Later et al. [1984]
58.	4-4′-methylenebis (o-ethylaniline)	−0.55	0.47	−1.02	3.66	0.605	−8.181	Rao et al. [1982]

Continued

TABLE IIIa. Mutagenicity (*S. typhimurium* TA100 With Metabolic Activation) of Aromatic and Heteroaromatic Amines and Their Physico-Chemical Parameters Used to Derive Equation 5 (Continued)

No.	Compounds	log Rev./nmol Obs.	log Rev./nmol Pred.	Dev.	log P	ϵ_{LUMO}	ϵ_{HOMO}	Reference
59.	4-aminophenylether	−0.27	−1.28	1.01	1.36[a]	0.317	−8.167	LaVoie et al. [1979]
60.	4-ethoxyaniline (p-phenetidine)	−0.61	−1.65	1.04	1.24[a]	0.513	−8.182	Zeiger et al. [1988]
61.	5-aminoquinoline	0.14	0.90	1.04	1.16[a]	−0.395	−8.395	MBA*
62.	2-methyl-4-chloroaniline	0.38	−0.67	1.05	2.43	0.289	−8.508	Zimmer et al. [1980]
63.	1-aminoaphthalene	−1.00	0.20	−1.20	2.25[a]	−0.195	−8.108	Connor et al. [1983]
64.	2, 4-dimethylaniline[b] (2, 4-xylidine)	−0.23	−1.47	1.24	1.68[a]	0.605	−8.288	Zeiger et al. [1988]
65.	2, 4-difluoroaniline	−2.52	−1.26	−1.25	1.54	−0.085	−8.695	Zeiger et al. [1988]
66.	4, 4′-methylenedianiline[b]	−0.15	−1.51	1.36	1.59[a]	0.576	−8.274	LaVoie et al. [1979]
67.	9-aminophenanthrene	2.79	1.61	1.36	3.56[a]	−0.364	−8.099	Later et al. [1984]
68.	3, 4′-diaminobiphenyl[b]	0.65	−0.86	1.51	1.58	0.103	−8.187	Nohara et al. [1985]
69.	3-aminophenanthrene	2.66	1.12	1.54	3.26	−0.203	−8.122	Later et al. [1984]
70.	2-aminophenanthrene	2.74	1.18	1.56	3.26	−0.365	−8.233	Later et al. [1984]
71.	1-aminoanthracene[b]	0.36	2.57	−2.21	3.69[a]	−0.804	−7.820	Later et al. [1984]
72.	1-aminopyrene[b]	1.05	3.29	−2.24	4.31[a]	−0.700	−7.580	Later et al. [1984]
73.	9-aminoanthracene[b]	−0.24	2.32	−2.56	3.26	−0.712	−7.600	Later et al. [1984]

[a]Experimental log P.
[b]These data points were not included in deriving Eq. 5.
*These compounds were assayed by Microbiological Associates, Rockville, MD (see Methods).

TABLE IIIb. Mutagenicity (*S. typhimurium* TA100 With Metabolic Activation) of Aromatic Amines and Their Physico-Chemical Parameters Used to Derive Equation 6

No.	Compounds	log Rev./nmol Obs.	log Rev./nmol Pred.	Dev.	log P	ϵ_{LUMO}	ϵ_{HOMO}	Reference
1.	2, 4-diaminotoluene	−1.66	−1.70	0.04	0.14[a]	0.697	−8.117	Haworth et al. [1983]
2.	3, 3′-diaminobenzidine	−1.11	−1.15	0.04	−0.88	0.184	−7.738	Messerly et al. [1987]
3.	1, 3-phenylenediamine	−1.40	−1.44	0.04	−0.33[a]	0.740	−8.226	Zieger et al. [1988]
4.	3, 4-diaminotoluene	−2.10	−1.97	−0.13	0.66[a]	0.567	−8.086	Zieger et al. [1988]
5.	1, 2-phenylenediamine	−1.89	−1.70	−0.19	0.15	0.578	−8.186	Zieger et al. [1988]
6.	3-amino-6-methylphenol	−1.82	−2.02	0.20	0.75	0.513	−8.334	Zieger et al. [1988]
7.	2, 4-diaminoethylbenzene[b]	−1.21	−2.01	0.80	0.72	0.705	−8.133	Shahin et al. [1980]

[a]Experimental log P.
[b]This compound was not used in deriving Eq. 6.

lieved to operate for the two families of mutagens (Scheme 1). The following paper [Debnath et al., submitted for publication], which also contains a new QSAR for the action of nitroarenes in TA100, presents just such an analysis, and lends additional support for the relationships expressed in Eqs. 3 and 5.

The customary method for evaluating regression equations such as Eqs. 3 and 5 is to inspect the statistics describing the regression. Though each term in Eqs. 3 and 5 is highly significant by the F-test, the overall correlation coefficients are not extremely high. Equations 3 and 5 account for only 80% and 77% of the variation in log TA98 and log TA100, respectively. Steric effects and other structural factors which have not been built into the QSARs must be responsible for some of the observed variation in activity. Nevertheless, it is important to keep in mind the likely magnitude of such effects; a structural change that is not covered by the parameters in Eqs. 3 and 5, and which causes a doubling of mutagenic activity or which causes 50% of the starting compound to be lost, will lead to an error of 0.3 (log 2) in the predicted activity. An error of this magnitude is much smaller than the standard deviations of Eqs. 3 and 5 and would have little effect on the form of these equations, since they are designed to model much larger variations in activity.

It should also be kept in mind that the mutagenicity data used to derive these equations come from many separate experiments carried out in several different laboratories. One can be certain that the data set is contaminated by systematic variations in experimental technique which will magnify the uncertainty already present in each data point. Indeed, a perfect fit of the data under these conditions would be suspect, since the precision of the QSAR would exceed the precision of each individual data point. On the other hand, we have shown that an excellent fit of Ames test results is possible when the mutagenicity data is obtained

TABLE IIIc. Predicted Activity (log Rev./nmole) and the Physico-Chemical Parameters of Inactive Aromatic Amines in *S. typhimurium* TA100(+S9) Using Equation 5

No.	Compounds	Pred	log P	ϵ_{HOMO}	ϵ_{LUMO}	References
1.	4, 4′-methylenebis (2, 6-diisopropylaniline)	5.37	7.31	−8.059	0.666	Rao et al. [1982]
2.	4, 4′-methylenebis (2, 6-diethylaniline)	3.50	5.72	−8.111	0.643	Rao et al. [1982]
3.	4, 4′-methylenebis (2-methyl-6-t-butylaniline)	4.18	6.26	−8.058	0.673	Rao et al. [1982]
4.	4, 4′-methylenebis (2-methyl-6-isopropylniline)	3.23	5.46	−8.097	0.641	Rao et al. [1982]
5.	4, 4′-methylenebis (2-methyl-6-ethylaniline)	2.31	4.66	−8.113	0.636	Rao et al. [1982]
6.	4, 4′-methylenebis (2, 6-dimethylaniline)	1.11	3.60	−8.120	0.624	Rao et al. [1982]
7.	3-aminobiphenyl	−0.03	2.80	−8.498	−0.003	Nohara et al. [1985]
8.	2, 3-diaminobiphenyl	−1.19	1.58	−8.305	0.167	Nohara et al. [1985]
9.	2-methyl-4-chloroaniline	−0.81	2.43	−8.633	0.224	Haworth et al. [1983]
10.	2-chloro-4-methylaniline	−0.72	2.43	−8.559	0.274	Haworth et al. [1983]
11.	4-methoxyaniline	−1.95	0.95[a]	−8.212	0.429	Haworth et al. [1983]
12.	3-methoxyaniline	−2.56	0.93[a]	−8.527	0.597	Haworth et al. [1983]
13.	Aniline	−2.61	0.90[a]	−8.530	0.615	Haworth et al. [1983]
14.	3-chloroaniline	−1.62	1.88[a]	−8.739	0.246	Zieger et al. [1987]
15.	3-ethoxyaniline	−1.84	1.55	−8.504	0.621	Zieger et al. [1988]
16.	2-ethoxyaniline	−1.51	1.55	−8.344	0.525	Zieger et al. [1988]
17.	4-aminophenol	−3.01	0.04[a]	−8.269	0.408	Zieger et al. [1988]
18.	3-aminophenol	−3.35	0.17[a]	−8.523	0.504	Zieger et al. [1988]
19.	2, 4, 6-trimethylaniline	−0.40	2.41	−8.222	0.616	Zimmer et al. [1980]
20.	2, 4, 6-tribromoaniline	1.05	4.03	−8.803	−0.319	Zimmer et al. [1980]
21.	2, 4, 6-trichloroaniline	0.69	3.69[a]	−8.767	−0.258	Zimmer et al. [1980]
22.	2, 6-diethylaniline	0.02	2.97	−8.359	0.594	Zimmer et al. [1980]
23.	3, 5-dimethylaniline	−1.31	1.91	−8.440	0.597	Zimmer et al. [1980]
24.	2, 6-dimethylaniline	−1.18	1.91	−8.370	0.582	Zimmer et al. [1980]
25.	2, 4-dibromoaniline	−0.11	3.05	−8.741	−0.069	Zimmer et al. [1980]
26.	2, 4-dichloroaniline	−0.21	2.91[a]	−8.682	0.001	Zimmer et al. [1980]
27.	4-iodoaniline	−0.99	2.34[a]	−8.685	0.211	Zimmer et al. [1980]
28.	2-iodoaniline	−1.00	2.32[a]	−8.684	0.204	Zimmer et al. [1980]
29.	2-fluoroaniline	−2.04	1.26[a]	−8.560	0.275	Zimmer et al. [1980]
30.	2-bromoaniline	−1.19	2.11[a]	−8.656	0.203	Zimmer et al. [1980]
31.	4-ethylaniline	−1.18	1.96[a]	−8.389	0.613	Zimmer et al. [1980]
32.	2-ethylaniline	−1.49	1.74[a]	−8.438	0.593	Zimmer et al. [1980]
33.	4-methylaniline	−1.77	1.39[a]	−8.362	0.606	Zimmer et al. [1980]
34.	3-methylaniline	−1.96	1.40[a]	8.486	0.589	Zimmer et al. [1980]
35.	2-methylaniline	−1.97	1.32[a]	−8.443	0.585	Zimmer et al. [1980]
36.	2, 2′-diaminobiphenyl	−1.34	1.34[a]	−8.193	0.267	Nohara et al. [1985]
37.	3, 3′-dimethylbenzidine	0.37	2.34[a]	−7.873	0.185	Haworth et al. [1983]
38.	9-aminofluorene	−0.77	2.43	−8.817	−0.326	Later et al. [1984]
39.	2, 4-diaminoisopropylbenzene	−1.75	1.12	−8.121	0.722	Shahin et al. [1980]
40.	2, 4′-diaminobiphenyl	−0.95	1.58	−8.144	0.215	Nohara et al. [1985]
41.	2-aminophenol	−2.53	0.62[a]	−8.356	0.448	Haworth et al. [1983]
42.	4, 4′-methylenebis (2 isopropylaniline)	2.01	4.46	−8.165	0.610	Rao et al. [1982]
43.	3, 3′-diaminobiphenyl	−1.30	1.58	−8.414	0.056	Nohara et al. [1985]
44.	2-methoxyaniline	−1.91	1.18[a]	−8.331	0.525	Haworth et al. [1983]
45.	3-trifluoromethylaniline	−1.38	2.29[a]	−9.032	−0.104	Haworth et al. [1983]
46.	4-bromoaniline	−0.97	2.26[a]	−8.620	0.212	Zieger et al. [1988]

[a]Experimental log P.

from a single laboratory using a small and relatively homologous set of compounds [Shusterman et al., 1989].

The physical reasonableness of a QSAR provides another method for judging its reliability. Equations 3 and 5 contain four structure-dependent parameters whose values correlate with mutagenic activity. While it is not possible to identify unambiguously the mechanistic significance of each term, plausible origin(s) can be proposed for each of the structure-activity relationships.

The principle factor affecting the relative mutagenicity of aminoarenes is their hydrophobicity (activity = 1.1 log P). Coefficients with log P of ~1 have turned out to be fairly common in QSARs: triazenes acting in TA92 [Shusterman et al., 1989] and nitroarenes acting in either TA98 [Debnath et al., 1991] or TA100 [Debnath et al., submitted] show similar relationships between activity and log P. On the other hand, no log P term appears in the QSAR describing the mutagenicity of a series of platinum(diamine) complexes

in TA92 [Hansch et al., 1980], and negative coefficients with log P are observed for the small set of hydrophilic diamines studied here (Eqs. 4 and 6).

A precise interpretation of the log P term in a QSAR is difficult, since hydrophobicity plays an important role in at least two different types of processes: transport of the chemicals to their sites of activation and chemical reaction, and binding of the chemicals to the bioreceptors responsible for activation. The log P dependence found in a given QSAR, therefore, reflects the overall effect of hydrophobicity on several different processes. The log P terms found in Eqs. 3 and 5 are reasonable, but their interpretation requires a detailed knowledge of the log P dependence of each of the steps shown in Scheme 1.

Since activation of the amino group involves oxidation by cytochrome P-450 (Scheme 1, step 1), electronic factors might also be expected to play a key role in determining relative mutagenicity. In fact, two electronic terms appear in Eqs. 3 and 5: activity $\approx 1.5\ \epsilon_{HOMO}$ and activity $\approx -0.7\ \epsilon_{LUMO}$. The positive correlation between activity and ϵ_{HOMO} seems reasonable; compounds with higher ϵ_{HOMO} are easier to oxidize and should be readily activated. The negative correlation between activity and ϵ_{LUMO}, indicating that amines that are better electron acceptors are also more mutagenic, lacks a simple explanation. One possible rationale for the ϵ_{LUMO} term can be found in a recent suggestion by Zhou and Parr [1990] that the barrier for the reaction of an electrophile with an aromatic compound is related to the "hardness" of the aromatic compound, where hardness or η, is defined as one half of the difference between the two frontier orbital energies (i.e., $\eta = (\epsilon_{LUMO} - \epsilon_{HOMO})/2$). Equations 7 and 8 show that increasing activity is correlated with decreasing hardness in the aminoarene.

$$\log TA98 = 1.02\ (\pm 0.26)\ \log P - 1.50\ (\pm 0.83)\ \eta + 1.67\ (\pm 0.54)\ I_L + 2.83\ (\pm 3.60)$$
$$n = 88,\ r = 0.892,\ s = 0.878 \qquad (7)$$

$$\log TA100 = 1.07\ (\pm 0.25)\ \log P - 1.86\ (\pm 0.74)\ \eta + 5.03\ (\pm 3.45)$$
$$n = 67,\ r = 0.873,\ s = 0.728 \qquad (8)$$

Loew and her colleagues [1979] have also addressed the problem of electronic effects on the mutagenicity of aminoarenes. Using MINDO/3-based calculations, they concluded that the mutagenic activity of nitroanilines can be explained by factors that enhance N-hydroxylation and nitrenium ion stability. They also concluded that ring epoxidation is unlikely to be involved. They interpret their results as indicating that it is the superdelocalizability of the electrons on nitrogen which is crucial, the most mutagenic compound in their study, 1,2,3-triaminobenzene, having the highest N-superdelocalizability. Paradoxically, the next most active congeners in their study, which was based on mutation rates in TA1538, are the nitroanilines, which were calculated to have the lowest superdelocalizabilities. A better explanation for their results might be found in the combined use of ϵ_{HOMO} and ϵ_{LUMO} as in Eqs. 3 and 5 (or by "hardness" as in Eqs. 7 and 8). A high ϵ_{HOMO} should be found for electron-rich 1,2,3-triaminobenzene, while a low ϵ_{LUMO} should be found for the electron-poor nitroanilines. Either tendency according to Eqs. 3 and 5 would tend to enhance mutagenicity.

More perplexing than the question of which electronic index to use in QSAR development is the observation that the electronic terms in Eqs. 3, 5, 7, and 8 account for only about 4–11% of the total variation in mutagenic activity. That is, it appears that electronic effects are of minor importance in determining the relative mutagenicity of aminoarenes. It is important to remember, however, that the electronic terms, like the log P term, represent the overall effect of several activation steps. For example, while the initial oxidation step may be favorable for more electron-rich amines, electron-poor hydroxylamines are more likely to survive the subsequent journey from S9 to their activation site inside the bacterial cell [Vance et al., 1988]. The opposing electronic demands of these two steps may tend to reduce the overall importance of electronic factors. It should also be noted that esterification of the hydroxylamine is also believed to play an important role in the activation mechanism [King and Philips, 1968; DeBaum et al., 1970; Weisberger et al., 1972; Duffel and Jakoby, 1981; McCoy and Rosenkranz, 1982; McCoy et al., 1983; Bryant et al., 1984] and little is known about the role of electronic effects on esterification or on the reaction of an esterified hydroxylamine with DNA.

A final warning is also in order regarding the interpretation of electronic effects. It may not be possible to attribute the electronic properties calculated for the aminoarene to each of its metabolic products. For example, an aminoarene with a low ϵ_{LUMO} need not necessarily give rise to an electron-poor hydroxylamine, and so on. We are currently investigating this point by performing electronic structure calculations on the different intermediates shown in Scheme 1.

Equation 3 also contains an indicator variable, I_L, which is strongly correlated with the activity of aminoarenes in TA98 + S9 ($r = 0.771$). I_L does not appear in Eq. 5, the TA100 + S9 QSAR. In fact, Eq. 3 is nearly identical to Eq. 5, aside from the I_L term. Equations 9a and 9b show the correlation between log TA98 and log TA100 for the 60 amines that are listed in both Table IIa and Table IIIa. The coefficient with log TA100 in Eq. 9b is nearly one, and the coefficient with I_L is well within the confidence interval of Eq. 3.

$$\log TA98 = 1.22\ (\pm 0.15)\ \log TA100 - 0.25\ (\pm 0.24)$$
$$n = 60,\ r = 0.903,\ s = 0.897,\ F_{1,58} = 257 \qquad (9a)$$

$$\log TA98 = 0.95\ (\pm 0.19)\ \log TA100 + 1.22\ (\pm 0.58)\ I_L - 0.70\ (\pm 0.30)$$
$$n = 60,\ r = 0.927,\ s = 0.791,\ F_{1,57} = 17.5 \qquad (9b)$$

Equation 9b indicates that, aside from the I_L term, any change in aminoarene structure affecting mutagenicity in TA100 will, on the average, produce an identical effect in TA98. Therefore, it is likely that the structural factors that control activity in TA100 (i.e., hydrophobicity and electronic effects) exert their influence primarily during the activation and transport processes that the two bacterial strains have in common. The "I_L-effect," on the other hand, must occur during a process that is unique to TA98. The only significant difference between the two strains lies in the structure of a single gene (the DNA in this gene contains a frameshift mutation in TA98 and a basepair substitution in TA100); thus, the I_L term is probably associated with a difference in how the activated amine species reacts with the DNA in this gene. Klopman et al. [1985] have previously noted that larger amines show enhanced frameshift mutagenicity. Equations 3 and 5 support this proposal and refine it to indicate that enhancement of frameshift mutagenicity is a characteristic of aromatic systems containing three or more fused rings. The QSARs also demonstrate that this effect, which may be due to intercalation of larger compounds into the DNA, is separate from the increased hydrophobicity associated with such compounds.

Tables IIc and IIIc list aminoarenes that were found to be inactive in the mutagenicity assay. Also listed are the relevant physico-chemical parameters for each amine and the activity that would be expected from Eqs. 3 and 5, respectively. Most of the compounds in Table IIc, aside from the methylenebis(aniline)s, are predicted to be weakly active. An exception is 4-aminophenanthrene, which is predicted to be relatively active. This compound, though inactive in TA98, does show weak activity in TA100, but this activity is about six times less than expected. It would be interesting to test other structures of this type to see if the 4-position is sterically hindered. Steric hindrance due to the alkyl groups in the 2- and 6-positions of the inactive methylenebis(aniline) derivatives is almost certainly responsible for their behavior. Table IIIc contains many more inactive compounds than IIc, most of which are aniline derivatives for which only weak activity is predicted. It is not clear why the TA100 system is less sensitive to these compounds.

In deriving Eqs. 3 and 5, a number of data points have not been included and need special consideration. Most notable are the hydrophilic amines ($\log P < 1$) used to derive Eqs. 4 and 6. As these two QSARs show, the mutagenicity of these compounds increases with decreasing log P, suggesting an entirely different mechanism of action. Since these hydrophilic amines are unlikely to undergo oxidation in the lipophilic microsomes, we believe that these compounds may be undergoing activation by oxidases located in an aqueous compartment. The hydrophilic nitrophenylenediamines listed in Table IIb do not conform to Eq. 4. While we do not understand this behavior, we find once again that the QSAR approach is effective in sorting out specific outliers which need more detailed attention.

The following compounds in Table IIa were not utilized in the derivation of Eq. 3: 2,7-diaminophenazine, 4,4′-methylenebis(o-isopropylaniline), 1-aminopyrene, 6-aminochrysene, 4-aminocarbazole, 1-aminocarbazole, and 9-aminoanthracene. The aminochrysene is less active than expected and this may be due, at least in part, to its very high log P (4.98). We have previously found that there is an optimal log P value for mutagenic nitroarenes (log $P_0 = 4.93$ in Eq. 1) beyond which activity rapidly falls. Since the aminochrysene is the only highly hydrophobic compound in the aminoarene data set, the existence of an optimal log P for the action of aminoarenes could not be evaluated. Though most of the other outliers were much less active than predicted (the phenazine is more active than predicted), there is no clear reason why these compounds fail to conform to Eq. 3.

The following compounds in Table IIIa were not used in the derivation of Eq. 5: 9-aminoanthracene, 1-aminopyrene, 1-aminoanthracene, 2,4-xylidine, 4,4′-methylenedianiline, and 3,4′-diaminobiphenyl.

Steric hindrance might account for the especially low activity of 9-aminoanthracene in TA98 and TA100. We have previously found [Compadre et al., 1990], as had others [Fu et al., 1985, 1988], that the 9-nitroanthracene-type congeners are much less active than expected. It has also been pointed out that while 6-nitrobenzo[a]pyrene (having a 9-nitroanthracene type structure) is very weakly mutagenic, the corresponding nitroso compound is quite active [Fu, 1990]. This would suggest that the steric effect occurs at the site of initial nitro reduction, rather than in the reaction with DNA. It may be that initial oxidation of 9-aminoanthracene is similarly hindered, accounting for its low activity.

The QSAR for the aromatic amines are less satisfactory than for the nitro compounds in that inactive or weakly active amines are not as well predicted. This is especially true for TA100. This may be due to the inherent toxicity of the amino group to micro organisms illustrated by QSAR 10.

$$\log 1/C = 0.60 \log P + 3.94$$
$$n = 15,\ r = 0.940,\ s = 0.137 \qquad (10)$$

Equation 10, correlating the inhibition of growth of *S. typhosa* (Lien et al., 1968), and others like it show that arylamines are much more toxic to microorganisms than other simple organic compounds, such as phenols [Lien et al. 1968]. Thus, arylamines might inhibit TA98 and TA100 at sublethal concentrations which would not be readily detected. We plan to investigate this point.

CONCLUSION

QSARs describing the mutagenic activity of amines in TA98 + S9 and TA100 + S9 show that activity is primarily dictated by the hydrophobicity of the amine; activity increases with hydrophobicity for amines with $\log P > 1$.

Electronic effects also play a role in determining mutagenic activity, but they are considerably less important, possibly due to the conflicting electronic demands of different activation steps. Steric effects were not incorporated into the QSARs.

Hydrophobic and electronic effects play essentially identical roles in determining mutagenic activity in TA98 and TA100, suggesting that these effects are exerted during the activation process. An additional effect, the number of aromatic rings in the amine, affects activity in TA98 only. This effect, which must be associated with the particular structure of the DNA around the mutation site in TA98, suggests that large ring systems are especially well-suited to induce frameshift mutations, possibly via intercalation.

While the QSARs can be validated in several ways they are far from ideal. Several compounds had to be excluded in the derivation of each QSAR, and research into the role of steric effects and the design of superior methods for modeling electronic effects is underway.

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