Factors that influence tadpole narcosis. An LFER analysis

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Application of the new solvation equation to the results of Overton on tadpole narcosis yield the correlation given by eqn. (*a*), where C_{nar} is the narcotic concentration of solute in mol dm^{-3} and the

$$\log (1/C_{nar}) = 0.579 + 0.824R_2 - 0.334\pi_2^H - 2.871\Sigma\beta_2^O + 3.097V_X \quad (a)$$

descriptors are R_2 the solute excess molar refraction, π_2^H the solute dipolarity/polarizability, $\Sigma\beta_2^O$ the solute hydrogen-bond basicity and V_X the solute volume. For 84 solutes, the correlation coefficient, ρ, is 0.9730 and the standard deviation, sd, is 0.246 log units. The above equation shows that the two main factors that influence tadpole narcosis are solute hydrogen-bond basicity that decreases toxicity and solute volume that increases toxicity. Solute hydrogen-bond acidity has no effect at all.

The use of water–octanol partition coefficients as log *P*(oct) leads to the regression equation [eqn. (*b*)]

$$\log (1/C_{nar}) = 1.129 + 0.833\log P(\text{oct}) \quad (b)$$

where $\rho = 0.9212$ and sd = 0.407 for the same 84 solutes. This equation is not improved by addition of $[\log P(\text{oct})]^2$ as a descriptor, but it is if V_X is included [eqn. (*c*)]. Now $\rho = 0.9400$ and sd = 0.359

$$\log (1/C_{nar}) = 0.621 + 0.743\log P(\text{oct}) + 0.668V_X \quad (c)$$

log units. When additional data are added to the Overton set, similar equations to the above are obtained for 114 varied solutes, showing that tadpole narcosis is a general phenomenon.

Results on the narcosis of α,ω-diols and cycloalkanols can be interpreted using the analysis of Franks and Lieb. It is suggested that the anaesthetic binding site on the primary protein target is a large or flexible pocket, extending inward from the external water-facing surface, that is rather aqueous and of limited hydrophobicity.

Around the turn of the century, Overton and Meyer independently studied the narcotic activity of aqueous compounds towards the tadpole.[1–3] Overton was particularly active and gathered data on a large number of compounds (or solutes) in terms of their narcotic concentration, *i.e.* the minimum concentration required for narcosis, C_{nar}, in mol dm^{-3}. Both Overton and Meyer noted that narcotic concentration was related to the partition coefficient of the solute between water and olive oil; this was the foundation of the 'lipoid' theory of anaesthesia that has dominated the field ever since. Oddly enough, neither Overton nor Meyer set out any mathematical relationship between narcotic concentration and the partition coefficient. This was left to K. H. Meyer and Gottlieb-Billroth,[4] some 20 years later, who put forward the relationship (1), where *P* is the water–olive oil partition

$$C_{nar} \times P = \text{constant} \quad (1)$$

coefficient. For 39 varied compounds studied by Overton and by Meyer, the product was reasonably constant. A few years later, K. H. Meyer and Hemmi[5] showed that water–oleyl alcohol partition coefficients could be used in eqn. (1), this time using their own determined values of C_{nar}. Eqn. (1) can be expressed in logarithmic form as eqn. (2), or more generally as eqn. (3), where SP can be a biological property such as $1/C$

$$\log (1/C) = \log P + \text{constant} \quad (2)$$

$$\log \text{SP} = a \times \log P + c \quad (3)$$

for tadpole narcosis or a physicochemical property, such as another partition coefficient. Eqn. (3) is now referred to as the Overton–Meyer relationship, or sometimes just as the Overton rule, and has been applied to numerous series of biological results in aqueous solution.[6]

There have been several studies of Overton's data, although often restricted to particular compound sets. Hansch *et al.*[7] examined results for 10 alkan-1-ols, but later analysed much larger data sets of 53 compounds[8] and 57 compounds.[9] Kamlet *et al.*[10] also used the same 53 solute set, but the lack of descriptors reduced the number to 42 in their correlation, further reduced to 39 after leaving out acetonitrile, nitromethane and acetophenone. The same 39 solutes were examined by Wilson and Famini[11] using their theoretical descriptors. Magee[12] used a rather large data set of 52 solutes, similar to the Hansch 53 data set. A rather different approach was used by Lipnick,[13] who did not attempt to correlate Overton's data in total, but restricted his analysis to alcohols, ketones and aromatic hydrocarbons. This yielded a 'base-line' quantitative structure–activity relationship, or QSAR, for 18 solutes. A summary of the statistical fits of the various correlation equations in $\log (1/C_{nar})$ is given in Table 1.

In this table, and elsewhere, *n* is the number of data points, ρ is the overall correlation coefficient, sd is the standard deviation, *F* is the F-statistic and *D* is the number of descriptors used. The regression equations do not vary much in the goodness-of-fit and yield sd values of *ca.* 0.2–0.3 log units, probably not far from the experimental error.

In some cases, the descriptors lend themselves to chemical interpretation. From the correlation equations obtained by Kamlet *et al.*[10] and by Wilson and Famini,[11] using Overton's data, it appears that solute hydrogen-bond basicity decreases solute toxicity and solute volume increases toxicity. This

Table 1 Results of correlations of Overton's data

n	ρ	sd	F	D	Ref.
10	0.987	0.311	302	1[a]	7
10	0.995	0.215	347	2	7
53	0.956	0.343	535	1[a]	8
57	0.962	0.312	683	1[a]	9
42	0.9788	0.244	211	4	10
39	0.9899	0.168	414	4	10
41	0.970	0.290	141	4	11
39	0.969	0.300	129	4	11
52	0.976	0.262	316	3	12
18	0.986	0.227	560	1[a]	13

[a] Log P(oct).

was also found by Abraham *et al.*[14] who used a different data set. Other work on tadpole narcosis has focussed on the cut-off point observed at tridecan-1-ol[15] and on the use of particular series of solutes such as cycloalcohols,[16] alkane-α,ω-diols,[17] and bromododecane-1,12-diols[18] to probe the molecular dimensions of the anaesthetic target site in tadpoles. Much of this has been reviewed by Franks and Lieb[19] who conclude that anaesthetics act directly on proteins, probably by binding to pockets or clefts, rather than acting on lipids.

Although up to 57 solutes of the Overton data set have been examined (Table 1), this still does not represent the full list of solutes studied by Overton. It was our aim to examine as many solutes as possible out of the Overton set and then to see if the set could be extended even further by incorporation of data obtained by other workers, including the various series of Franks and Lieb and co-workers.[16–18]

Methodology

Our method is based[20,21] on the linear free energy relationship (LFER), or solvation equation [eqn. (4)]. Here, SP is a biological

$$\log \mathrm{SP} = c + rR_2 + s\pi_2^{\mathrm{H}} + a\Sigma\alpha_2^{\mathrm{H}} + b\Sigma\beta_2 + vV_{\mathrm{X}} \quad (4)$$

or chemical property of a series of solutes in a given system and the independent variables are solute descriptors as follows:[20,21] R_2 is the solute excess molar refraction, π_2^{H} is the solute dipolarity/polarizability, $\Sigma\alpha_2^{\mathrm{H}}$ is the solute overall or effective hydrogen-bond acidity, $\Sigma\beta_2$ is the solute overall or effective hydrogen-bond basicity and V_{X} is McGowan's characteristic volume[22] in units of (cm^3 mol^{-1})/100. For most solutes, the effective hydrogen-bond basicity descriptor is constant over all solvent systems and is denoted as $\Sigma\beta_2^{\mathrm{H}}$ or $\Sigma\beta_2^{\mathrm{O}}$ (the two being identical). In the case of certain specific solutes, including anilines and pyridines, the effective hydrogen-bond basicity varies with the solvent system. For partition between water and rather non-aqueous solvent systems, the $\Sigma\beta_2^{\mathrm{H}}$ descriptor is used, but for partition between water and aqueous solvent systems such as wet octanol and wet ether, an alternative $\Sigma\beta_2^{\mathrm{O}}$ descriptor is used (the two now not being identical). Eqn. (4) has been applied to numerous water–solvent partitions,[23–25] so it is by now a well established equation. Two examples are water–isobutanol and water–hexadecane partition coefficients, as log P(Bu$^{\mathrm{i}}$OH) and log P(16). In the former case, the $\Sigma\beta_2^{\mathrm{O}}$ descriptor is used,[25] but for the water–hexadecane partition the $\Sigma\beta_2^{\mathrm{H}}$ descriptor is employed.[24]

$$\log P(\mathrm{Bu^iOH}) = 0.227 + 0.514R_2 - 0.693\pi_2^{\mathrm{H}} + 0.020\Sigma\alpha_2^{\mathrm{H}} - 2.258\Sigma\beta_2^{\mathrm{O}} + 2.776V_{\mathrm{X}} \quad (5)$$
$$n = 37,\ \rho = 0.9911,\ \mathrm{sd} = 0.119,\ F = 345$$

$$\log P(16) = 0.087 + 0.667R_2 - 1.617\pi_2^{\mathrm{H}} - 3.587\Sigma\alpha_2^{\mathrm{H}} - 4.869\Sigma\beta_2^{\mathrm{H}} + 4.433V_{\mathrm{X}} \quad (6)$$
$$n = 370,\ \rho = 0.9982,\ \mathrm{sd} = 0.124,\ F = 20\,236$$

The coefficients in eqns. (5) and (6) can be regarded as characteristic of the particular system investigated, so that here they contain information of the differences between water and the two organic solvents. The positive r-coefficient shows that the solvents are more polarizable than water and the negative s-coefficient shows that water is much more dipolar than the solvents. The negative a-coefficient in eqn. (6) shows that water is a much stronger hydrogen-bond base than hexadecane, but the almost zero a-coefficient in eqn. (5) implies that water and (wet) isobutanol must have the same hydrogen-bond basicity. The negative b-coefficient in both equations means that water is more acidic than either solvent and the positive v-coefficients show that larger solutes favour the organic solvents, the latter are thus more hydrophobic than water.

Results and discussion

The Overton data set

We assembled values of log $(1/C_{\mathrm{nar}})$ for 110 solutes, taken from Lipnick's translation[26] of Overton's book,[1] or from the paper of Lipnick.[13] Descriptors were available or could be calculated for 89 of these compounds as shown in Table 2. Of these, five compounds (acetamide, urea, *N*-methylurethane, nicotine and 2-propylpiperidine) were outliers, to leave 84 compounds. Use of the alternative $\Sigma\beta_2^{\mathrm{O}}$ descriptor in eqn. (4) led to the following regression.

$$\log(1/C_{\mathrm{nar}}) = \underset{(0.087)}{0.609} + \underset{(0.086)}{0.866}R_2 - \underset{(0.110)}{0.347}\pi_2^{\mathrm{H}} - \underset{(0.106)}{0.174}\Sigma\alpha_2^{\mathrm{H}} - \underset{(0.138)}{2.808}\Sigma\beta_2^{\mathrm{O}} + \underset{(0.115)}{3.054}V_{\mathrm{X}} \quad (7)$$
$$n = 84,\ \rho = 0.9739,\ sd = 0.244,\ F = 287$$

In eqn. (7), the standard deviation of the coefficient is given in parentheses below the coefficient. Even though this is a much larger data set than previously studied, the goodness-of-fit of eqn. (7) is about the same as that for the equations in Table 1. We checked the descriptors in eqn. (7) for possible strong interrelations; the most marked cross-correlations were between R_2 and π_2^{H} ($\rho = 0.705$ and $\rho^2 = 0.497$) and between π_2^{H} and $\Sigma\beta_2^{\mathrm{O}}$ ($\rho = 0.637$ and $\rho^2 = 0.406$), which can be tolerated. In addition, the range of values of the descriptors for the 84 compounds is very large, so that the set covers compounds of very varied chemical type and structure.

Hence eqn. (7) shows that narcosis towards the tadpole *Rana temporaria*, the species used by Overton, is a very general phenomenon indeed, with only five compounds out of the 89 not fitting eqn. (7). Lipnick[13] has discussed some of the reasons why compounds may not fit correlation equations. Apart from the well known 'cut-off' effect, some compounds are so insoluble in water, that their anaesthetic concentration is never reached and hence appear to be less toxic than expected. A number of very water-soluble compounds such as urea, acetamide and succinimide may act through osmotic effects at the high aqueous concentrations used. Finally, compounds can undergo reactions in the biological system, of the Michael addition type, that lead to enhanced toxicity. A difficulty not mentioned by Lipnick may occur with compounds that are strong proton acids or strong proton bases and which are partially ionised in aqueous solution. Ionised species either do not distribute from water into organic phases or distribute to a much smaller extent than the corresponding neutral species. The analytical concentration of the total [(inactive) ionised species plus

Table 2 Descriptors and values of log $(1/C_{nar})$ used

Compound	R_2	Π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^O$	V_X	Log P(oct)	Log $(1/C_{nar})$ Obs	Log $(1/C_{nar})$ Calc[b]
Overton's data[a]								
Pentane	0.000	0.00	0.00	0.00	0.8131	3.39	2.55	3.30
2-Methylbut-2-ene	0.159	0.08	0.00	0.07	0.7701	2.67	2.64	3.04
Trichloromethane	0.425	0.49	0.15	0.02	0.6167	1.97	2.85	2.62
Tetrachloromethane	0.458	0.38	0.00	0.00	0.7391	2.83	3.14	3.14
Chloroethane	0.227	0.40	0.00	0.10	0.5128	1.43	2.35	1.93
1,2-Dichloroethane	0.416	0.64	0.10	0.11	0.6352	1.48	2.63	2.32
Bromoethane	0.366	0.40	0.00	0.12	0.5654	1.61	2.57	2.16
Iodoethane	0.640	0.40	0.00	0.15	0.6486	2.00	2.96	2.58
Diethyl ether	0.041	0.25	0.00	0.45	0.7309	0.89	1.47	1.72
Propanone	0.179	0.70	0.04	0.49	0.5470	−0.24	0.54	0.80
Butanone	0.166	0.70	0.00	0.51	0.6879	0.29	1.04	1.20
Pentan-2-one	0.143	0.68	0.00	0.51	0.8288	0.84	1.72	1.67
Pentan-3-one	0.154	0.66	0.00	0.51	0.8288	0.82	1.54	1.69
Camphor	0.450	0.85	0.00	0.56	1.3161	2.53	2.88	3.28
Ethyl formate	0.146	0.66	0.00	0.38	0.6057	0.27	1.16	1.27
Methyl acetate	0.142	0.64	0.00	0.45	0.6057	0.18	1.10	1.10
Ethyl acetate	0.106	0.62	0.00	0.45	0.7466	0.73	1.52	1.56
Propyl acetate	0.092	0.60	0.00	0.45	0.8875	1.24	1.96	2.04
Butyl acetate	0.071	0.60	0.00	0.45	1.0284	1.82	2.30	2.49
Isobutyl acetate	0.052	0.57	0.00	0.47	1.0284	1.60	2.24	2.44
Pentyl acetate	0.067	0.60	0.00	0.45	1.1693	2.01	2.72	2.95
Ethyl propanoate	0.087	0.58	0.00	0.45	0.8875	1.21	1.96	2.05
Ethyl butanoate	0.068	0.58	0.00	0.45	1.0284	1.73	2.37	2.50
Ethyl pentanoate	0.049	0.58	0.00	0.45	1.1693	2.26	2.72	2.96
Butyl pentanoate	0.033	0.56	0.00	0.45	1.4511	3.32	3.60	3.90
Ethyl isobutanoate	0.034	0.55	0.00	0.47	1.0284	1.51	2.24	2.44
Triacetin	0.136	1.30	0.00	1.35	1.5999	0.25	1.64	1.63
Acetonitrile	0.237	0.90	0.07	0.32	0.4042	−0.34	0.44	0.67
Nitromethane	0.313	0.95	0.06	0.31	0.4237	−0.33	1.09	0.79
Pentanamide	0.400	1.30	0.50	0.62	0.9286	0.35	1.30	1.60
N-Ethylurethane	0.236	0.82	0.24	0.61	0.9873	1.02	1.40	1.97
Methanol	0.278	0.44	0.43	0.47	0.3082	−0.74	0.24	
Ethanol	0.246	0.42	0.37	0.48	0.4491	−0.30	0.54	
Propan-1-ol	0.236	0.42	0.37	0.48	0.5900	0.25	0.96	
Propan-2-ol	0.212	0.36	0.33	0.56	0.5900	0.05	0.89	1.09
Butan-1-ol	0.224	0.42	0.37	0.48	0.7309	0.84	1.42	
2-Methylpropan-1-ol	0.217	0.39	0.37	0.48	0.7309	0.76	1.35	1.77
2-Methylpropan-2-ol	0.180	0.30	0.31	0.60	0.7309	0.35	0.89	1.47
3-Methylbutan-1-ol	0.192	0.39	0.37	0.48	0.8718	1.28	1.64	2.22
2-Methylbutan-2-ol	0.194	0.30	0.31	0.60	0.8718	0.89	1.24	1.96
Octan-1-ol	0.199	0.42	0.37	0.48	1.2950	3.07	3.40	
Menthol	0.400	0.48	0.32	0.61	1.4677	3.31	3.97	3.96
Ethane-1,2-diol	0.404	0.90	0.58	0.78	0.5078	−1.36	0.19	0.08
Ethanethiol	0.392	0.35	0.00	0.24	0.5539	1.18	2.09	1.87
Carbon disulfide	0.876	0.26	0.00	0.03	0.4905	1.94	3.28	2.64
Triethyl phosphate	0.000	1.00	0.00	1.06	1.3934	0.80	1.96	1.79
Benzene	0.610	0.52	0.00	0.14	0.7164	2.13	2.68	2.72
m-Xylene	0.623	0.52	0.00	0.16	0.9982	3.20	3.42	3.62
Naphthalene	1.340	0.92	0.00	0.20	1.0854	3.30	4.19	4.08
Phenanthrene	2.055	1.29	0.00	0.26	1.4540	4.46	5.25	5.45
Methyl phenyl ether	0.708	0.75	0.00	0.29	0.9160	2.11	2.82	2.91
1,3-Dimethoxybenzene	0.816	1.01	0.00	0.45	1.1160	2.21	3.35	3.07
1,4-Dimethoxybenzene	0.806	1.00	0.00	0.50	1.1160	2.03	3.05	2.94
Acetophenone	0.818	1.01	0.00	0.48	1.0139	1.63	3.04	2.65
Aniline	0.955	0.96	0.26	0.50	0.8162	0.90	1.96	2.14
N,*N*-Dimethylaniline	0.957	0.84	0.00	0.47	1.0980	2.31	2.85	3.19
Diphenylamine	0.700	0.88	0.60	0.38	1.4240	3.50	4.43	4.58
Azobenzene	0.680	1.20	0.00	0.44	1.4808	3.82	4.74	4.85
Acetanilide	0.870	1.40	0.50	0.67	1.1133	1.16	2.31	2.38
p-Methoxyacetanilide	0.970	1.63	0.48	0.86	1.3133	1.05	2.09	2.47
p-Ethoxyacetanilide	0.940	1.60	0.48	0.84	1.4542	1.58	2.55	2.99
Phenol	0.805	0.89	0.60	0.30	0.7751	1.50	2.28	2.54
o-Cresol	0.840	0.86	0.52	0.30	0.9160	1.98	2.92	3.04
m-Cresol	0.822	0.88	0.57	0.34	0.9160	1.98	2.75	2.92
p-Cresol	0.820	0.87	0.57	0.31	0.9160	1.97	2.75	3.00
2-Isopropyl-5-methylphenol	0.822	0.79	0.52	0.44	1.3387	3.30	4.26	4.13
4-*tert*-Pentylphenol	0.810	0.89	0.56	0.41	1.4796	3.83	4.52	4.61
2-Methoxyphenol	0.837	0.91	0.22	0.52	0.9747	1.32	2.57	2.56
Catechol	0.970	1.07	0.85	0.52	0.8338	0.88	2.12	2.23
Resorcinol	0.980	1.00	1.10	0.58	0.8338	0.80	1.64	2.19
Hydroquinone	1.000	1.00	1.16	0.60	0.8338	0.59	2.12	2.17
Vanillin	1.040	1.04	0.32	0.67	1.1313	1.21	2.48	2.78

Table 2 (*contd.*)

Compound	R_2	Π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^O$	V_X	Log P(oct)	Log $(1/C_{nar})$ Obs	Log $(1/C_{nar})$ Calc[b]
Eugenol	0.946	0.99	0.22	0.51	1.3544	2.99	3.91	3.88
Phenylthiourea	1.250	1.72	0.49	0.78	1.1774	0.73	2.18	2.38
Coumarin	1.060	1.79	0.00	0.46	1.0619	1.32	3.24	2.51
Phthalide	0.950	1.90	0.00	0.46	0.9640	0.80	2.37	2.02
Piperonal	0.990	1.60	0.00	0.52	1.0227	1.05	2.78	2.30
Paraldehyde	0.136	0.68	0.00	0.68	1.0215	0.67	1.60	1.87
Pyridine	0.631	0.84	0.00	0.47	0.6753	0.65	1.60	1.52
Quinoline	1.268	0.97	0.00	0.51	1.0443	2.03	2.72	3.05
Antipyrine	1.320	1.50	0.00	1.48	1.5502	0.23	1.89	1.90
Caffeine	1.400	1.55	0.00	1.34	1.3632	−0.07	1.92	1.68
Morphine	2.200	2.34	0.86	1.79	2.0648	0.76	2.76	3.11
Phenylurea	1.110	1.40	0.77	0.77	1.0726	0.83	2.34	2.24
Acetamide	0.460	1.30	0.54	0.68	0.5059	−1.26	0.77	(0.07)
Methyl urethane	0.263	0.82	0.24	0.61	0.8464	0.34	0.57	(1.52)
Nicotine	0.865	1.34	0.00	0.94	1.3710	1.17	3.51	(2.46)
2-Propylpiperidine	0.364	0.44	0.10	0.69	1.2270	2.15	3.48	(2.91)
Urea	0.500	1.00	0.50	0.90	0.4648	−2.11	0.60	(−0.40)
Acetaldoxime							0.93	
Acetoxime							1.12	
Chloral formamide							1.76	
Chloral hydrate							2.22	
Chloralose							2.49	
3-Chloropropane-1,2-diol							0.77	
1,3-Dichloropropan-2-ol							1.95	
Ethyl acetoacetate							1.72	
Ethyl citrate							2.04	
Ethyl nitrate							2.14	
Ethyl tartrate							1.22	
Methyl acetyl urea							0.76	
N-Phenylurethane							3.22	
Pinacol							0.81	
Sparteine							3.45	
Strychnine							4.34	
Succinamide							0.70	
Sulfonal							2.06	
Triethyl thiourea							1.62	
Trional							2.16	
Turpentine							3.30	
Additional data								
N-Ethylurethane[c]	0.236	0.82	0.24	0.61	0.9873	1.02	1.46	1.97
N-Propylurethane[c]	0.225	0.82	0.24	0.61	1.1282	1.55	2.18	2.43
N-Isobutylurethane[c]	0.202	0.79	0.24	0.61	1.2691	2.02	2.50	2.91
N-Isopentylurethane[c]	0.190	0.79	0.24	0.61	1.4100	2.64	2.93	3.37
Methanol[d]	0.278	0.44	0.43	0.47	0.3082	−0.74	0.23	0.40
Ethanol[d]	0.246	0.42	0.37	0.48	0.4491	−0.30	0.72	0.82
Propan-1-ol[d]	0.236	0.42	0.37	0.48	0.5900	0.25	1.14	1.29
Butan-1-ol[d]	0.224	0.42	0.37	0.48	0.7309	0.84	1.97	1.75
Pentan-1-ol[d]	0.219	0.42	0.37	0.48	0.8718	1.51	2.54	2.22
Hexan-1-ol[d]	0.210	0.42	0.37	0.48	1.0127	2.03	3.24	2.68
Heptan-1-ol[d]	0.211	0.42	0.37	0.48	1.1536	2.62	3.64	3.16
Octan-1-ol[d]	0.199	0.42	0.37	0.48	1.2950	3.07	4.24	3.62
Nonan-1-ol[d]	0.193	0.42	0.37	0.48	1.4354	3.77	4.43	4.08
Decan-1-ol[d]	0.191	0.42	0.37	0.48	1.5763	4.18	4.90	4.55
Undecan-1-ol[d]	0.181	0.42	0.37	0.48	1.7170	4.72	5.09	5.01
Dodecan-1-ol[d]	0.175	0.42	0.37	0.48	1.8580	5.13	5.33	5.48
Butan-2-ol[e]	0.217	0.36	0.33	0.56	0.7309	0.65	1.77	1.57
Pentan-2-ol[e]	0.195	0.36	0.33	0.56	0.8718	1.25	2.32	2.03
Hexan-2-ol[e]	0.187	0.36	0.33	0.56	1.0127	1.76	2.86	2.49
Heptan-2-ol[e]	0.188	0.36	0.33	0.56	1.1536	2.31	3.48	2.96
Octan-2-ol[e]	0.158	0.36	0.33	0.56	1.2950	2.90	4.21	3.41
Cyclohexanol[f]	0.460	0.54	0.32	0.57	0.9040	1.23	2.30	2.18
Cycloheptanol[f]	0.513	0.54	0.32	0.58	1.0450	1.80	2.89	2.67
Cyclooctanol[f]	0.578	0.54	0.32	0.58	1.1860	2.36	3.41	3.18
Cyclodecanol[f]	0.621	0.54	0.32	0.58	1.4677	3.48	4.08	4.17
Pentane-1,5-diol[g]	0.388	0.95	0.72	0.92	0.9305	−0.25	1.72	1.15
Hexane-1,6-diol[g]	0.385	0.95	0.75	0.92	1.0714	0.25	1.60	1.59
Heptane-1,7-diol[g]	0.381	0.95	0.75	0.92	1.2123	0.78	2.48	2.06
Octane-1,8-diol[g]	0.380	0.95	0.75	0.92	1.3532	1.32	3.02	2.53
Nonane-1,9-diol[g]	0.370	0.95	0.75	0.92	1.4941	1.85	3.19	3.00
Decane-1,10-diol[g]	0.370	0.95	0.75	0.92	1.6350	2.39	3.60	3.47
Dodecane-1,12-diol[g]	0.360	0.95	0.75	0.92	1.9168	3.46	4.41	4.40

Table 2 (*contd.*)

Compound	R_2	Π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^O$	V_X	Log *P*(oct)	Log $(1/C_{nar})$ Obs	Calc[b]
Acetal[h]	0.000	0.67	0.00	0.76	1.0714	0.84	1.92	1.41
Benzamide[h]	0.990	1.50	0.49	0.67	0.9728	0.64	2.52	1.93
Benzyl alcohol[i]	0.803	0.87	0.39	0.56	0.9160	1.10	2.70	2.30
2-Bromododecane-1,2-diol[j]							4.47	
3-Bromododecane-1,2-diol[j]							4.25	
5-Bromododecane-1,12-diol[j]							4.54	
6-Bromododecane-1,12-diol[j]							4.01	

[a] Refs. 13 and 26. [b] By eqn. (10); values in parentheses are for the outlying compounds. [c] Ref. 28. [d] Ref. 15. [e] Ref. 27. [f] Ref. 17. [g] Ref. 16. [h] Ref. 5. [i] Ref. 29. [j] Ref. 18.

Table 3 Term-by-term analysis of eqn. (8) to show the factors that influence tadpole narcosis[a]

Solute	rR_2	$s\pi_2^H$	$b\Sigma\beta_2^O$	vV_X	log $(1/C_{nar})$ Obs	Calc
2-Methylbut-2-ene	0.13	−0.03	−0.20	2.39	2.64	2.87
Benzene	0.50	−0.17	−0.40	2.21	2.68	2.72
Naphthalene	1.10	−0.31	−0.57	3.36	4.19	4.16
m-Cresol	0.67	−0.29	−0.98	2.84	2.75	2.82
Diethyl ether	0.03	−0.08	−1.29	2.26	1.47	1.50
Morphine	1.81	−0.79	−5.14	6.40	2.76	2.86

The constant term is 0.58 and the $a\Sigma\alpha_2^H$ term is zero.

(active) neutral species] will then be larger than the concentration of the (active) neutral species, and the compound will appear to be less toxic than it actually is. In principle, the proportion of the ionised species can be calculated and a correction for this inactive proportion can be made. This requires a knowledge of the pH of the system at equilibrium, which is not known for the Overton experiments, however.

Overton[1] and Lipnick[13] have suggested that the outliers to eqn. (7), acetamide, urea and *N*-methylurethane, probably exert their influence through osmotic effects at the high solute concentration employed. However, other solutes used at high concentration, *e.g.* ethane-1,2-diol, methanol and acetonitrile, fit eqn. (7) quite well. The calculated values of log $(1/C_{nar})$ for the other two outliers, nicotine and 2-propylpiperidine, are much smaller than those observed; 2.44 *vs.* 3.51 for nicotine and 2.58 *vs.* 3.48 for 2-propylpiperidine. This is in the wrong direction if ionisation was the cause [note that in eqn. (10) the calculated values are 2.46 and 2.91, see Table 2], and we feel that there are no convincing explanations for the five outliers we have identified. Two other solutes, pentane and carbon disulfide are outliers to eqn. (7) by 0.54 and −0.59 log units (calc − obs), but this may be due to the experimental difficulty in the measurement of the concentration of these hydrophobic substances. We have retained pentane and carbon disulfide in eqn. (7), however.

In eqn. (7), the $\Sigma\alpha_2^H$ descriptor is significant only at the 89% level and if it is omitted we find eqn. (8). This is our

$$\log(1/C_{nar}) = \underset{}{0.579} + \underset{(0.085)}{0.824R_2} - \underset{(0.083)}{0.334\pi_2^H} - \underset{(0.111)}{2.871\Sigma\beta_2^O} + \underset{(0.134)}{3.097V_X} \quad (8)$$

$$n = 84,\ \rho = 0.9730,\ \text{sd} = 0.246,\ F = 351$$

preferred equation for the Overton data set. It shows that solute hydrogen-bond basicity markedly reduces the solute narcotic activity and solute excess molar refraction (slightly) and solute volume (greatly) increase the solute narcotic activity, as found before with smaller data sets.[10,11,14] We can illustrate the solute factors that influence narcosis, through a term-by-term analysis of eqn. (8) for a number of solutes, as shown in Table 3. For non-polar compounds such as 2-methylbut-2-ene, only the vV_X term makes any real contribution. The volume effect is very large, however; even for as small a compound as the alkene it contributes 2.39 log units. Aromatic compounds have a slightly increased toxicity because they all have larger values of R_2, but for benzene and naphthalene by far the most dominant effect is again through the vV_X term. Solute dipolarity/polarizability diminishes toxicity, but the effect is not very large: for morphine, with a large π_2^H value of 2.35, the $s\pi_2^H$ term is only −0.79 log units. Hydrogen-bond acidity plays no part. In the case of *m*-cresol, a moderately strong hydrogen-bond acid, with $\Sigma\alpha_2^H$ = 0.57, other factors such as basicity and especially volume are those that influence toxicity. Hydrogen-bond basicity is very important in reducing the solute toxicity. Even with diethyl ether, where $\Sigma\beta_2$ is only 0.45 units, the hydrogen-bond basicity contributes −1.29 log units. The most striking effect is with morphine, a large solute with very high hydrogen-bond basicity. Now $b\Sigma\beta_2^O$ contributes no less than −5.14 log units and vV_X as much as 6.40 log units to the toxicity, log $(1/C_{nar})$.

It is of interest to compare our methodology with that of Lipnick[13] who used a restricted data set to obtain a 'base-line' toxicity equation, Table 1, and then calculated an excess toxicity defined as eqn. (9). For 39 additional compounds $Te < 2$ and

$$Te = C_{nar}(\text{pred})/C_{nar}(\text{obs}) \quad (9)$$

for 67 additional compounds $Te < 3$, corresponding to deviations of 0.30 and 0.48 log units, respectively. However by our methodology and those of Kamlet *et al.*[10] and of Wilson and Famini,[11] any excess toxicity must be restricted to the outliers, by definition. Now a deviation of 0.30 log units is of the same order as the various regression standard deviations [see Table 1 and eqns. (7) and (8)], and is not likely to be significant. Larger deviations in the order of 0.48 log units could at least in part be due to the fact that the water–octanol system is not an exact mimic of tadpole narcosis, as we show later. Hence our finding that 84 out of 89 compounds fit eqns. (7) and (8), and

so do not have any excess toxicity, does not conflict with the conclusions of Lipnick.[13]

An extended data set

Following Overton, a large number of workers subsequently studied tadpole narcosis, sometimes using the same species as Overton, *Rana temporaria*, sometimes using other tadpole species. Furthermore, various narcotic end points were used, thus making it difficult to compare results with those of Overton. There seemed little point in replacing Overton's results by other data sets, especially if they refer to only a few compounds. A notable exception is the important work on alkan-1-ols by Alifimoff and co-workers,[15] who used *Rana pipiens* tadpoles. We have therefore amended the Overton list by using the values of Alifimoff *et al.*[15] on alkan-1-ols instead of the Overton data and by addition of data[27] on the alkan-2-ols. The other large set of data we include is that of Franks and Lieb and co-workers[16–18] on α,ω-diols and cycloalkanols (towards *Xenopus laevis* tadpoles) and bromododecane-1,12-diols (towards *Rana temporaria* tadpoles). The α,ω-diols are particularly important, because Franks and Lieb and co-workers[17] interpreted their narcotic activity in terms of an interesting model for general anaesthesia. They suggest that the anaesthetic binding site on the primary protein target is a long, narrow hydrophobic pocket extending inward from the external water-facing surface. When an α,ω-diol binds, one of the hydroxy groups becomes anchored to the polar mouth of the pocket and the second hydroxy group is forced into an unfavourable hydrophobic environment of the pocket.

We also included a few more unusual compounds from the work of Vernon,[28] Meyer and Hemmi,[5] and Kita *et al.*[29] Of the additional compounds, descriptors were not available for the four bromododecane-1,12-diols. The same five compounds as in the Overton set were outliers, leaving 114 solutes in the following correlation equation. The $\Sigma\alpha_2^H$ descriptor in eqn. (10) is only significant at the 94% level and omission leads to

$$\log(1/C_{nar}) = \underset{(0.104)}{0.582} + \underset{(0.108)}{0.770}R_2 - \underset{(0.137)}{0.696}\pi_2^H + \underset{(0.126)}{0.243}\Sigma\alpha_2^H - \underset{(0.172)}{2.592}\Sigma\beta_2^O + \underset{(0.114)}{3.343}V_X \quad (10)$$

$$n = 114, \rho = 0.9536, \text{sd} = 0.337, F = 217$$

eqn. (11). There is little difference between eqns. (10) and (11)

$$\log(1/C_{nar}) = \underset{(0.105)}{0.595} + \underset{(0.107)}{0.805}R_2 - \underset{(0.135)}{0.725}\pi_2^H - \underset{(0.166)}{2.489}\Sigma\beta_2^O + \underset{(0.115)}{3.341}V_X \quad (11)$$

$$n = 114, \rho = 0.9520, \text{sd} = 0.341, F = 263$$

and eqns. (7) and (8), showing again how general is the phenomenon of narcotic activity. The largest difference in the coefficients between eqns. (10) and (7) is 0.35 for the *s*-coefficient and 0.42 for the *a*-coefficient, but if these are compared with the *sd* values for the coefficients (0.14 and 0.11 for *s*, and 0.13 and 0.11 for *a*), it is doubtful if the differences are significant.

It is not possible to assign a precision to the results of Overton, because either single experiments were carried out, or multiple experiments were conducted with different solute concentrations. However, Alifimoff *et al.*[15] give more details of their own experiments from which an error of *ca.* 0.05 log units may be deduced. This seems rather low and from the careful work of Franks and Lieb and co-workers[16–18] an error somewhat over 0.10 log units is more likely. Interlaboratory errors are, as usual, much more. Alifimoff *et al.*[15] listed results from four workers which lead to *sd* values in log $(1/C_{nar})$ of 0.24 for ethanol, 0.14 for propan-1-ol and 0.21 for butan-1-ol, *i.e.* an average of 0.20 log units. Inclusion of Overton's results leads to the same average *sd* value of 0.20 log units, as for five workers. We conclude that the standard error in log $(1/C_{nar})$ cannot be less than 0.10 log units, and over an extended series of compounds, possibly with data from several sources, is likely to be nearer to 0.20 log units. The sd values of 0.34 log units in eqns. (10) and (11), and the values of 0.24 and 0.25 log units for the Overton data set in eqns. (7) and (8) are therefore quite reasonable. The results listed in Table 1 suggest also that the sd value for log $(1/C_{nar})$ in the Overton experiments must be *ca.* 0.1–0.2 log units.

Our interpretation of eqns. (10) and (11) follows exactly that of eqns. (7) and (8), and is similar to our previous interpretation using a much smaller and different data set.[14] As regards the two main sets of additional data, the alkanols of Alifimoff *et al.*[15,27] fit eqns. (10) and (11), with the exception of the inactive solute tridecan-1-ol. But this is a manifestation of the well known 'cut off' point.[15] More interesting than the fit of the alkan-1-ols, is the observation that the α,ω-diols also fit eqns. (10) and (11). At first sight, this seems contrary to the model of Franks, Lieb and co-workers.[17] If one hydroxy group binds to a polar region and the second hydroxy group is positioned near to a hydrophobic region, then by our methodology, this would lead to the diols being considerable outliers. We attempt to resolve this anomaly through a more detailed analysis.

The anaesthetic binding site

Previously, we compared transfer from water to the anaesthetic binding site with transfer from water to a number of solvents.[14] Since then, considerably more results have been obtained on partitions between water and organic solvents and in Table 4 we compare the constants in eqn. (10) with those for water–solvent partitions;[23–25] we use eqn. (10) rather than eqn. (11), because the water–solvent partitions retain all the terms in the general eqn. (4). The coefficients for eqn. (10) markedly resemble those for (wet) isobutanol or (wet) pentanol. Since these solvents contain 17.0 and 9.0 wt% water, respectively, they are quite a way from archetypal hydrophobic solvents such as hexane or hexadecane. If the *v*-coefficient is taken as a rough measure of the solvent hydrophobicity, then the anaesthetic binding site is about as hydrophobic as wet pentanol. As shown before,[14] the binding site is somewhat dipolar (the *s*-coefficient is close to those for isobutanol and pentanol), of the same hydrogen-bond basicity as bulk water (because the *a*-coefficient is almost zero), but is much less acidic than water ($b = -2.37$). All this relates to the general, or overall, property of the binding site.

At first sight, water–ester partitions are not quite as good model processes as water–alcohol partitions, see Table 4. The nearest water–ester partition is the butyl acetate system; as with the alcohols, the long chain compounds such as olive oil or propylene glycol dipelargonate (PGDP) are not good models. As regards linear relationships between log $(1/C_{nar})$ and log *P* for any water–solvent system, the absolute values of the coefficients are less important than their relative values in eqn. (4). We give in Table 5 values of the coefficients for various processes, all relative to $v = 3.34$, as in eqn. (10). Now there is little to choose between isobutanol, pentan-1-ol and butyl acetate as models for the anaesthetic binding site.

Franks, Lieb and co-workers[17] probed the properties of the binding site in firefly luciferase and bacterial luciferase in some detail by the use of a series of α,ω-diols. They compared the potency of a diol to that of an alkan-1-ol of the same carbon number and compared the alkan-1-ol to the corresponding alkane. The energetics of transfer of $-CH_2OH$ from water to the binding site by comparison with $-CH_3$ will give an indication of the polar nature of the site at which $-CH_2OH$ binds, both for

Table 4 Coefficients in eqn. (4) for water–solvent partitions[23–25]

Solvent	c	r	s	a	b	v
Isobutanol	0.23	0.51	−0.69	0.02	−2.26	2.78
Pentanol	0.17	0.57	−0.79	0.02	−2.84	3.25
Hexanol	0.14	0.72	−0.98	0.14	−3.21	3.40
Octanol	0.09	0.56	−1.05	0.03	−3.46	3.81
Decanol	0.01	0.48	−0.97	0.01	−3.80	3.94
Olive oil	−0.09	0.57	−0.86	−1.45	−4.95	4.30
Butyl acetate	−0.47	0.71	−0.40	0.01	−3.74	3.86
PGDP	0.29	0.34	−0.64	−0.91	−5.04	4.09
Oleyl alcohol	−0.36	−0.27	−0.53	−0.03	−4.04	4.20
Alkane	0.29	0.65	−1.66	−3.52	−4.82	4.28
Hexadecane	0.09	0.67	−1.62	−3.59	−4.87	4.43
Eqn. (7)[a]	0.61	0.87	−0.35	−0.17	−2.81	3.05
Eqn. (10)[b]	0.58	0.77	−0.70	0.24	−2.59	3.34

[a] Tadpole narcosis; the Overton data set. [b] Tadpole narcosis; an extended data set.

Table 5 Relative coefficients in eqn. (4) for water–solvent partitions

Solvent	r	s	a	b	v
Isobutanol	0.61	−0.83	0.02	−2.72	3.34
Pentanol	0.59	−0.81	0.02	−2.92	3.34
Octanol	0.49	−0.92	0.03	−3.03	3.34
Oleyl alcohol	−0.21	−0.42	−0.02	−3.21	3.34
Butyl acetate	0.61	−0.35	0.01	−3.24	3.34
Olive oil	0.44	−0.67	−1.13	−3.84	3.34
Eqn. (7)	0.95	−0.38	−0.19	−3.07	3.34
Eqn. (10)	0.77	−0.70	0.24	−2.59	3.34

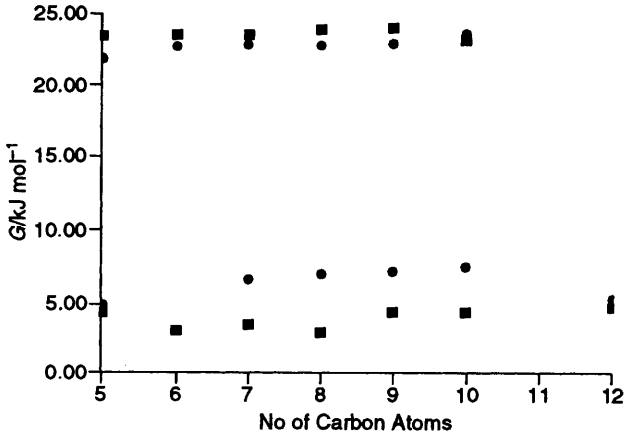

Fig. 1 Relative Gibbs' energies of transfer of α,ω-diols to alkan-1-ols [●] and alkan-1-ols to alkanes [■] as a function of carbon number. Top: from water to hexadecane. Bottom: from water to the anaesthetic binding site.

the first OH and for the second OH group. Unfortunately, such an analysis could not be carried out for anaesthesia towards the tadpole, because of a lack of data on the activity of the alkanes.[17] However, we are now in a position to calculate log $(1/C_{nar})$ for alkanes *via* eqn. (11) and to obtain the relative Gibbs energies of transfer through eqns. (12) and (13), where T is 298

$$\Delta G^\circ(\text{1st OH}) = -RT \ln [(1/C_{nar}, \text{alcohol})/(1/C_{nar}, \text{alkane})] \quad (12)$$

$$\Delta G^\circ(\text{2nd OH}) = -RT \ln [1/C_{nar}, \text{diol})/(1/C_{nar}, \text{alcohol})] \quad (13)$$

K, and ΔG° is in kJ mol^{-1}. Plots of ΔG° against carbon number are shown in Fig. 1, and show that the second OH is bound *ca.* 3–4 kJ mol^{-1} less favourably than the first. Hence the environment around the second OH must be 'less polar' or 'more hydrophobic' than around the first OH, exactly as

Table 6 Gibbs energies of transfer of nonan-1-ol relative to nonane and nonane-1,9-diol relative to nonan-1-ol, from water to water-saturated solvents, in kJ mol^{-1} at 298 K[a]

Solvent	1st OH	2nd OH
Isobutanol	6.4	6.4
Pentanol	7.9	7.8
Hexanol	8.9	8.8
Octanol	9.9	9.9
Decanol	10.8	10.6
Butyl acetate	9.1	8.6
Alkane	22.5	22.6
Hexadecane	22.6	22.7
Anaesthetic site	4.4	7.1

[a] Values from partition coefficients observed, or calculated using the regression coefficients in Table 4, see text.

suggested by Franks, Lieb and co-workers.[17] But also shown in Fig. 1 are the corresponding energetics of transfer from water to hexadecane, obtained either from experimental log P values, or by calculation using the regression constants in Table 4. Now the environment around the second OH in hexadecane is the same as that around the first OH, as expected, but the energetics of the transfer are quite different to those for the anaesthetic binding site. The $-CH_3$ to $-CH_2OH$ conversion is unfavourable to the extent of 23 kJ mol^{-1} for both the first and the second OH groups, whereas in anaesthesia, the values are only 4 and 6 kJ mol^{-1} on average. Thus although the environment around the second OH group in the anaesthetic pocket is less polar or more hydrophobic than that around the first OH, the difference is actually very small, both the OH groups in the diols must be in environments that can only be described as dipolar, strongly basic, somewhat acidic and of intermediate hydrophilicity/hydrophobicity. It is because the environments around the first and second OH groups in the diols are so similar, that the diols fit eqns. (10) and (11). We can construct similar figures to Fig. 1 for the energetics of the first and second OH group transfers from water to various solvents, but since the variation with carbon number is small, we give results for derivatives of nonane only, Table 6. As expected from the coefficients in Table 4, the transfers of the first and second OH groups in solvents such as wet isobutanol and wet pentanol are the closest to those for the anaesthetic pocket.

Note that all the ΔG° values as calculated through eqns. (12) and (13) are uncorrected for any statistical effect. Since all the log $(1/C_{nar})$ values we have used in the various correlation equations are uncorrected, it would not be consistent to use values corrected for possible orientations in the anaesthetic pocket. The corrections are not large, in any case,[17] and would lead to a decrease in ΔG° of 1.7 kJ mol^{-1} for the first OH and an increase of 1.7 kJ mol^{-1} for the second OH.

Our conclusions based on the energetics of transfer of alkanes, alkan-1-ols and α,ω-diols therefore do not conflict with those of Franks and Lieb and co-workers.[17] However, we do not feel that the anaesthetic pocket can be sterically very constrained, because solutes of all shapes and sizes fit eqns. (10) and (11), *e.g.* phenanthrene, quinoline, caffeine and morphine. The pocket must either be quite large or flexible, or both.

Log *P*(oct) descriptor

As is evident from Table 1, the water–octanol partition coefficient, log *P*(oct), is the most widely used single descriptor for the correlation of aqueous narcosis. Although often restricted to small data sets, the 53 and 57 data sets in Table 2 represent a substantial portion of the Overton data. We thought it useful to compare log *P*(oct) as a descriptor for the exact 84 compound set we used in eqn. (7) and eqn. (8). The values of log *P*(oct) that we have used are in Table 2. For a few compounds experimental values were not available, and so we calculated log *P*(oct) values through eqn. (4) with the coefficients in Table 4 and the given descriptors in Table 2. A quite good correlation equation is found [eqn. (14)]. This is not surprising in view of

$$\log(1/C_{nar}) = 1.129 + 0.833\log P(oct) \quad (14)$$
$$(0.071)\ (0.039)$$
$$n = 84, \rho = 0.9212, \text{sd} = 0.407, F = 460$$

the coefficients in Table 4 and the relative coefficients in Table 5, which indicate that the water–octanol system is a reasonable model for tadpole narcosis. Eqn. (14) is comparable to those for the 53 and 57 data sets given in Table 1 as regards of goodness-of-fit and is as good as eqn. (8) in terms of the F-statistic, although not as regards the standard deviation. Of course, eqn. (14) suffers in comparison with eqn. (8) in that it conveys no detailed chemical information about the factors that influence narcosis. Hansch and co-workers[7,30] suggested that for the biological activity of a series of compounds where electronic and steric effects were constant, a two term equation would apply [eqn. (15)]. They examined a number of congeric series[7] and

$$\log(1/C) = c + a\log P(oct) + b[\log P(oct)]^2 \quad (15)$$

showed that in a large number of cases the squared term in eqn. (15) was important. Dearden[6] has given further examples of the parabolic relationship and has provided an explanation based on rates of partitioning between various compartments (or layers). We can now use Overton's data to examine whether or not the parabolic relationship is general, or is likely to be restricted to congeric series. For the 84 compounds we have studied, we find,

$$\log(1/C_{nar}) = 1.131 + 0.829\log P(oct) +$$
$$(0.079)\ (0.093)$$
$$0.001[\log P(oct)]^2 \quad (16)$$
$$(0.026)$$
$$n = 84, \rho = 0.9212, \text{sd} = 0.410, F = 227$$

It is quite clear that the parabolic relationship does not hold for the wide variety of compounds in the Overton set. The squared term is not significant and neither the overall sd nor the correlation coefficient have improved. Exactly the same conclusions can be reached with the 114 compound data set [eqns. (17) and (18)].

$$\log(1/C_{nar}) = 1.210 + 0.835\log P(oct) \quad (17)$$
$$(0.062)\ (0.031)$$
$$n = 114, \rho = 0.9301, \text{sd} = 0.414, F = 718$$

$$\log(1/C_{nar}) = 1.206 + 0.842\log P(oct) -$$
$$(0.073)\ (0.078)$$
$$0.002[\log P(oct)]^2 \quad (18)$$
$$(0.019)$$
$$n = 114, \rho = 0.9301, \text{sd} = 0.406, F = 356$$

Why a parabolic relationship should hold for a congeric series and not for a structurally varied series is none too clear, especially as the various models used to demonstrate the possibility of parabolae do not depend explicitly on any requirement for a congeric series.[6] In the 114 data set are 11 alkan-1-ols that form a typical congeric series. For the single descriptor we find eqn. (19), and for the parabolic relationship,

$$\log(1/C_{nar}) = 1.190 + 0.868\log P(oct) \quad (19)$$
$$(0.128)\ (0.042)$$
$$n = 11, \rho = 0.9892, \text{sd} = 0.242, F = 432$$

eqn. (20).

$$\log(1/C_{nar}) = 0.976 + 1.226\log P(oct) -$$
$$(0.075)\ (0.071)$$
$$0.073[\log P(oct)]^2 \quad (20)$$
$$(0.042)$$
$$n = 11, \rho = 0.9977, \text{sd} = 0.120, F = 883$$

Now, certainly, the parabolic relationship is much the better, although we should note that the correlation coefficient between the two descriptors in eqn. (20) is no less than 0.9554 for the particular set of compounds studied. We conclude that the parabolic relationship in tadpole anaesthesia holds for a congeric series, but collapses when applied to a structurally diverse series of compounds.† As regards predictions for further compounds, the parabolic relationship, limited to an homologous series, is of little practical use. For predictions of the Overton data, either the one-parameter eqn. (12), or the four-parameter eqn. (8) could be used. The one-parameter equation is the simpler, but cannot be better than 0.41 log units, whereas the four-parameter equation is good to *ca.* 0.25 log units. As regards interpretation of tadpole narcosis, only the multiparameter eqn. (8)–(11) lead to specific information on the factors that influence the narcotic activity of solutes.

Recently, we have shown[31] that although the log *P*(oct) parameter can be used as a descriptor for water–sodium dodecyl sulfate (SDS) micelle partition coefficients, a two-parameter equation in V_X and log *P*(oct) is a better predictive equation, as shown by eqns. (21) and (22).

$$\log K_X(\text{SDS}) = 2.01 + 0.69\log P(oct) \quad (21)$$
$$n = 132, \rho = 0.9345, \text{sd} = 0.346, F = 896$$

$$\log K_X(\text{SDS}) = 1.13 + 0.50\log P(oct) + 1.22V_X \quad (22)$$
$$n = 132, \rho = 0.9755, \text{sd} = 0.215, F = 1269$$

To some extent this is true in the present case,

$$\log(1/C_{nar}) = 0.621 + 0.743\log P(oct) + 0.668V_X \quad (23)$$
$$n = 84, \rho = 0.9400, \text{sd} = 0.359, F = 308$$

$$\log(1/C_{nar}) = 0.700 + 0.724\log P(oct) + 0.670V_X \quad (24)$$
$$n = 114, \rho = 0.9455, \text{sd} = 0.360, F = 468$$

Since correlations against log *P*(oct) alone are reasonable, we

† A referee has enquired about the interpretation of the $[\log P(oct)]^2$ descriptor through eqn. (4). Our view is that equations that contain this squared term are no longer linear free energy relationships and hence interpretation through LFERs is difficult or impossible.

regard eqns. (23) and (24) simply as empirical, but useful, equations for the estimation of log ($1/C_{nar}$). Both log P(oct) and V_X can be calculated,[22,32] so that eqns. (23) and (24) can be used to estimate log ($1/C_{nar}$) from the structure of a compound. In particular, eqn. (24) compares favourably with eqns. (11) and (17) for the extended data set.

Acknowledgements

This work was carried out whilst Dr Clara Rafols was on leave from the Department de Quimica Analitica, Universitat de Barcelona, Diagonal 647, 08028 Barcelona, Spain; Dr Rafols gratefully acknowledges support from the Catalan Government. We thank Dr Nicolas P. Franks and Dr William R. Lieb for helpful discussion and Dr Robert Lipnick for his interest in this work.

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Paper 5/02384J
Received 13*th April* 1995
Accepted 18*th May* 1995