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Authors

Abraham, Michael H.
Kumarsingh, Rachel
Cometto-Muniz, J. Enrique
et al.

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A Quantitative Structure-Activity Relationship (QSAR) for a Draize Eye Irritation Database

M. H. Abraham[1], R. Kumarsingh[1], J. E. Cometto-Muñiz[2] and W. S. Cain[2]

[1]Department of Chemistry, University College London, 20 Gordon Street, London, WC1H OAJ, UK

[2]Chemosensory Perception Laboratory, Department of Surgery (Otolaryngology), University of California, San Diego, Mail Code 0957, La Jolla, California, 92093-0957, USA

Running head: A QSAR for Draize eye scores

Abstract

A collection of data on the Draize rabbit eye test (Cronin et al., 1994) has been analysed using a set of physicochemical descriptors that we have previously put forward. These descriptors are compound (or solute) parameters as follows: R_2 is an excess molar refraction, π_2^H is the polarizability/dipolarity, $\sum\alpha_2^H$ and $\sum\beta_2^H$ are the effective hydrogen bond acidity and basicity, and $\log L^{16}$ is a descriptor where L^{16} is the vapour-hexadecane solubility at 25°C. When applied to Draize eye scores (DESs) for 38 pure bulk liquids, a very poor equation was obtained. However, when the DES values were correlated as $\log(DES/P^o)$, where P^o is the liquid vapour pressure, an excellent equation was found. On transforming the calculated $\log(DES/P^o)$ values back to calculated DES values, there was good agreement with the original DES values. It is suggested that the DES/P^o values refer to transfer of the irritant from the vapour phase to the biophase, and that the success of the present treatment demonstrates that for the pure liquids studied, a major factor in the Draize eye test is simply the transfer of the liquid (or the vapour) to the biological system.

Abbreviations: DES=Draize eye score; MDES=molar Draize eye score; QSAR=quantitative structure-activity relationship; VOC=volatile organic compound.

INTRODUCTION

Eye irritation is one of the main factors in indoor air pollution, and a knowledge of eye irritation potential is required for many volatile organic compounds (VOCs) that are released into the atmosphere at home and at the workplace. Most of these VOCs are only mild irritants, but their sum may make a significant contribution to poor indoor air quality (Cometto-Muñiz et al., 1997). At present, the Draize rabbit eye irritation test (Draize et al., 1944) is a major source of data. In the Draize test the substance under study is applied to the eye of a living rabbit. The effects of the substances on the cornea, iris and conjunctivae are graded on individual scales and given weighted scores. The final eye irritation score is the sum of the weighted scores for the cornea, iris and conjunctivae. There are important scientific and ethical reasons for developing in vitro alternatives for the Draize test. Not surprisingly, there have been numerous attempts to relate DESs to other measures of eye irritation (Balls et al., 1995; Devillers and Chessel, 1995; Kalweit et al., 1990; Spielmann et al., 1993 and 1995), so far with limited success (Balls et al., 1995). One major reason for the lack of correlation between the Draize test and other tests is that the various studies have been conducted on a completely heterogeneous set of substances, including pure organic liquids, aqueous solutions (such as aqueous sodium hydroxide and silver nitrate) and solids. If transport from the applied substance to the eye is a factor in the observed eye irritation, one would not expect different tests, with different transport requirements, to be well correlated over such a disparate set of substances. Other workers have narrowed the range of substances to pure organic liquids in attempts at physicochemical modelling of the DESs. Principal components analysis has been used (Barratt, 1995; Chamberlain and Barratt, 1995) in the investigation of 46 such liquids. No quantitative results were given, but compounds were classed as irritant or non-irritant on a plot of the two first principal components. Descriptors used in the principal components were the logarithm of the calculated water-octanol partition coefficient (ClogP), the dipole moment, and the principal moments of inertia.

A quantitative structure-activity relationship (QSAR) has been carried out on a set of 38 organic liquids (Cronin et al., 1994). The DES was first altered by converting it into a molar eye score (MDES) through

$$MDS = DES.MW/1000d \qquad [1]$$

where the molecular weight and density of the liquid are MW and d, respectively. The descriptors used were ClogP, the energy of the lowest unoccupied molecular orbit LUMO, and the zero order kappa alpha index $\kappa\alpha 0$. However, only very poor correlations were obtained, with values of the F-statistic between 7.6 and 8.2, and with r^2 0.35 at best. It was concluded (Cronin et al., 1994) that the lack of success of the QSAR analysis was perhaps due to varying mechanisms of toxic action of the different chemical groups of the 38 compounds. It is possible that

another reason for the poor results obtained in the principal components analysis and the QSAR analysis may be that the descriptors used (Barratt, 1995; Chamberlain and Barratt, 1995; Cronin et al., 1994) are not necessarily the relevant ones. For example, in both sets of analyses, ClogP was used as a descriptor. This relates to the partition of a solute from dilute solution in water to dilute solution in octanol. Even if octanol were a good model for the biological phase (i.e. the relevant structures in the eye), the state of dilute solution in water is an extremely poor model for the irritant stimulus (i.e. a pure organic liquid). What is needed is a model for the process of transferring an irritant from the state of a pure organic liquid to a state in which the irritant is distributed in an organic biophase (the biological structures of the eye). A possible model process is that of transfer of a pure organic liquid to a dilute solution in an organic solvent phase. The equilibrium constant governing such a model process is known as the activity coefficient, γ^o , which may be defined for a sparingly soluble liquid as the reciprocal of the solubility of the liquid in the organic solvent phase. Various chemical engineering methods are used to estimate activity coefficients of liquids in organic solvents, but these all require some knowledge of the physicochemical properties of the solvent phase. In the present case, the latter is a biological phase with unknown physicochemical properties, and such calculations of activity coefficients are not possible. Some other method of investigation of the model process is needed.

MATERIALS AND METHODS

Our method of data analysis uses the stratagem (Abraham et al., 1994) employed in the prediction of the solubility of organic liquids in polymeric solvents, that is, the prediction of the transfer of a pure organic liquid to a dilute solution in an organic solvent (our model process), from data on the solubility of the corresponding vapours in the polymeric solvents. Let the solubility of a vapour into a solvent phase be denoted as L, defined as

$$L = \frac{\text{[concn of the solute in the solvent phase]}}{\text{[concn of the solute in the gas phase]}} \qquad [2]$$

Then if the solubility of the pure liquid in the solvent phase is $1/\gamma^o$, we have from equation [2] that $L = (1/\gamma^o)/P^o$, where P^o is the saturated vapour pressure of the pure liquid, so that

$$1/\gamma^o = L*P^o \qquad [3]$$

The relationship between $1/\gamma^o$, L and P^o is shown in Fig. 1a. Now if DES relate to a transport-driven mechanism, the transfer process (on our model) will be from the pure organic liquid to an initial biophase that will be the tear film and cell membranes on the surface of the eye. The more soluble the organic liquid in the initial biophase, the larger will be the DESs, so that DES values will be

proportional to $1/\gamma^o$, the physicochemical solubility. Then we can write an equivalent equation to equation [3], as explained in Fig. 1b.

$$DES = L*P^o \quad [4]$$

and then obtain

$$\log(DES/P^o) = \log L \quad [5]$$

where P^o is the saturated vapour pressure in ppm at 25°C. We use vapour pressures at 25°C rather than at 37°C because many compilations list values at the standard temperature of 25°C; 106 ppm=1 atm.

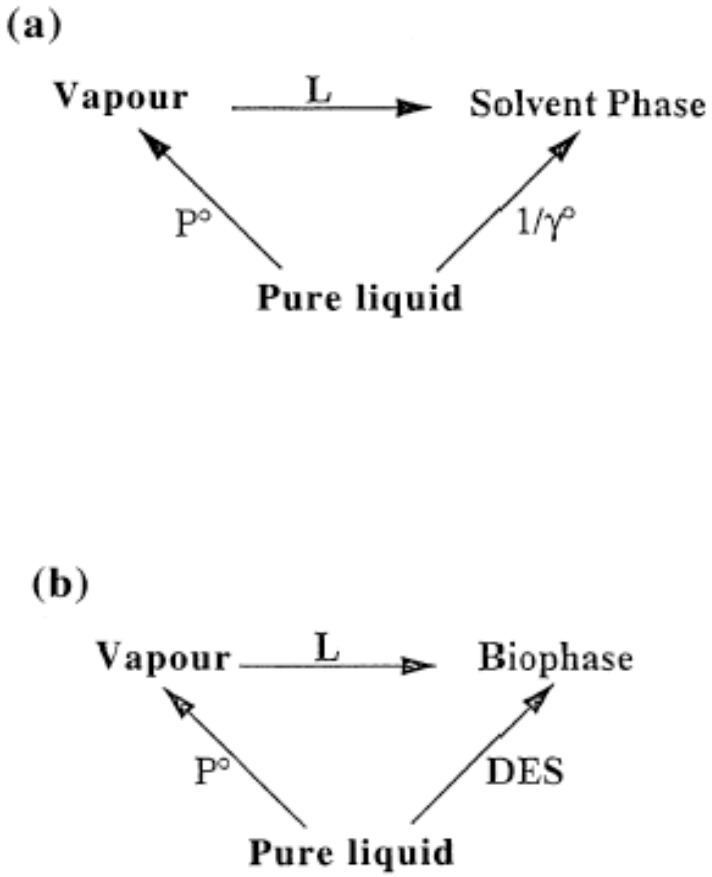

Fig. 1. (a) The relationship between $1/\gamma^o$, L and P^o for solubility in a solvent phase. (b) The relationship between DES, L and P^o for solubility in a biophase.

It is important to emphasize that the present model, leading to equation [5], can only account for effects that are `transport driven'. By this we mean processes where the key step is the distribution of a solute (i.e. the irritant) between phases

(in the present context between the pure liquid or vapour, and the various structures of the eye).

The reason for setting out equation [5] is that we have already constructed a general equation for the correlation and prediction of a series of logL values for solutes into a given condensed phase

$$\text{Log SP} = c + r \cdot R_2 + s \cdot \pi_2^H + a \cdot \sum\alpha_2^H + b \cdot \sum\beta_2^H + l \cdot \log L^{16} \qquad [6]$$

LogSP is the dependent variable (e.g. logL) and the independent variables are solute descriptors as follows (Abraham, 1994): R_2 is the excess molar refraction, π_2^H is the dipolarity/polarizability, $\sum\alpha_2^H$ is the hydrogen bond acidity, $\sum\beta_2^H$ is the hydrogen bond basicity and L^{16} is the solubility of the vapour in hexadecane at 25°C. It should be noted that these descriptors are for the monomeric compounds and are not for compounds as pure liquids. Hence the relevant dependent variable is $\log(DES/P^o)$ and not log(DES) or DES.

Statistics

Equations of the form of equation [6] were solved by the method of multiple linear regression analysis, using either an in-house package or release 7.1 of the software package Minitab. The regression coefficients were calculated, together with their standard deviations, and their *t* ratios by both packages. The regression standard deviation, SD, the overall correlation coefficient, r, and the regression F statistic, F, were also calculated. In all cases, the Student's test residuals, TRESID, and the DFITs were obtained for all the data points to check for possible outliers. A criterion of $DFIT>2(p/n)^{0.5}$ was taken as a measure of an unusual observation; p is the number of independent variables, and n is the number of data points. A correlation matrix between the independent variables was also calculated as a check against covariance.

RESULTS

We use the same 38 liquids selected previously (Cronin et al., 1994); the compounds, their DESs and values of $\log P^o$ are in Table 1. As expected, application of equation [6] to log(DES) values themselves yielded just as poor a correlation (r^2=0.274, F= 2.4) as those before (Cronin et al., 1994) but when $\log(DES/P^o)$ was used as the dependent variable a reasonable correlation was found

$$\log(DES/P^o) = -6.97 - 0.17\,R_2 + 0.88\pi_2^H + 3.83\sum\alpha_2^H + 1.41\sum\beta_2^H + 0.80 \log L^{16} \qquad [7]$$

$$n = 38,\ r^2 = 0.894,\ SD = 0.46,\ F = 54.0$$

Table 1. Values of DESs, $\log P^o$/ppm and compound descriptors

Compound	DES	$\log P^o$	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	$\log L^{16}$
Methyl trimethylacetate	2.67	4.59	0.049	0.54	0.00	0.45	2.932
Ethyl trimethylacetate	4.17	4.13	–0.010	0.52	0.00	0.45	3.481
Butyl acetate	7.50	4.18	0.071	0.60	0.00	0.45	3.353
Ethyl acetate	15.00	5.09	0.106	0.62	0.00	0.45	2.314
Cellosolve acetate	15.00	3.38	0.099	0.79	0.00	0.79	3.747
Ethyl 2-methylacetoacetate	18.00	2.84	0.205	0.83	0.00	0.80	4.250
Methyl acetate	39.50	5.45	0.142	0.64	0.00	0.45	1.911
2,2-Dimethylbutanoic acid	44.67	2.30	0.170	0.54	0.60	0.50	3.600
Glycerol	1.67	–0.63	0.512	0.90	0.70	1.14	3.200
Propan-2-ol	30.50	4.75	0.212	0.36	0.33	0.56	1.764
2-Ethylhexan-1-ol	50.00	2.28	0.209	0.39	0.37	0.48	4.433
Isobutanol	60.25	4.14	0.217	0.39	0.37	0.48	2.413
Butanol	60.75	3.91	0.224	0.42	0.37	0.48	2.601
Hexanol	64.75	2.94	0.210	0.42	0.37	0.48	3.610
Butyl cellosolve	68.67	3.16	0.201	0.50	0.30	0.83	3.806
Cyclohexanol	79.75	2.90	0.460	0.54	0.32	0.57	3.758
4-Bromophenetole	1.33	2.00	0.965	0.90	0.00	0.24	5.540
1,3-Diisopropylbenzene	2.00	2.62	0.605	0.46	0.00	0.20	5.170
sec-Butylbenzene	2.00	3.38	0.603	0.48	0.00	0.16	4.506
3-Ethyltoluene	2.33	3.59	0.630	0.51	0.00	0.18	4.275
2,4-Difluoronitrobenzene	3.67	2.50	0.604	1.15	0.00	0.20	4.440
Styrene	6.75	3.94	0.849	0.65	0.00	0.16	3.856
Toluene	9.00	4.57	0.601	0.52	0.00	0.14	3.325
m-Xylene	9.00	4.06	0.623	0.52	0.00	0.16	3.939
4-Fluoroaniline	69.83	2.90	0.760	1.09	0.28	0.40	4.007
3-Methylhexane	0.67	4.91	0.000	0.00	0.00	0.00	3.044
2-Methylpentane	2.00	5.45	0.000	0.00	0.00	0.00	2.503
Deca-1,9-diene	2.00	3.47	0.220	0.20	0.00	0.10	4.380
Dodecane	2.00	2.19	0.000	0.00	0.00	0.00	5.696
1,5-Dimethylcyclooctadiene	2.83	3.35	0.600	0.35	0.00	0.20	5.040
cis-Cyclooctene	3.33	4.00	0.460	0.24	0.00	0.10	4.119
Methylcyclopentane	3.67	5.26	0.225	0.10	0.00	0.00	2.907
Hexa-1,5-diene	4.67	5.47	0.191	0.20	0.00	0.10	2.480
Methyl isobutyl ketone	4.75	4.41	0.111	0.65	0.00	0.51	3.089
Heptan-2-one	16.25	3.70	0.123	0.68	0.00	0.51	3.760
Butanone	50.00	5.08	0.166	0.70	0.00	0.51	2.287
Propanone	65.75	5.48	0.179	0.70	0.04	0.49	1.696

If the point for propylene glycol is excluded, there is a considerable improvement in the correlation

$$\log(DES/P^o) = -7.00 - 0.35\,R_2 + 1.30\pi_2^H + 4.62\sum\alpha_2^H + 1.09\sum\beta_2^H + 0.78\log L^{16} \quad [8]$$

$$n = 37,\ r^2 = 0.953,\ SD = 0.32,\ F = 125.5$$

and if the R_2 descriptor is dropped as statistically not significant, as judged by the

t test, we obtain the final equation, where the sd values of the coefficients themselves are also given

$$\log(DES/P^o) = -(6.955 \pm 0.230) + (1.046 \pm 0.249)\pi_2^H + (4.437 \pm 0.352)\sum\alpha_2^H + (1.350 \pm 0.321)\sum\beta_2^H + (0.754 \pm 0.056)\log L^{16} \quad [9]$$

$$n = 37,\ r^2 = 0.951,\ SD = 0.32,\ F = 155.9$$

Equation [9] is quite reasonable statistically, with r^2=0.951 and F =155.9. A plot of the observed and calculated log(DES/P^o) values on equation [9] is given in Fig. 2; the plot is a straight line with random scatter about the line of identity.

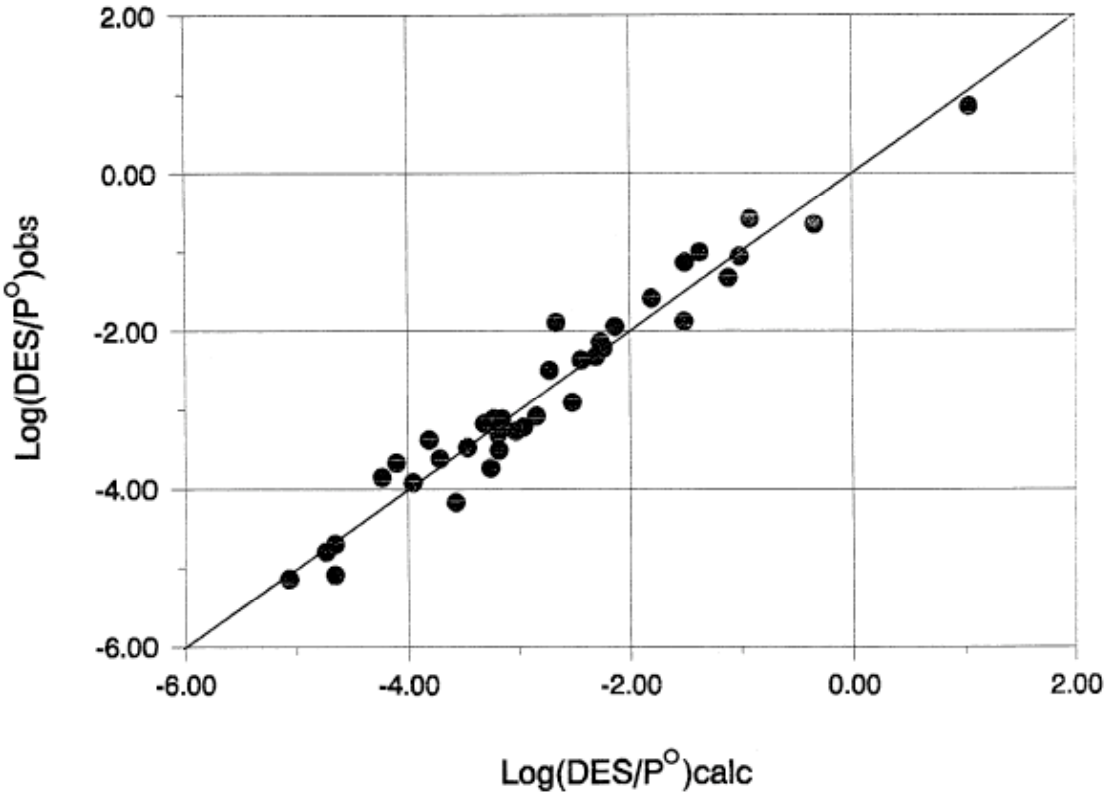

Fig. 2. Plot of log(DES/P^o) observed against log(DES/P^o) calculated from equation [9].

However, as regards the Draize test itself, equation [9] yields little information as to the irritancy of pure liquids when applied to the rabbit eye. We have therefore converted the calculated log(DES/P^o) values from equation [9] back into calculated DESs. These are in Table 2, together with the observed DESs, and also SD values for the observed DESs (Balls et al., 1995). It might be noted that although the DES values are quoted to two decimal places (Balls et al., 1995; Cronin et al., 1994), only differences between calculated and observed values of around 10 units are likely to be significant. A comparison of results in terms of DES with those in terms of log(DES/P^o) cannot fruitfully be made; it is more

appropriate to compare results in terms of logDES values with the log(DES/P^o) results. For the 37 compounds in equation [9] we find

Log(DES)obs = 0.022 + 0.979log(DES)calc [10]

N = 37, r2 = 0.771, SD = 0.30, F = 117.6

Table 2. Calculated DESs from equation [9], observed DESs and SD values for observed DESs

Compound	DES calc	DES obs	DES SD
Methyl trimethylacetate	10.45	2.67	
Ethyl trimethylacetate	9.00	4.17	3.82
Butyl acetate	9.71	7.50	4.36
Ethyl acetate	13.82	15.00	3.56
Cellosolve acetate	14.00	15.00	
Ethyl 2-methylacetoacetate	10.91	18.00	4.36
Methyl acetate	16.48	39.50	17.71
2,2-Dimethylbutanoic acid	90.46	44.67	14.49
Glycerol	2.57	1.67	
Propan-2-ol	52.70	30.50	20.63
2-Ethylhexan-1-ol	22.91	50.00	19.75
Isobutanol	50.82	60.25	8.10
Butanol	44.64	60.75	
Hexanol	27.29	64.75	16.26
Butyl cellosolve	13.06	68.67	
Cyclohexanol	33.86	79.75	
4-Bromophenetole	3.07	1.33	
1,3-Diisopropylbenzene	2.07	2.00	
sec-Butylbenzene	3.45	2.00	
3-Ethyltoluene	4.28	2.33	
2,4-Difluoronitrobenzene	2.32	3.67	
Styrene	6.11	6.75	
Toluene	7.21	9.00	2.00
m-Xylene	6.85	9.00	
4-Fluoroaniline	77.41	69.83	
3-Methylhexane	1.77	0.67	
2-Methylpentane	2.38	2.00	
Deca-1,9-diene	1.45	2.00	
Dodecane	0.34	2.00	
1,5-Dimethylcyclooctadiene	6.78	2.83	
cis-Cyclooctene	3.43	3.33	
Methylcyclopentane	3.97	3.67	2.34
Hexa-1,5-diene	5.32	4.67	
Methyl isobutyl ketone	14.11	4.75	2.99
Heptan-2-one	9.50	16.25	
Butanone	18.44	50.00	17.51
Propanone	23.87	65.75	4.50
Propylene glycol		1.33	

DISCUSSION

Equation [9], which results after dropping the outlier propylene glycol represents an excellent fit. There are good statistical grounds for excluding propylene glycol because in the two comparable equations, the F statistic increases markedly from 54 in equation [7] to 156 in equation [8]. Furthermore, both the Student's test residual (-6.24) and the DFIT (-3.12) of propylene glycol in equation [7] are so large in magnitude that there is no question that it is a very pronounced outlier. The final equation [10] is quite reasonable, and represents the best match of observed and calculated Draize scores yet reported. The apparent better fit of equation [9] over equation [10] is due to the considerable scale change on incorporation of the vapour pressure; $\log(DES/P^o)$ varies over a range of 6.0 log units, whereas log(DES) varies only over a range of 2.1 log units. A regression SD value of 0.3 log units in a range of 6.0 log units, as in equation [9], will always lead to a better correlation coefficient and F statistic than a regression SD value of 0.3 log units in a range of 2.1 log units, as in equation [10]. However, it follows from equation [10], that equation [9] is not simply a fit of $\log P^o$ to the descriptors, with log(DES) adding some noise. Neither is it an artefact due to transformation of the data because when the $\log(DES/P^o)$ values are reconverted into log(DES) values, equation [10] results. Hence, equation [9] contains a real connection between log(DES) and the descriptors. A plot of log(DES)obs against log(DES)calc on equation [10] is shown in Fig. 3. Although there is considerable random scatter about the line of identity, probably only the point for dodecane might be regarded as an outlier. Even here, we do not feel that log(DES) values of -0.5 or +0.3 are significantly different.

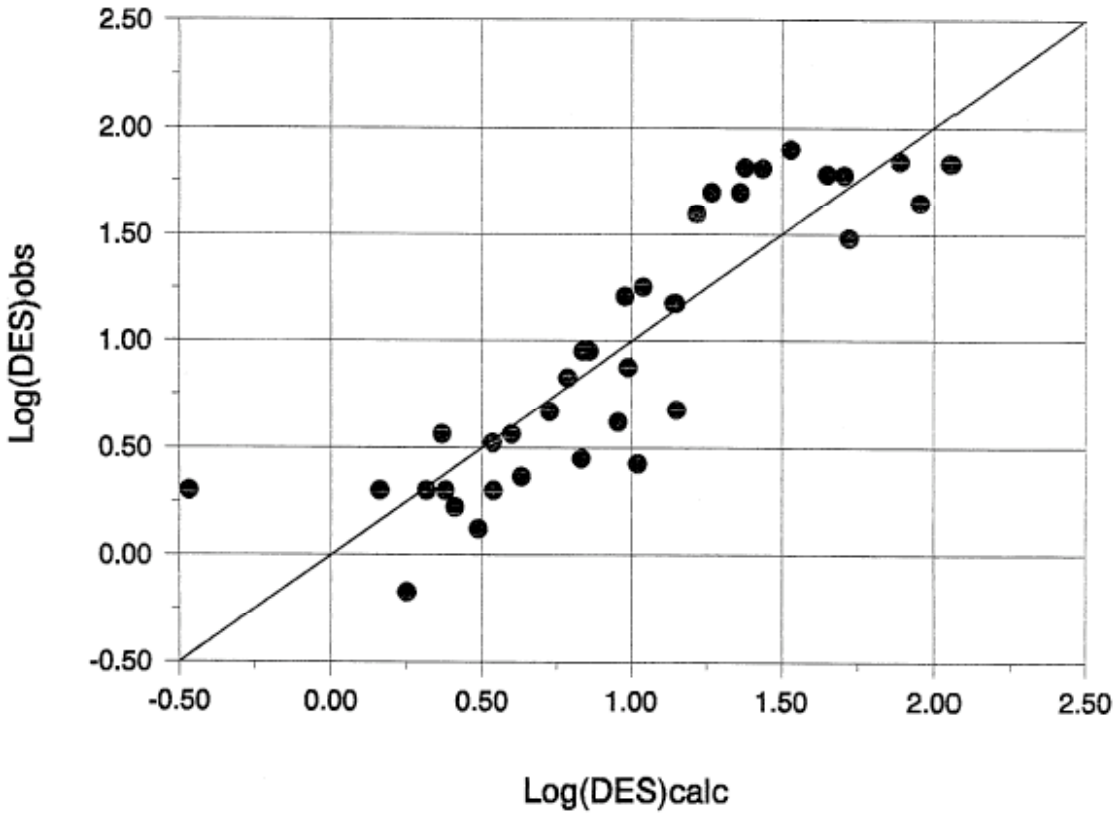

Fig. 3. Plot of log(DES) observed against log(DES) calculated from equation [10].

Because of the incorporation of P^o, equation [9] refers to the transport of compounds from the vapour phase to the biophase. Hence, the sign and magnitude of the coefficients should be compatible with what is found for transport of compounds from the vapour phase to various solvent phases. Coefficients in the general equation [6], with logSP = logL, are collected in Table 3 for the solubility of vapours in solvents (Abraham, 1996). It is clear that the coefficients in equation [9] are quite comparable to those found in equations for the solubility of vapours in organic solvents, as regards their sign and magnitude. Note that the c coefficient in equation [9] cannot be compared in this way because it depends on the units of P^o.

Table 3. Coefficients in equation [6] for the solubility, as logL values, of vapours in solvents

Phase	c	r	s	a	b	l
Water (37°C)	–1.36	1.05	2.63	3.74	4.50	–0.25
NFM (25°C)*	–0.53	—	2.57	4.32	—	0.73
NFM (40°C)	–0.56	—	2.39	3.92	—	0.68
EHP (25°C)†	–0.07	–0.26	0.91	3.74	—	0.95
EHP (37°C)	–0.09	–0.19	0.83	3.41	—	0.89
OCT (25°C)‡	–0.18	—	0.62	3.73	1.36	0.86
Oil (37°C)§	–0.24	–0.02	0.81	1.47	—	0.89
Hexadecane	0.00	0.00	0.00	0.00	0.00	1.00

It is further possible to describe general chemical characteristics of the biophase in the Draize eye test, by noting how the coefficients for the vapour-to-biophase transfer relate to those for the vapour-to-solvent transfers (Table 3). The biophase appears to be moderately dipolar, with an s coefficient near that for a phosphate ester. The biophase is highly basic, with a coefficient as large as that for an amide, and is also quite acidic (close to wet octanol). The hydrophobicity of the phase, judging by the l coefficient, is about the same as the amide N-formylmorpholine, and somewhat less than olive oil. All these properties are chemically reasonable, and so we believe that, for the first time, we have been able to analyse the results of the Draize rabbit eye test in a chemically meaningful way. What is very important is that if the DESs as log(DES/P^o), can be correlated through equation [9], then this implies that for the 37 irritants in equation [9], either transport from the pure liquid to the receptor phase is a main feature of the process, or if there are various receptor sites then these sites all have quite similar physicochemical properties. Furthermore, there is no need to

postulate that the 37 irritants act through different mechanisms. Of course, this may be so for other pure liquid irritants, which would then be identified as outliers to equation [9]. It is possible that propylene glycol, which is such an outlier, exerts irritancy through another mechanism; we have no other explanation as to why this compound is an outlier.

What is of considerable importance from the point of view of indoor air pollution is that equation [9] represents the effect of the vapour of pure liquids, that is of vapour concentrations of VOCs. There is no need to convert $\log(DES/P^o)$ back into DESs; the $\log(DES/P^o)$ values themselves relate to the effect of VOC vapours on the rabbit eye.

Of course, reasonable calculations of DESs, using equation [9] or [10] can only be made for a restricted data set, specifically for compounds tested in the form of pure liquids, and for which reliable vapour pressures at 25°C are available. Even with this restriction, we feel that equation [9] especially might be very useful in the assessment of eye irritation due to VOCs in the atmosphere, particularly in indoor air.

Although we have used a quite varied selection of irritants for our calculations, the resulting QSARs still need to be validated. Our approach will first be to incorporate other measures of eye irritation into our QSARs, and second to use Draize scores for other pure organic liquids as a test set.

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