

Further evaluation of the quantitative structure-activity relationship for skin-sensitizing alkyl transfer agents

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The biological activity of skin-sensitizing chemicals can be expressed in terms of physicochemical properties which relate to the propensity of those chemicals to behave as electrophiles and which describe their ability to partition into the epidermis and between compartments within it. For defined series of chemicals, it has proved possible to express such structure-activity relationships quantitatively. Such quantitative relationships can provide valuable insights into the mechanisms of skin sensitization and/or are of use in predictive toxicology. In the present work the quantitative structure-activity relationship (QSAR) previously derived for a series of alkyl transfer agents based on alkanesulfonate leaving groups has been critically examined in the light of skin sensitization data obtained for new members of that series and also for alkyl transfer agents based on different leaving groups. The QSAR predictions were broadly accurate, but demonstrated that further refinement was both necessary and possible. In particular, the physicochemical parameters which relate to the disposition of the chemical in the epidermis, i.e., its penetration through the stratum corneum, cell surface/cytoplasmic distribution and the associated dynamics, will need to be understood more fully in order to enhance the precision of the QSAR and its predictive power.

Key words: quantitative structure-activity relationship; QSAR; alkyl transfer agents; alkyl alkane-sulfonates; guinea pig sensitization; modified single adjuvant test; MSIAT; cross-sensitivity.

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The capacity of a chemical to behave as a contact allergen depends on its ability to partition into the viable epidermis and there to react with skin proteins (1). Chemical structural alerts can be combined with simple physicochemical parameters modelling skin permeation to predict whether or not a chemical may behave as a contact allergen (2). However, to predict the potency of a skin sensitizer simply on the basis of physicochemical properties is more difficult. So far this has proven successful only for restricted sets of compounds (e.g., (3-5)). Even then, the quantitative structure-activity relationship (QSARs) that have been derived have not been subjected to further testing.

Alkyl alkanesulfonates act as electrophiles which by transferring methyl or longer chain alkyl groups to protein nucleophiles groups are skin sensitizers. They were found to be mutually cross-reactive, indicating that the specificity of sensitization is essentially independent of the structure of

the alkyl group transferred. In earlier publications (4, 6) we have presented guinea pig sensitization test data for alkyl alkanesulfonates and shown how the data can be correlated quantitatively by a combined QSAR/dose-response relationship based on the relative alkylation index (RAI) model (3). This relationship is shown in eq. (1):

$$PRS = 2.24RAI_i - 0.26(RAI_i)^2 + 0.54RAI_c - 2.23. \quad (1)$$

PRS is the probit value corresponding to the skin response on challenge. RAI_i and RAI_c are terms which model the relative extent to which hapten reacts with skin protein at induction and challenge. These terms include measure of dose, chemical reactivity and lipophilicity.

To assess the scope and limitations of eq. (1) and gain further insights into the dependence of skin sensitization potential on physicochemical parameters, we have tested the predictions of eq. (1)

against sensitization test and cross-challenge data on further compounds of the same type, and using a small data set on primary alkyl bromides which are also skin sensitizers by virtue of their ability to behave as alkylating agents (7).

Materials and Methods

Chemicals

All the alkyl alkanesulfonates were synthesized following the principles described previously (4, 6). Purity was assessed to be >95% by chromatography and nuclear magnetic resonance. Dimethyl sulfococoate and dimethyl sulfostearate were prepared at the Port Sunlight Laboratory; residual material consisted largely of unsulfonated fatty acid and its methyl ester. The details relating to purity and structure are detailed in the Results section. All the alkyl bromides were purchased from Aldrich Chemical Co., Poole, Dorset, UK. Purity was >98%, with the residual material being contamination with nearby carbon chain lengths. The logarithm of the octanol-water partition coefficient ($\log P$) was calculated using the Leo & Hansch (8)

method, except where indicated otherwise in the text.

Modified single adjuvant test (MSIAT)

This assay was carried out as described previously (4). In brief, preliminary skin irritation tests were carried out to determine concentrations of test substances suitable for the induction of sensitization and for epicutaneous challenge. Sensitization was then induced in groups of 10 guinea pigs by a series of 6 0.1 ml intradermal injections of the test substance in combination with Freund's complete adjuvant in the neck/shoulder region. After a 2-week interval, the initial challenge was conducted by topical application of the sensitizing chemical at the maximum non-irritant concentration using 8 mm Finn Chambers (Epitest Oy, Finland) applied for 6 h to clipped and shaved flank skin. Reactions were scored for erythema (scale 0–3) and the presence of oedema approximately 24 h and 48 h after removal of the patches. A control group ($n=5$) were treated exactly as the test animals, except that the test substance was omitted during the

Table 1. Alkyl alkanesulfonates – sensitization test data

Compound	$\log P$	PRS calc ^{a)}	PRS obs ^{b)}	% TES calc ^{c)}	% TES obs ^{d)}
C ₉ H ₁₉ SO ₃ Me	3.87				
induction (ppm)	5000				
challenge (ppm)	25000	5.47	5.76	63.3	77.5
	2500	4.93	4.09	46.0	18.3
	250	4.39	3.16	28.6	3.3
	25	3.85	3.04	11.3	2.5
C ₆ H ₁₃ SO ₃ Me	2.25				
induction (ppm)	5000				
challenge (ppm)	25000	5.11	6.19	51.9	88.3
	2500	4.57	4.57	33.3	33.3
	250	4.03	≤2	17.2	0
C ₉ H ₁₉ SO ₃ C ₇ H ₁₅	7.11				
induction (ppm)	5000				
challenge (ppm)	25000	5.44	5.3	62.6	61.7
	2500	4.90	4.94	45.2	47.5
	250	4.36	2.87	27.9	1.7
C ₉ H ₁₉ SO ₃ C ₄ H ₉	5.49				
induction (ppm)	5000				
challenge (ppm)	25000	5.04	5.17	49.8	56.7
	10000	4.83	4.96	42.8	48.3
	1000	4.29	4.96	25.5	48.3
	100	3.75	3.61	8.2	8.3
	10	3.21	≤2	0	0
				mean	SD
Residuals					
on probits, all data				–0.18	0.73
on probits, heptyl and butyl nonanesulfonates only				–0.12	0.67
on probits, methyl hexane- and nonane-sulfonates only				–0.36	0.84
comparison from original eq. (1)					0.48

^{a)} Calculated probit score.

^{b)} Probit score derived from the observed results.

^{c)} Total erythema score predicted by the QSAR (as % of maximum theoretically fonible).

^{d)} The observed total erythema score (as % of maximum theoretically fonible).

Table 2. Cross-challenges

Sensitizer	Chal- lenger	TES (%) ^{a)}	PRS (Obs) ^{b)}	PRS (Calc) ^{c)}	Residual
16/1	12/1	36 (37)	4.64	4.47	0.17
16/1	16/1	39 (27)	4.72	5.01	-0.29
16/1	1/16	66.67	5.43	4.34	1.09
16/1	1/12	42.67	4.81	3.83	0.98
1/12	1/18'	50.00	5.00	5.12	-0.12
1/16	12/1	37.67	4.69	5.89	-1.20
1/16	1/12	30.00	4.48	5.25	-0.77
1/16	1/18'	50.00	5.00	5.88	-0.88
1/18'	1/12	40.00	4.75	4.69	0.06
12/1	16/1	3.33	3.16*	6.21	-3.05
12/1	16/1	56.67	5.17	5.75	-0.58
16/1	12/1	70.00	5.52	5.32	0.20
16/1	16/1	0.00	3 or less*	6.33	-4.33
mean (all)					-0.59
SD (all)					1.33
mean (omitting asterisked data)					-0.12
SD (omitting asterisked data)					0.73
9/7	9/1	25.83	4.35	5.48	-1.13
6/1	9/1	77.50	5.76	5.48	0.28
9/1	9/7	9.17	3.37	5.43	-2.06

^{a)} The observed total erythema score (as for Table 1).

^{b)} Probit score derived from the observed results.

^{c)} Calculated probit score.

induction process. Subsequent challenges and cross-challenges were usually carried out at weekly intervals on alternate flanks. Specific details of test concentrations are given in Tables 1-4.

Results and Discussion

Alkyl alkanesulfonates

Table 1 shows sensitization results for 4 alkyl alkanesulfonates not tested in the original study together with the predictions of eq. (1). Overall the new data are fitted by equation 1 with a degree of scatter not much greater than for the original data used to derive it, as indicated by comparison of the standard deviation of the residuals with that for eq. (1). The 2 methyl alkanesulfonates have lower log *P* values than the compounds originally used. Therefore the data for these compounds provide an extrapolation test of eq. (1). There are indications that for this pair the dose-response relationships for challenge are somewhat steeper than for the compounds originally studied, since the highest challenge dose responses are overestimated and the lowest challenge dose responses underestimated by eq. (1). The other compounds, butyl and heptyl nonanesulfonate, have log *P* values within the range covered by the original compound set, so data for these compounds provide an interpolation test of eq. (1). These compounds differ from those originally studied in that both their alkyl groups are larger than methyl. This should not affect their electrophilic reac-

tivity - kinetic experiments show that heptyl nonanesulfonate reacts at the same rate as dodecyl methanesulfonate (9). Their calculated log *P* values are identical to those of their isomers in which one group is methyl. As expected, the standard deviation of the residuals for the interpolation cases is better than that for the extrapolation cases (Table 1).

Table 2 shows the results of cross-challenges involving compounds used in the development of eq. (1) and some of the further compounds shown in Table 1. Among the cross-challenges involving only the compounds used originally to develop eq. (1), the 2 in which methyl hexadecanesulfonate is used for challenge (data asterisked in Table 2) stand out as giving markedly lower responses than predicted from eq. (1), even though in each case both the induction and challenge compounds were methyl transfer agents. We have previously drawn attention to the anomalously low cross-challenge elicitation potential of methyl hexadecanesulfonate, which may possibly be attributable to the compound's high melting point, a property which is related to aqueous solubility and the kinetics of stratum corneum penetration (10).

Apart from these 2 cases, the remaining results for the original compounds are reasonably well predicted by eq. (1), as shown by the comparison between the standard deviations of residuals. There seems no evidence of an effect seen previously (with a different class of sensitizers) in which cross-challenges give lower responses than self-challenges, even when the compounds involved both transfer the same hapten to skin protein (11). There also seems little evidence for any specificity for the alkyl group transferred. The mean residual for cross-challenges in which both compounds are methyl transfer agents (even excluding the 2 in which methyl hexadecanesulfonate is used for challenge) is 0.03, whereas that for cross-challenges in which only 1 compound is a methyl transfer agent is more positive, 0.29.

The 3 cross-challenges involving the compounds not used in the development of eq. (1) show a rather different pattern. One of the cross-challenges, in which both compounds are methyl transfer agents, have lower log *P* values than any of the original set of compounds and differ in carbon number by only 3 (corresponding to a calculated log *P* difference of 1.62), gives reasonable agreement between observed and calculated responses. However, for the other 2 cross-challenges, in which 1 compound is heptyl nonanesulfonate (log *P* within the range covered by the original set of compounds) and the other is methyl nonanesulfonate (log *P* below the range covered by the original set of compounds), the observed responses are sub-

stantially lower than predicted from eq. (1). It may be significant that for these cross-challenges the difference between log *P* values of the 2 compounds (3.24) is larger than for any of the other cross-challenges shown in Table 2.

We now turn to alkyl transfer agents which are structurally different from those used to establish eq. 1, but which can sensitize by the same chemical reaction mechanism, transferring a simple linear chain alkyl hapten onto skin protein.

Sulfocarboxylic acid diesters

Table 3 shows sensitization and cross-challenge data for diesters of α -sulfocarboxylic acids. These esters were isolated as by-products from laboratory scale sulfonation of fatty acid esters. The di-

methyl sulfococoate in Table 3 was a crude by-product containing 35% impurities (composed of coco fatty acid and coco fatty acid methyl ester, which would not be expected to interfere with the sensitization test), the remaining 65% having the structure $RCH(CO_2Me)SO_3Me$, where $R=C_{10}H_{21}$, 43%; $R=C_{12}H_{25}$, 22%. For this mixture RAI values were calculated separately for the 2 homologues, the dose term being adjusted to allow for the different concentrations of each homologue. In the RAI calculations for these sulfocarboxylic acid diesters, it is assumed that the reactivity of alkyl alkanesulfonates is not significantly altered by the presence of the carboxyalkyl group. The log *P* values used in the RAI calculations were calculated by the Leo & Hansch (8), method modified by application of a proximity factor cut-off and sup-

Table 3. Sulfocarboxylic acid diesters

Compound		log <i>P</i>	PRS calc ^{a)}	PRS obs ^{b)}	% TES calc ^{c)}	% TES ^{d)} obs
Dimethyl sulfococoate		5.37/6.45				
all challenges	50000 ppm					
induction (ppm)	1000		5.98	5.84	83.6	80.0
	100		5.87	5.25	80.8	60.0
	10		5.20	4.86	57.9	44.3
	1		4.06	4.01	17.4	16.0
Dimethyl sulfostearate		8.61				
induction (ppm)	100					
challenge (ppm)	100000		6.77	5.17	96.2	56.7
	10000		6.23	4.29	89.0	24.0
cross challenges with:						
dimethyl sulfococoate,	10000 ppm		5.53	3.16	65.2	3.3
methyl hexadecane sulfonate,	50000 ppm		6.32	4.88	90.8	45.3

^{a)} Calculated probit score.

^{b)} Probit score derived from the observed results.

^{c)} Total erythema score predicted by the QSAR (as for Table 1).

^{d)} The observed total erythema score (as for Table 1).

Table 4. Alkyl bromides

Hexyl bromide		log <i>P</i>	PRS calc ^{a)}	PRS obs ^{b)}	% TES calc ^{c)}	% TES ^{d)} obs
induction	50,000 ppm	3.72				
Challenge	100,000 ppm		5.06	5.25	28.5	60
Cross-challenges with:						
dimethyl sulfococoate,	500 ppm		4.92	4.22	39.4	21.7
hexadecyl methane			5.76	4.51	55.6	31.3
sulfonate,	5000 ppm					

Cross challenges of alkyl bromides onto methyl dodecane sulfonate (5000 ppm) test animals

		log <i>P</i>	PRS calc	PRS obs	% TES calc	% TES obs
Self-challenge,	25,000 ppm	5.49	5.58	5.34	71.9	63.3
Alkyl bromide:						
C9	50,000 ppm	5.31	5.22	4.16	31.2	20
C12	50,000 ppm	6.9	5.59	4.57	45.2	33.3
C15	50,000 ppm	8.48	5.96	3.29	60.3	4.4
C18	50,000 ppm	10.01	6.32	≤2	73.2	0

^{a)} Calculated probit score.

^{b)} Probit score derived from the observed results.

^{c)} Total erythema score predicted by the QSAR (as for Table 1).

^{d)} The observed total erythema score (as for Table 1).

pression of the branching factor (Roberts, Marshall and Hodges, unpublished).

The sensitization test results for dimethyl sulfocooate are quite well predicted by eq. (1), supporting the robustness of the relationship and demonstrating its applicability to mixtures of homologues. However the test results for dimethyl sulfostearate, including cross-challenges, are all substantially overpredicted by eq. (1). It seems clear that this compound did not induce as high a degree of sensitization as predicted by eq. (1). It should be noted that dimethyl sulfostearate has a higher calculated log *P* value than any of the other sulfonate esters considered here or used in the original development of eq. (1). It is likely that its skin penetration is lower than estimated.

Primary alkyl bromides

Table 4 shows sensitization and cross-challenge test data for alkyl bromides. These compounds, like the alkyl alkanesulfonates, are electrophilic alkyl transfer agents. They are somewhat less reactive than higher alkyl alkanesulfonates, which transfer alkyl groups higher than methyl. In calculation of RAI values for the alkyl bromides, the reactivity term was based on kinetic data for reactions of *n*-butylamine with dodecyl bromide and with dodecyl methanesulfonate (D W Roberts, R S Ward and P Hughes, unpublished).

The alkyl bromides are cross reactive with the alkyl alkanesulfonates, as would be expected. The sensitization potency of hexyl bromide is somewhat underpredicted by eq. (1), although the cross-challenges of the sulfonate esters onto the hexyl bromide sensitized animals gave slightly lower responses than predicted. With the higher alkyl bromides, used in cross-challenges on methyl dodecane sulfonate test animals, the responses are quite well predicted for the C₉ and C₁₂, but the C₁₅ and C₁₈ bromides give very slight (zero in the case of the C₁₈ bromide) responses. The C₁₅ and C₁₈ bromides are, on the basis of their log *P* values, more hydrophobic than any of the sulfonate esters considered here. It is possible that the poor ability of these compounds to elicit responses reflects their low skin penetration. However, this would imply a different dependence on log *P* of guinea pig and mouse skin, since for the latter the evidence suggests that the log *P* value of the C₁₅ bromide is optimum for sensitization (7).

It is worth bearing in mind that although the primary alkyl bromides are closely related to the alkyl alkanesulfonates in terms of their reaction chemistry, they may be substantially different in their partitioning properties and in their mass-transfer characteristics in the epidermis and the stratum corneum. The use of log *P* to model such

properties is based on the implicit assumption that the same linear relationships between log *P* (octanol/water) and log *P* (biological lipid phase/water) apply to all of the compounds considered. There is increasing evidence (e.g., ref. (12)) that this is not the case when 1 group contains a polar grouping (such as the -SO₂O- group of the alkyl alkane-sulfonates) and another group (such as the alkyl bromides) does not.

The derivation of quantitative structure-activity relationships for skin sensitizers is complicated by the quality of the biological data obtained from guinea pig predictive tests, which is only partly truly cardinal in nature, i.e., the proportion of the test group sensitized. Nevertheless, it has proved possible to develop equations relating biological activity to physicochemical properties and the dose applied for specific series of skin sensitizers (e.g., (3)-(6)). However, it is important to assess the robustness of a QSAR by determining how well it can predict the sensitizing potency of related chemicals. The QSAR equation previously derived for alkyl alkane sulfonates provided reasonable predictions of sensitizing potency for each 6 compounds from the same chemical family. As expected, interpolations were better than extrapolations outside the original training set. Predictions for dose responses were not quite so good, especially at lower challenge doses, but in practice the observed data will be substantially affected by technical aspects of assay conduct. This is due in part to the sequential nature in which the dose responses are generated; preceding challenges at higher doses can modify the sensitization of the test animals. Once compounds from rather different chemical families were examined, the predictions became less reliable, even though they transferred the same haptens onto skin proteins and demonstrated cross-reactivity. Nevertheless, many of the predictions were still fairly accurate and provide encouragement that the approach taken is reasonable. Thus, the challenge for the future will be to continue to refine this and other QSARs in the light of new data and to test it with further compounds. In particular, the physicochemical parameters which relate to the disposition of the chemical in the epidermis, i.e., its penetration through the stratum corneum, migration into the viable layers of epidermis, cell surface/cytoplasmic distribution and the associated dynamics, will need to be understood more fully in order to enhance the precision of the QSAR and its predictive power.

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