

A QUANTITATIVE STRUCTURE–ACTIVITY/DOSE RELATIONSHIP FOR CONTACT ALLERGENIC POTENTIAL OF ALKYL GROUP TRANSFER AGENTS

D. W. ROBERTS* and D. A. BASKETTER††

*Unilever Research, Port Sunlight, The Wirral, Cheshire and

†Environmental Safety Laboratory, Colworth House, Sharnbrook, Bedford MK44 1LQ, UK

Abstract—Since no *in vitro* model is available, or indeed likely, to predict or investigate skin sensitization potential of substances, an approach based on a model using physicochemical criteria is the most likely route to a reduced requirement for animal testing. As part of the investigation of structure–activity relationships in contact allergy, it was shown that methyl transfer agents are capable of acting as skin sensitizers. This work has now been extended to a more general examination of alkyl transfer reactions. *In vivo* dose responses to challenge and the patterns of cross reactivity between C₁₂, C₁₆, unsaturated C₁₆ and methyl transfer agents were examined to form the basis of the mathematical model. All alkyl transfer agents examined were potent sensitizers, with evidence of mutual cross reactivity between them. The sensitization data have been accurately modelled using a mathematical equation. Analysis in terms of a modified relative alkylation index model showed evidence of an overload effect.

Introduction

Roberts and Williams (1982) have described a mathematical model of skin sensitization potential. This model has been used to rationalize conflicting data on the sensitizing activity of acrylates (Roberts, 1987). *In vivo* data showed methyl transfer agents (RSO₃Me) to have a high skin sensitization potential (Roberts *et al.*, 1988). This report combines those results with data on related higher alkyl and alkenyl methane sulphonates (MeSO₃R), which act as R group transfer agents, and provides an analysis in terms of a modified relative alkylation index (RAI) model.

Materials and Methods

Materials. Hexadecyl methane sulphonate (MeSO₃C₁₆H₃₃, 98%) was purchased from Aldrich Chemicals, Gillingham, UK. Oleyl methane sulphonate (MeSO₃C₁₈H₃₅, >95%) and dodecyl methane sulphonate (MeSO₃C₁₂H₂₅, >95%) were prepared by reactions of methane sulphonyl chloride with oleyl and dodecyl alcohols, respectively.

Sensitization test method. Sensitization tests were carried out by the modified single injection adjuvant test (Goodwin and Johnson, 1985). Initial challenge was always conducted with the sensitizing chemical at its maximum non-irritant concentration.

Calculation of RAI values. The RAI expresses the relative extent to which carrier protein is alkylated following application of test material. Independent values can be calculated for the induction (RAI_i) and challenge (RAI_c) phases of sensitization tests. As originally defined, the RAI is expressed as:

$$\log \frac{kD}{P_{a/h} + P_{a/h}^2}$$

where k is the relative rate constant for reaction of the sensitizer with a standard nucleophile, D is the molar dose, and $P_{a/h}$ is the aqueous methanol/hexane partition coefficient (originally chosen for experimental convenience). A more widely applicable partition coefficient is that between octanol and water, whose logarithm ($\log P$) can be calculated from structure by, *inter alia*, the fragment method of Hansch and Leo (1979). $\log P$ values for different pairs of solvents tend to be linearly related, at least for related series of compounds, and for a series of cyclic and non-cyclic sulphonate esters we have found the following correlation:

$$\log P_{a/h} = -0.48 \log P + 2.33$$

For the highly hydrophobic compounds discussed in this paper, the $P_{a/h}$ term will dominate over the $P_{a/h}^2$ term in the RAI expression. Thus, since the RAI is dimensionless, the expression can be redefined as:

$$\text{RAI} = \log k + \log D + 0.48 \log P$$

This is the definition used in this report, with calculated $\log P$ values. D is the concentration of test material for induction (D_i) or challenge (D_c). The $\log k$ values are taken as zero for the methyl sulphonates and -1.48 for the higher alkyl and alkenyl methane sulphonates, based on the generalization that SN2 reactions of primary alkyl groups larger than methyl are about 30 times slower than those of methyl groups (Lewis *et al.*, 1987).

Results

Sensitization test data

Dodecyl, hexadecyl and oleyl transfer agents, like methyl transfer agents, were strong sensitizers. They cross reacted with one another and with methyl group transfer agents.

†To whom all correspondence should be addressed.
Abbreviations: PR = probit value; RAI = relative alkylation index.

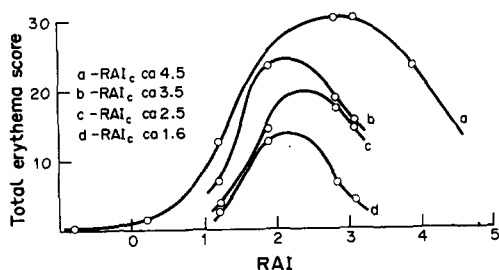


Fig. 1. RAI plots based on similar RAIC values.

RAI plots

Sensitization responses expressed as the total erythema score—the multiple of the incidence score and the mean erythema score—were plotted against calculated RAI values at matching RAIC values. Data previously reported from experiments with methyl transfer agents have been included (Roberts *et al.*, 1988). The curve has the double sigmoid shape theoretically predicted and subsequently demonstrated for several other types of sensitizer (Andersen *et al.*, 1985; Roberts, 1987; Roberts *et al.*, 1988). Several data points fall in the overload region, and it appears that maximum sensitivity is obtained with an RAI value of around 2.5–3.

Quantitative structure–activity relationship analysis

The plots in Fig. 1 indicate that the sensitization response depends on: (1) RAI—initially increasing then decreasing with increasing RAI; and (2) RAIC—increasing with increasing RAIC. These relationships can be put on a more quantitative basis by means of multiple linear regression. For this purpose, the sensitization response is expressed as a probit value (PR), with the probit transformation (Bliss, 1935) being designed to linearize sigmoid dose-response curves. To model the correlation with RAI, a quadratic expression is appropriate for initial evaluation, that is using both RAI and RAI^2 as variables in the regression. One of the RAI values in the data set is negative (−0.78), so in order that the squared term should increase monotonically with increasing RAI, $(RAI + 0.78)^2$ is used rather than RAI^2 . This parameter is subsequently denoted by RAI^{i2} . Translocation ability in biological systems is often correlated bilinearly with $\log P$. However, for the highly hydrophobic compounds under present consideration, migratory ability is expected to be negatively correlated with $\log P$. Regression analysis using these variables gives:

$$PR = 1.82 RAI - 0.22 RAI^{i2} + 0.62 RAIC - 0.57 \log P + 4.35 \quad (\text{equation } 2)$$

where $n = 27$, $R = 0.907$, $s = 0.48$ and $F = 25.4$.

The $\log P$ term is significant at a level $P < 0.001$. The other terms on the right-hand side are all significant at $P < 0.001$. The R^2 value is 0.82, that is the regression explains 82% of the variation in the data. The extent to which the equation fits the data is depicted graphically in Fig. 2, which shows a plot of observed against calculated PR values. The equation is a hybrid dose-response/structure–activity relationship.

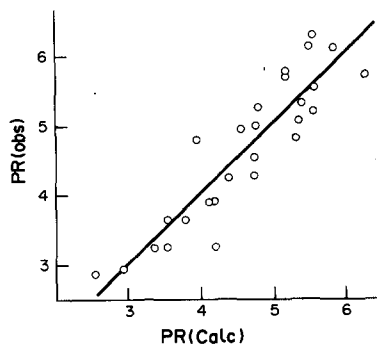


Fig. 2. Plot of observed *v.* calculated PR values.

Discussion

Previous studies (Roberts *et al.*, 1988) and the present report have shown that simple alkyl transfer agents are potent sensitizers. In the earlier paper a possible mechanism of sensitization was suggested, which implicated modification to carrier protein structure rather than hapten as the target for the T-lymphocyte antigen receptor. The evidence of cross challenge reactions between alkyl groups of various chain lengths supports the proposed mechanism. By making simple generalizations on the alkylation activity of the materials studied, it has proved possible to make a further quantitative evaluation of the relationship between physicochemical parameters and skin sensitization potential. The data fit a quadratic expression relating an index of the degree of observed sensitization to RAI values for induction and challenge plus a partition coefficient term. The results suggesting antigen overload leading to tolerance are also modelled by this expression.

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