Pergamon

0887-2333(94)00137-5

Toxic. in Vitro Vol. 8, No. 5, pp. 1053–1060, 1994
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0887-2333/94 $7.00 + 0.00

AN EXPERT SYSTEM RULEBASE FOR IDENTIFYING CONTACT ALLERGENS

M. D. BARRATT, D. A. BASKETTER, M. CHAMBERLAIN, G. D. ADMANS* and J. J. LANGOWSKI*

Unilever Environmental Safety Laboratory, Sharnbrook, Bedford MK44 1LQ and *LHASA UK, School of Chemistry, University of Leeds LS2 9JT, UK

Abstract—There are currently no *in vitro* methods suitable for the prospective identification of skin sensitizers (contact allergens). Knowledge relating chemical structure to toxicity can be programmed into expert systems. An historical database, containing results of 294 defined single substances tested in the guinea pig maximization test (GPMT) carried out according to a single protocol, has been used to derive a set of structural alerts for skin sensitization. Where possible, the approach used was to group the substances according to their most likely mechanism of reaction with skin proteins. Where no mechanism could be identified, structural alerts were derived empirically for groups of molecules with similar chemical functionality. This process has currently resulted in the production of 40 structure–activity rules which have been incorporated into the expert system DEREK. This system forms an integral part of a strategic approach to the identification of contact allergens.

Introduction

There are currently no *in vitro* methods suitable for the prospective identification of skin sensitizers (contact allergens), although methods that can be useful in fundamental research have been described (Hauser and Katz, 1988). To fill this gap, a rulebase is under development based on the correlation of the protein reactivity of chemicals with their skin sensitization potential (Dupuis and Benezra, 1982).

Knowledge relating chemical structure to toxicity can be programmed into expert systems. DEREK (an acronym for 'Deductive Estimation of Risk from Existing Knowledge') is one such expert system (Sanderson and Earnshaw, 1991), being developed by LHASA UK at the School of Chemistry, University of Leeds. DEREK embodies both a controlling programme and a chemical rulebase. The chemical rulebase consists of descriptions of molecular substructures 'structural alerts', which have been found previously to correlate with specific toxicological endpoints. The user communicates with DEREK through interactive computer graphics by drawing the two-dimensional chemical structure of the query molecule on the screen.

An historical database, which contains the results of testing 294 defined chemical substances in the guinea pig maximization test (GPMT) carried out according to a single protocol (Magnusson and Kligman, 1970), has been used to derive a set of structural alerts for skin sensitization.

Methods

The Magnusson and Kligman guinea pig maximization test

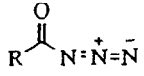
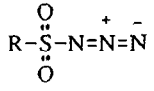
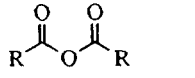
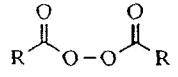
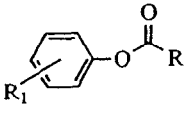
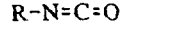
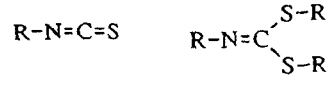
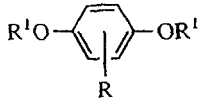
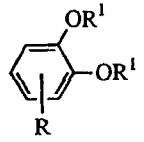
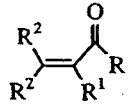
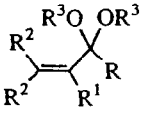
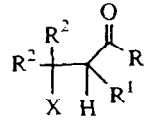
The GPMT carried out was based on that described by Magnusson and Kligman (1970). Guinea pigs were albino Dunkin–Hartley strain and weighed approximately 350 g at the start of testing. Preliminary irritation tests were carried out to determine the concentrations of test substances that were suitable for induction of sensitization and for sensitization challenge. Guinea pigs were then treated by a series of six intradermal injections in the shoulder region to induce sensitization. After 6–8 days the sensitization was boosted by a 48-hr occluded patch placed over the injection site, and 12–14 days later the animals were challenged on one flank by a 24-hr occluded application in 8-mm Finn chambers (Epitest, Oy) at the maximum non-irritant concentration. Challenge sites were scored for erythema and oedema 24 and 48 hr after removal of the patches.

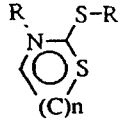
Rationale

The chemicals tested were grouped into categories on the basis of the GPMT results: strong sensitizers (70–100% positive); moderate sensitizers (30–70% positive); weak sensitizers (up to 30% positive); non-sensitizers (negative).

To identify groups of chemicals with common reaction mechanisms, initially only chemicals that were classified as strong or moderate sensitizers were considered. These were the chemicals that currently

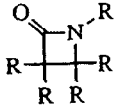
Table 1. Skin sensitization rulebase, rule titles and structures

No.	Title	Structure
401	Acid halides	R = any X = F, Cl, Br
402	Acid azides	 R = any
403	Sulphonyl halides	R = alkyl,aryl X = Cl, Br
404	Sulphonyl azides	 R = any
405	Acid anhydrides	 R = any
406	Diacyl peroxides	 R = any
407	Phenyl esters	 R = alkyl, aryl R_1 = any
408	Isocyanates	 R = any
409	Isothiocyanates	
417	Hydroquinones and O-alkyl precursors	 R = any R^1 = H, alkyl
418	Catechols and O-alkyl precursors	 R = any R^1 = H, alkyl
419	Alpha-, beta-unsaturated aldehydes, amides, esters and ketones	 R = H, C, N, O (not OH) R^1 = not a heteroatom R^2 = not aryl (unless R = H)
420	Precursors of alpha-, beta-unsaturated aldehydes, amides, esters and ketones	 R = H, C, N, O R^1 = not a heteroatom R^2 = not aryl (unless R = H) R^3 = any
421	Precursors of alpha-, beta-unsaturated aldehydes, amides, esters and ketones	



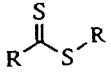
R = any

410 Beta lactams



R = any

411 Thioesters

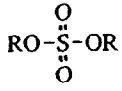


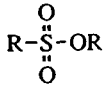
R = any

412 Haloalkanes

R = alkyl
X = Cl, Br, I

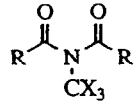
413 Dialkylsulfates/sulfonates





R = alkyl, aryl

414 *N*-Trihalomethyl imides

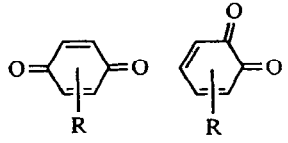


R = any
X = Cl, Br, I

415 Halogen containing di- or tri-nitro aromatics

R = any
X = F, Cl, Br

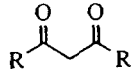
416 Quinones



R = any

R = H, C, N, O (not OH)
R^1 = not a heteroatom
R^2 = not aryl (unless R = H)
X = good leaving group

422 1,3-Diketones

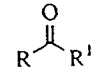


R = alkyl, aryl

423 Aldehydes

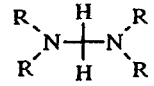
R = alkyl, aryl

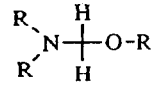
424 Ketones

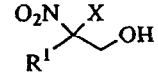


R, R^1 = alkyl, aryl

425 Formaldehyde donors

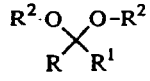






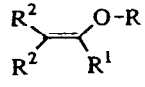
R, R^1 = any
X = halogen

426 Precursors of aldehydes and ketones



R, R^1 = alkyl, aryl
R^2 = any

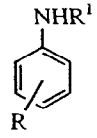
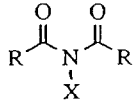
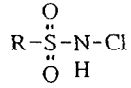
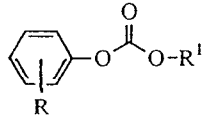
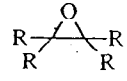
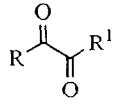
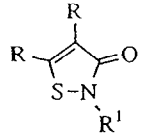
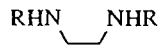
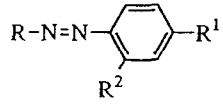
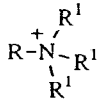
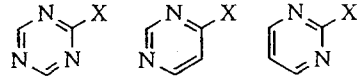
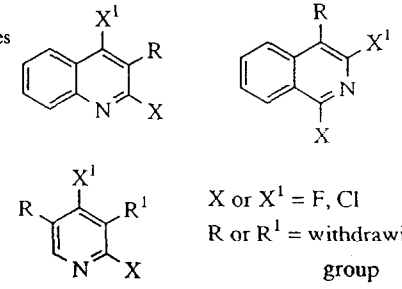
427 Enol precursor of aldehydes and ketones

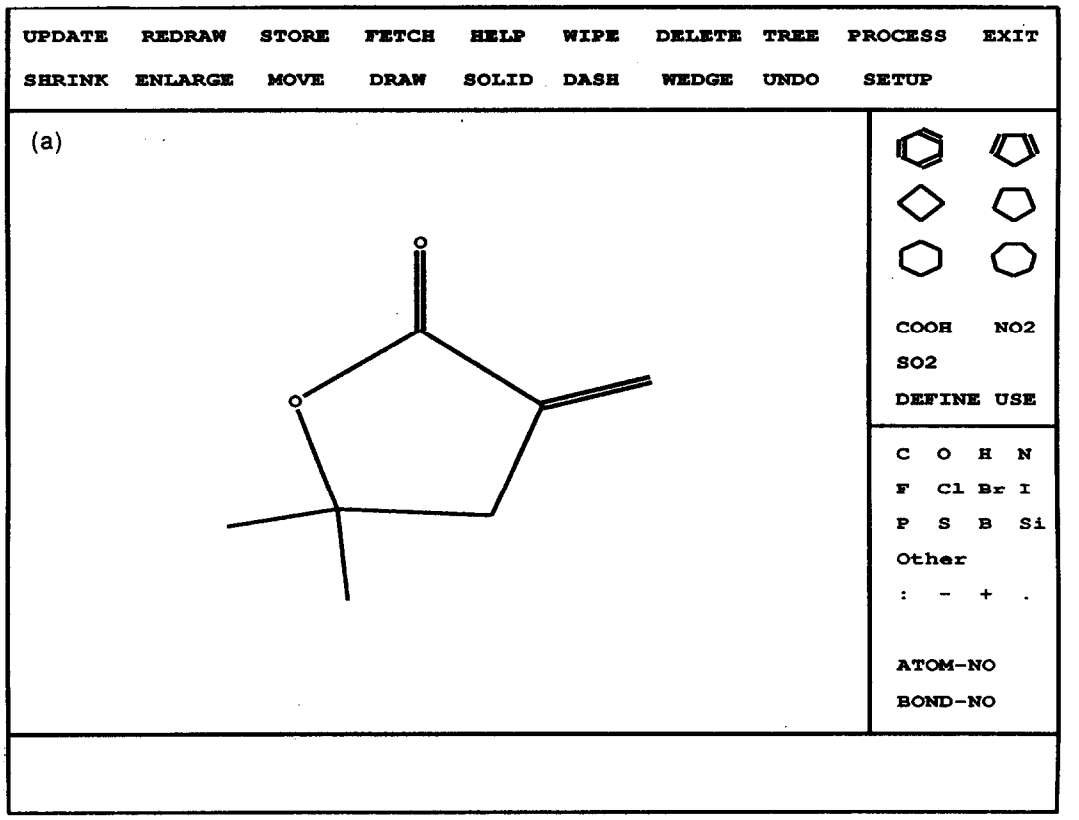


R, R^2 = any
R^1 = not a heteroatom

[contd]

Table 1—*contd*

No.	Title	Structure
428	Aromatic primary and secondary amines	 R = any; R^1 = alkyl, aryl
429	*N*-Haloimides	 R = any; X = Cl, Br
430	*N*-Chloro-sulfonamides	 R = any
431	Disulfides	R–S–S–R R = alkyl, aryl
432	Phenyl carbonates	 R = any; R^1 = alkyl, aryl
433	Epoxides	 R = any
434	1,2-Dicarbonyls	 R = alkyl, aryl; R^1 = H, alkyl, aryl
435	Thiazolones	 R, R^1 = alkyl, aryl
436	1,2-Diamines	 R = alkyl, aryl
437	Amine precursor - aromatic azo compound	 R = aryl; R^1 or R^2 = electron donating
438	Quaternary ammonium cation	 R, R^1 = aryl or alkyl
439	Activated *N*-heterocycle halides	 X = F, Cl
440	Activated halo-pyridines, quinolines, and isoquinolines	 X or X^1 = F, Cl; R or R^1 = withdrawing group



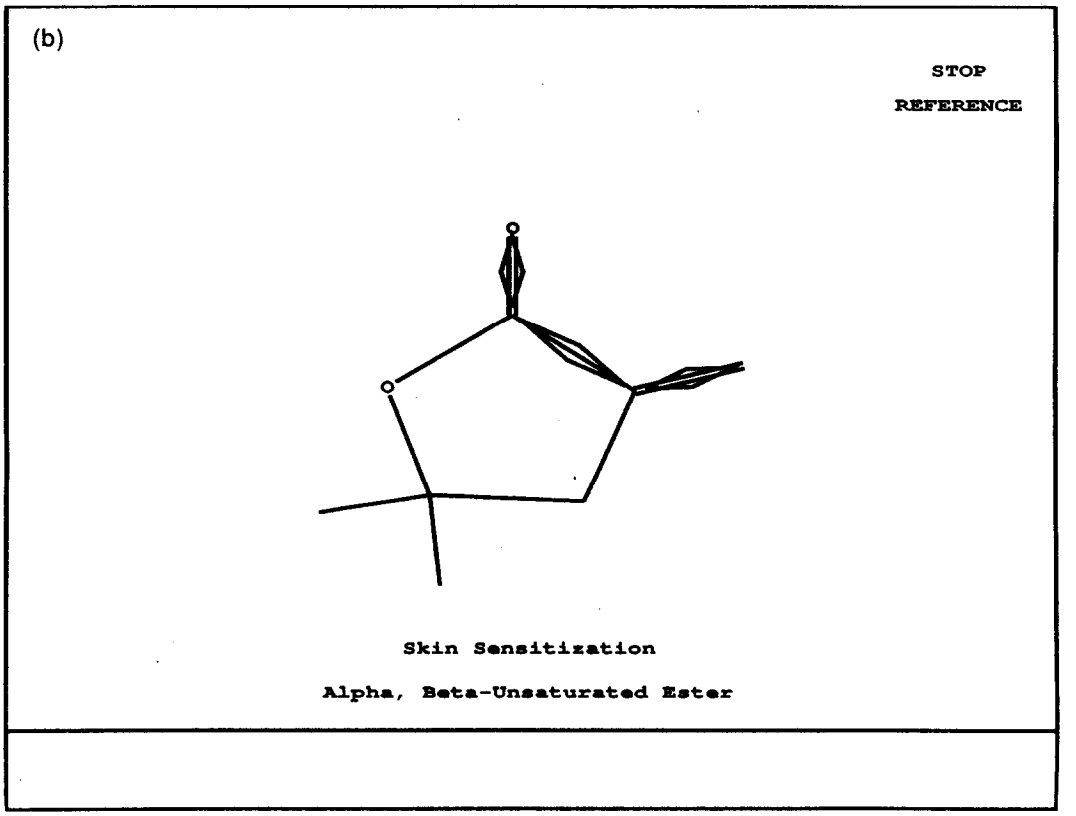


Fig. 1(a and b)—*legend overleaf.*

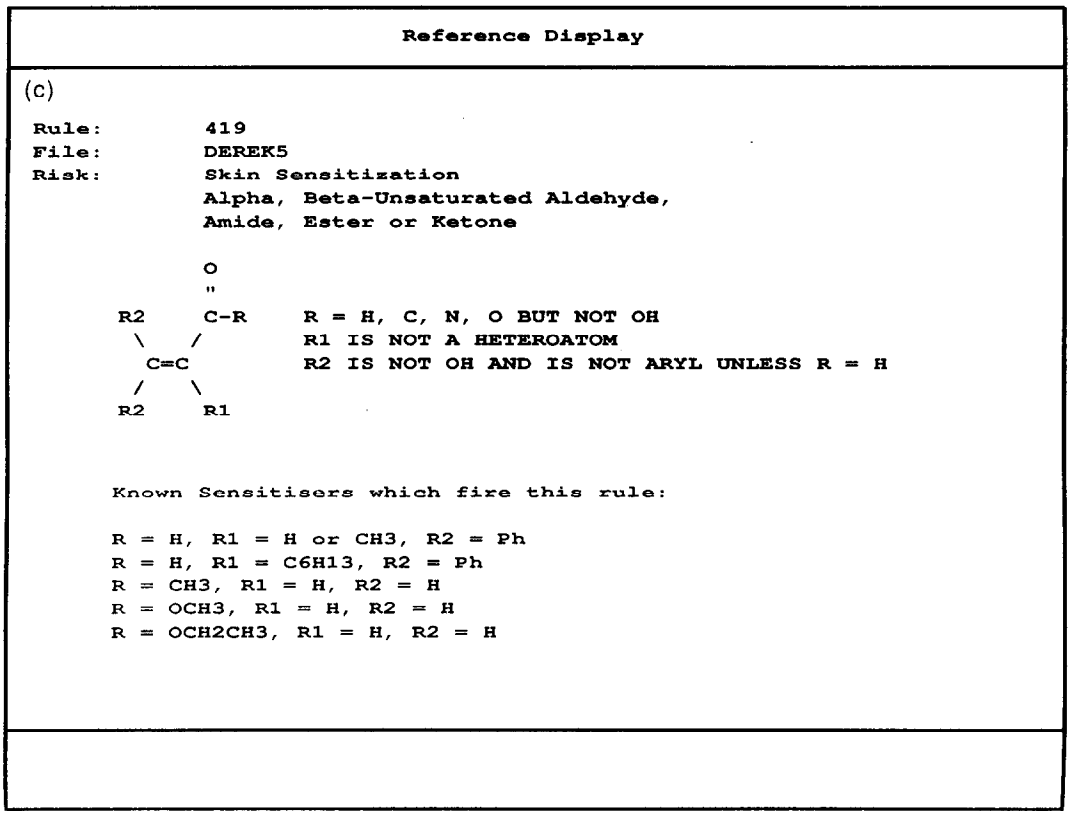


Fig. 1(c).

Fig. 1. Typical DEREK screens showing (a) a molecular structure (3-methylene-5,5-dimethyl-dihydro-2(3*H*)-furanone) prior to processing for structural alerts, (b) the structure with the structural alert identified and (c) the reference screen containing information about the rule which has been fired.

would be classified as skin sensitizers according to EC criteria (EEC, 1983).

For the purposes of identifying structural alerts, the chemicals were divided into groups either on the basis of reaction mechanisms or by empirical derivation: acylating agents; alkylating/arylating agents; "Michael" electrophiles and precursors; aldehydes and precursors; free radical generators; thiol-exchange agents; others (empirical).

40 structural alerts were then identified from these groups of compounds and programmed into the DEREK system.

Results

The 40 structural alerts for skin sensitization are shown in Table 1. Typical screens from the DEREK system showing (a) a chemical structure before processing for structural alerts, (b) the structure with the structural alert identified and (c) the reference screen containing information about the rule that has been triggered, are shown in Fig. 1.

Of the 294 molecules in the database, 135 had sensitization scores such that they would be classified as sensitizers according to Annex V of the EC Dangerous Substances Directive (i.e. strong and moderate, $\geqslant$30% positive) and 159 would be classified as non-sensitizers (made up of 120 negative and 39 weak).

Of the 135 sensitizers, DEREK identified 132 as containing a structural alert likely to lead to skin sensitization. Of the 120 negative chemicals DEREK found no structural alert for 98 molecules, but identified 22 as false positives. Of the 39 weak sensitizers (chemicals showing some response but not classifying as sensitizers) DEREK found structural alerts for 16.

The three sensitizers not identified by DEREK were abietic acid, cinnamic alcohol and anethole. The sensitization potential of abietic acid arises from sensitizing impurities (Karlberg, 1991) and cinnamic alcohol requires skin metabolism (Basketter, 1992). Anethole is believed to react by way of a phenolic radical mechanism (Barratt and Basketter, 1992). Refinements to the rulebase are in progress to accommodate these exceptions.

Discussion

While details of the biology of skin sensitization are only imperfectly understood, it is now widely accepted that the ability to react with a nucleophile either directly or after appropriate metabolism is a prerequisite for the large majority of skin sensitizers.

However, it should not be overlooked that skin permeability is another key factor in determining whether or not a chemical is in practice a sensitizer. A chemical that does not penetrate skin cannot cause allergic contact dermatitis. The octanol–water partition coefficient (P) of a molecule has been shown to be an important factor determining its skin permeability (Flynn, 1990), with higher logP values (i.e. greater lipophilicity) broadly leading to greater permeability. Consequently, quantitative structure–activity relationships of skin sensitization have been based on a measure of chemical reactivity and the inclusion of logP as independent variables (e.g. Basketter *et al.*, 1992a; Roberts and Basketter, 1990).

Therefore it seems reasonable that some molecules may contain a structural alert (i.e. be reactive, presumably by the same mechanism), but may not be skin sensitizers because their skin permeability is too low.

For example, 4-aminobenzoic acid (a zwitterionic compound) and sodium 4-benzoyloxybenzoate are weak or non-sensitizers, while 4-chloroaniline and phenyl benzoate are moderate or strong sensitizers. The former two molecules are both quite polar by virtue of possessing charged groups and will thus penetrate the skin much less readily than the latter two molecules. Several more of the 22 non-sensitizers identified by DEREK as containing structural alerts for sensitization appear to fall into this category. Conversely, where the skin penetration of molecules is similar, reactivity may be the dominant parameter (Roberts and Basketter, 1990).

Although it is still in a relatively early stage of development, the DEREK skin sensitization rulebase forms part of the first step in a strategic approach to the identification of contact allergens. Quantitative structure–activity relationships are also of value, but tend to apply only to homologous series of chemicals with a well defined single mode of action (e.g. Barratt *et al.*, 1994; Roberts and Basketter, 1990). At the current state of development, these data are not acceptable for classification of a substance as a skin sensitizer, but are valuable as a first screen and for the prioritization of testing of new chemicals.

In the absense of *in vitro* methods for skin sensitization, a local lymph node assay (LLNA) in the mouse (Kimber and Basketter, 1992) forms the second step in a strategic hierarchy. This assay uses fewer animals even than the 'half size' GPMT and is also less stressful to the animals, avoiding the use of Freund's complete adjuvant. Furthermore, it will provide dose–response data that may be of considerable value in relative potency estimation (Botham *et al.*, 1991). Whilst LLNA data are acceptable for classification of a substance as a skin sensitizer, non-classification would not be accepted. However, data from comparisons with other methods (Basketter and Scholes, 1992; Kimber *et al.*, 1990) and with OECD-recommended positive control substances provide powerful evidence for a change to this position (Basketter *et al.*, 1992b).

If the substance cannot be categorized as a skin sensitizer, the third step would be to conduct an OECD/Annex V acceptable assay such as the GPMT. This is likely also to be the only viable option if it is necessary to identify, and then conduct a risk assessment on, the very weakest allergens. If further evaluation is considered to be necessary, re-challenge studies on animals from the maximization test, further dose–response work, or human studies may be carried out (e.g. see Robinson *et al.*, 1989).

It is our intention to continue to develop the sensitization rulebase within DEREK in two ways: (a) to add additional rules (e.g. derived from sources such as classification data from the European List of New Chemical Substances), and (b) to incorporate an estimate of skin permeability in order to refine the identification of potential sensitizers. The potential of rulebases of this type for use in toxicological hazard screening can only increase and may ultimately lead to a reduction in the use of experimental animals.

Acknowledgements—The authors wish to thank Dr Martin P. Payne of the Occupational Medicine and Hygiene Laboratory, Health and Safety Executive, Sheffield for several helpful discussions in the generation of the rulebase.

REFERENCES

Barratt M. D. and Basketter D. A. (1992) Possible origin of the skin sensitization potential of isoeugenol and related compounds. *Contact Dermatitis* **27,** 98–104.

Barratt M. D., Basketter D. A. and Roberts D. W. (1994) Skin sensitization structure–activity relationships for phenyl benzoates. *Toxicology in Vitro* **8,** 823–826.

Basketter D. (1992) Skin sensitization to cinnamic alcohol: The role of skin metabolism. *Acta Dermatologica-venereologica* **72,** 264–265.

Basketter D. A., Roberts D. W., Cronin M. and Scholes E. W. (1992a) The value of the local lymph node assay in quantitative structure activity investigations. *Contact Dermatitis* **27,** 137–142.

Basketter D. A. and Scholes E. W. (1992) Comparison of the local lymph node assay with the guinea pig maximization test for the detection of a range of contact allergens. *Food and Chemical Toxicology* **30,** 65–69.

Basketter D. A., Scholes E. W., Cumberbatch M., Evans C. D. and Kimber I. (1992b) Sulphanilic acid: divergent results in the guinea pig maximization test and the local lymph node assay. *Contact Dermatitis* **27,** 209–213.

Botham P. A., Hilton J., Evans C. D., Lees D. and Hall T. J. (1991) Assessment of the relative skin sensitizing potency of 3 biocides using the murine local lymph node assay. *Contact Dermatitis* **25,** 172–177.

Dupuis G. and Benezra C. (1982) *Contact Dermatitis to Simple Chemicals: A Molecular Approach.* Marcel Dekker, New York.

EEC (1983) EEC Commission Directive of 29 July 1983 adapting to technical progress for the fifth time Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. (Annex V.) *Official Journal of the European Communities* **L257,** 1.

Flynn G. L. (1990) Physicochemical determinants of skin absorption. In *Principles of Route-to-Route Extrapolation for Risk Assessment.* Edited by T. R. Gerrity and C. J. Henry. pp. 93–127. Elsevier, New York.

Hauser C. and Katz S. I. (1988) Activation and expansion of hapten and protein specific T helper cells from non-sensitized mice. *Proceedings of the National Academy of Sciences of the U.S.A.* **85,** 5625–5628.

Karlberg A.-T. (1991) Air oxidation increases the allergic potential of Tall Oil Rosin. Colophony contact allergens also identified in Tall Oil Rosin. *American Journal of Contact Dermatitis* **2,** 43–49.

Kimber I. and Basketter D. A. (1992) The murine local lymph node assay: a commentary on collaborative studies and new directions. *Food and Chemical Toxicology* **30,** 165–169.

Kimber I., Hilton J. and Botham P. A. (1990) Identification of contact allergens using the murine local lymph node assay: comparisons with Buehler occluded patch test in the guinea pig. *Journal of Applied Toxicology* **10,** 173–180.

Magnusson B. and Kligman A. M. (1970) *Allergic Contact Dermatitis in the Guinea-pig. Identification of Contact Allergens.* Charles A. Thomas, Springfield, IL.

Roberts D. W. and Basketter D. A. (1990) A quantitative structure activity/dose response relationship for contact allergic potential of alkyl group transfer agents. *Contact Dermatitis* **23,** 331–335.

Robinson M. K., Stotts J., Danneman P. J., Nusair T. L. and Bay P. H. S. (1989) A risk assessment process for allergic contact sensitization. *Food and Chemical Toxicology* **27,** 479–489.

Sanderson D. M. and Earnshaw C. G. (1991) Computer prediction of possible toxic action from chemical structure; the DEREK system. *Human and Experimental Toxicology* **10,** 261–273.