

Structure–activity relationships for skin sensitization: recent improvements to Derek for Windows

KATE LANGTON¹, GRACE Y. PATLEWICZ^{2*}, ANTHONY LONG¹, CAROL A. MARCHANT¹ AND DAVID A. BASKETTER²

¹Lhasa Limited, 22-23 Blenheim Terrace, Woodhouse Lane, Leeds, UK and ²Safety and Environmental Assurance Centre (SEAC), Unilever Colworth, Colworth House, Sharnbrook, Bedford, UK

Derek for Windows (DfW) is a knowledge-based expert system that predicts the toxicity of a chemical from its structure. Its predictions are based in part on alerts that describe structural features or toxicophores associated with toxicity. Recently, improvements have been made to skin sensitization alerts within the DfW knowledge base in collaboration with Unilever. These include modifications to the alerts describing the skin sensitization potential of aldehydes, 1,2-diketones, and isothiazolinones and consist of enhancements to the toxicophore definition, the mechanistic classification, and the extent of supporting evidence provided. The outcomes from this collaboration demonstrate the importance of updating and refining computer models for the prediction of skin sensitization as new information from experimental and theoretical studies becomes available.

Key words: aldehydes; allergic contact dermatitis; Derek for Windows; 1,2-diketones; expert systems; isothiazolinones; skin sensitization; structure–activity relationship; toxicity prediction.
© Blackwell Munksgaard, 2006.

Accepted for publication 20 July 2006

Introduction

Allergic contact dermatitis is a skin disease that affects around 1%–4% of the global population at considerable cost to society and industry (1, 2). At present, there are no validated *in vitro* tests available that allow the identification of a chemical's potential to cause this condition despite legislative and ethical pressure to reduce animal testing (3). Computer methods can, however, offer an alternative approach to the predictive identification of chemicals possessing the ability to cause skin sensitization (4).

Derek for Windows (DfW) is a knowledge-based expert system designed to predict the toxicity of a chemical from its structure. Its skin sensitization predictions are the result of a reasoning process involving consideration of the presence of alerts, an estimation of skin permeability, and the existence of relevant toxicity data for the chemical of interest (5). Each alert describes a structural feature or toxicophore associated with the occurrence of skin sensitization which has been derived

by human expert interpretation of evidence including toxicity data for known compounds within the same chemical class and an understanding of the toxicological mechanism. Many DfW skin sensitization alerts describe structural features that have the potential for electrophilic binding to skin proteins either directly or following metabolism. This reflects the generally accepted view that in order for a chemical to induce skin sensitization, it must form a stable association (usually covalent) with a skin protein to form a macromolecular immunogen which is then recognized by the immune system (1).

The skin sensitization knowledge base in DfW was initially developed in collaboration with Unilever in 1993 using its historical database of guinea-pig maximization test (GPMT) data for 294 chemicals and contained approximately 40 alerts (6). Since that time, the knowledge base has undergone extensive improvements as more data have become available and scientific knowledge has increased (7). The current DfW knowledge base (version 9.0.0) contains 70 alerts for skin sensitization and the closely related end-point of photoallergenicity (8).

Unilever has had a long-standing interest in skin sensitization and has been involved in a number

*Current address: European Chemicals Bureau, Institute for Health and Consumer Protection, Joint Research Centre, European Commission, Ispra, Italy.

of studies involving chemical classes such as aldehydes (9, 10), isothiazolinones (11), and 1,2-diketones (12, 13). These have often taken place through sponsorship of university studentships or participation in European Union Framework Programmes. The current collaboration focused on using the outcomes and knowledge derived from such studies to refine the existing skin sensitization alerts in DfW.

Materials and Methods

The relevant results from earlier Unilever collaborative skin sensitization projects were collated (9–13). Supplementary data for the chemical classes of interest were also sought from the general literature.

Initial skin sensitization predictions were obtained from DfW version 7.0.0 using the default processing constraints. Modifications to the DfW version 7.0.0 knowledge base were then made as appropriate using the editor that accompanies the program. Performance was subsequently assessed using DfW version 9.0.0. DfW skin sensitization predictions were considered positive if they were associated with a level of likelihood of 'plausible' or higher (5); all other predictions were considered negative.

All DfW analyses were run on standard desktop PCs running Microsoft Windows 2000 or XP. Chemical structures were drawn in ISIS/Draw 2.3 or 2.4.

Results and Discussion

Improvements to DfW version 7.0.0 skin sensitization alerts were realized in three main areas: toxicophore definition, mechanistic classification, and supporting evidence. Selected examples for each area of improvement are outlined below.

Toxicophore definition

In an earlier study investigating the structure–activity relationships of aldehydes, skin sensitization data for 17 aldehydes were collected. Based on a detailed consideration of mechanistic chem-

istry, each of the chemicals was classified in accordance with its reactivity and likely mechanism of skin sensitization (9). Four classes of aldehyde were identified: (1) aryl-substituted aliphatic aldehydes, (2) aryl aldehydes, (3) aryl aldehydes with special features, and (4) α,β -unsaturated aldehydes. It was rationalized that class 1 and class 4 aldehydes were skin sensitizers on account of their potential for Schiff base formation and Michael addition with skin proteins, respectively (Fig. 1), whereas class 3 aldehydes, in contrast, were likely to induce skin sensitization through the presence of structural features other than the aldehyde group. All class 2 aldehydes were non-sensitizers.

When these 17 aldehydes were processed through DfW version 7.0.0, the 7 chemicals classed as aryl-substituted aliphatic aldehydes and the 5 chemicals classed as α,β -unsaturated aldehydes were all correctly identified as skin sensitizers based on the proposed mechanisms described above. Of the remaining 5 chemicals, the 3 aryl aldehydes were also predicted to be skin sensitizers by DfW on the basis of their potential for Schiff base formation (an additional alert for phenolic precursors was also found in one case); but in practice these chemicals were found to be non-sensitizers. This absence of activity was attributed to the lower reactivity of aryl aldehydes brought about by resonance stabilization of the aldehyde group by the aromatic ring (9). Chemicals in the aryl aldehydes with special features class were also identified by DfW as potential skin sensitizers on the basis of their aldehyde group and the fact that they are metabolic precursors of catechols. The two chemicals concerned were only weakly or non-sensitizing.

A search of the published literature was initiated to investigate further the basis for these false positive DfW predictions and to determine whether aryl aldehydes in general are not associated with skin sensitization activity as the results of the analysis suggested. This search identified a positive GPMT result for benzaldehyde (14). In the absence of additional experimental details, however, it was not possible to provide an unequivocal explanation for the apparent conflict between this result and the negative response

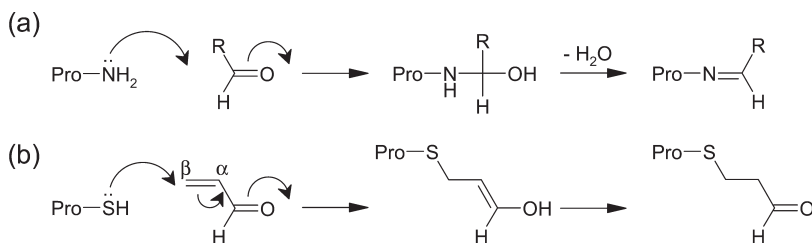


Fig. 1. Reaction of aldehydes with skin proteins via **a** Schiff base formation and **b** Michael addition. Simple aldehydes are harder electrophiles than α,β -unsaturated aldehydes so the 2 structure types are therefore shown reacting preferentially with hard and soft protein nucleophilic groups respectively. The Schiff base formation and Michael addition mechanisms are not exclusive and α,β -unsaturated aldehydes may therefore react to some extent by Schiff base formation also.

reported in the murine local lymph node assay (LLNA) (9). Two additional aryl aldehydes, atranol and chloroatranol, were also shown to elicit allergic contact dermatitis in appropriately sensitized patients, but the authors in these cases suggested activity may have resulted from an enzymic oxidation mechanism rather than the presence of the aryl aldehyde group (15, 16). Thus, these compounds can be considered as belonging to the class of aryl aldehydes with special features.

Because no significant evidence was found in the published literature supporting the skin sensitization activity of aryl aldehydes, the toxicophore definition for the DfW alert describing the skin sensitization of aldehydes was modified to exclude coverage for this compound class. As a consequence, aryl aldehydes identified by DfW version 7.0.0 as potential skin sensitizers on the basis of the presence of their aldehyde group are no longer predicted as such in DfW version 9.0.0. This modification in behaviour is illustrated in Fig. 2 for heliotropin, which is a non-sensitizer in the GPMT (9). Subsequent to this change to the DfW knowledge base, two additional aryl aldehydes (4-nitrobenzaldehyde and 4-chlorobenzaldehyde) were also reported as weakly or non-sensitizing in the LLNA, providing further support for the general exclusion of structures of this type (17).

The low skin sensitization potential of diaryl-substituted 1,2-diketones may also be attributed at least in part to resonance stabilization effects comparable to those observed in aryl aldehydes (17). Roberts et al. (12) derived a quantitative structure–activity relationship (QSAR) for 1,2-diketones relating their skin sensitization potency

in the LLNA to electronic and physicochemical (log P) properties. The results of this work form in part the basis of an alert in the DfW version 7.0.0 knowledge base describing the skin sensitization of 1,2-dicarbonyl compounds and their precursors. More recently, other 1,2-diketones investigated as part of a Unilever sponsored studentship suggested that diaryl-substituted structure types were generally weak or non-sensitizing (13, 17).

Table 1 summarizes the results of processing aryl-substituted 1,2-diketones from these studies through DfW both before (version 7.0.0) and after (version 9.0.0) excluding diaryl-substituted 1,2-diketones from the scope of the relevant alert. As a consequence of the improvement in toxicophore definition, 3 weakly or non-sensitizing chemicals in the data set are no longer identified as potential skin sensitizers in DfW version 9.0.0. The activity of the moderate skin sensitizer benzil is, however, no longer correctly predicted. Further work is required to gain insights into why this compound has an anomalously high skin sensitizing potency compared with the other diaryl-substituted 1,2-diketones examined.

Mechanistic classification

Patlewicz et al. (10) have since developed 2 distinct QSAR equations modelling the LLNA skin sensitization potency of aldehydes acting by either a Schiff base or a predominantly Michael addition mechanism (class 1 and class 4 aldehydes respectively). Although a QSAR equation may be used only to provide an indication, rather than proof, of mechanism, by comparing predicted potencies

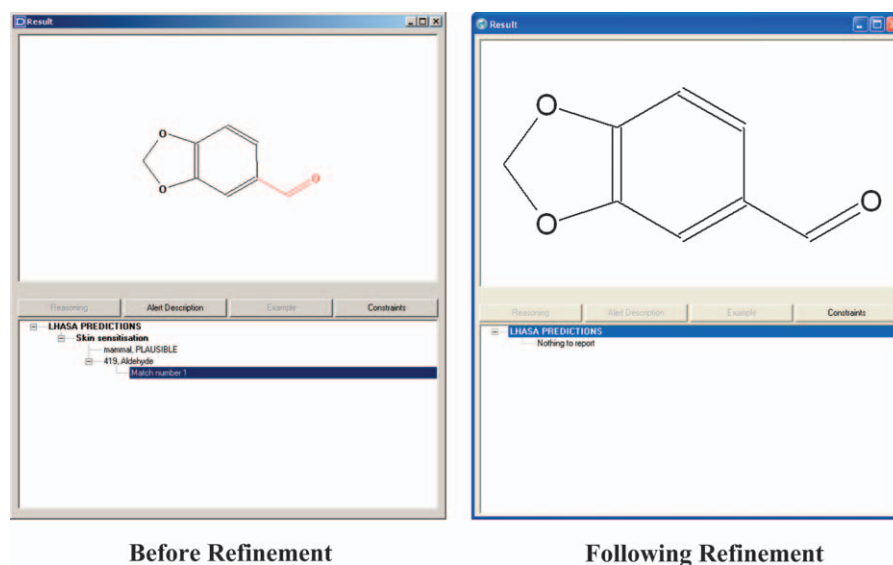
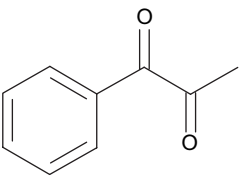
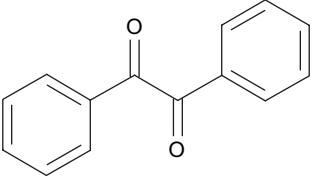
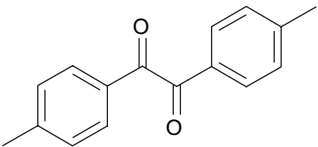
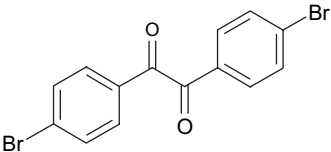
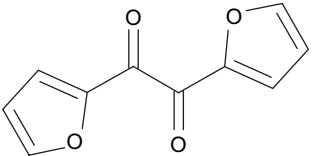


Fig. 2. DfW skin sensitization predictions for the non-sensitizer heliotropin (9). Before refinement of the alert describing the skin sensitization of aldehydes to exclude aryl aldehydes (DfW version 7.0.0), the chemical is incorrectly predicted as a plausible skin sensitizer in mammals. Following refinement (DfW version 9.0.0), skin sensitization activity is no longer predicted.

Table 1. LLNA EC3 values (12, 13, 17) and DfW version 7.0.0 and 9.0.0 skin sensitization predictions in mammals for aryl-substituted 1,2-diketones^a

Name	CAS Number	Structure	EC3 (%)	DfW 7.0.0	DfW 9.0.0
1-Phenylpropane-1,2-dione	579-07-7		0.85	Positive	Positive
Benzil	134-81-6		8.6	Positive	Negative
4,4'-Dimethylbenzil	3457-48-5		>50	Positive	Negative
4,4'-Dibromobenzil	35578-47-3		20.5	Positive	Negative
Furil	492-94-4		>25	Positive	Negative

^aThe generally low skin sensitization potency of diaryl-substituted 1,2-diketones may be attributed in part to resonance stabilization of the carbonyl groups by the aromatic rings and these compounds are no longer predicted as skin sensitizers in DfW version 9.0.0. The skin sensitization potency of benzil appears anomalously high in comparison with other diaryl-substituted 1,2-diketones. The basis for this observation remains unclear.

derived from these equations with actual experimental values, it was possible to propose likely mechanisms of skin sensitization for a number of aldehydes not included in the data set used to derive them.

Simple α,β -unsaturated aldehydes such as trans-2-hexenal were thought to act largely via a Michael addition mechanism, whereas β -disubstituted α,β -unsaturated aldehydes such as farnesal were considered to react preferentially via a Schiff base mechanism owing to steric hindrance of Michael addition (10). When these 2 chemicals were processed in DfW version 7.0.0, however, they were both predicted as skin sensitizers on the basis of the potential for Michael addition to the α,β -unsaturated aldehyde func-

tionality. In order to reflect the new mechanistic insights, the knowledge base coverage for β -disubstituted α,β -unsaturated aldehydes was moved from the DfW alert describing the Michael addition of α,β -unsaturated aldehydes to that describing the Schiff base potential of other aldehydes. As a consequence, farnesal is now identified as a skin sensitizer in DfW version 9.0.0 as a result of its potential for Schiff base formation between skin proteins and the aldehyde group (Fig. 3).

Supporting evidence

The supporting evidence for a DfW alert can be viewed in the alert description window when an alert is activated by a query chemical. This comprises a pictorial representation of the toxicophore

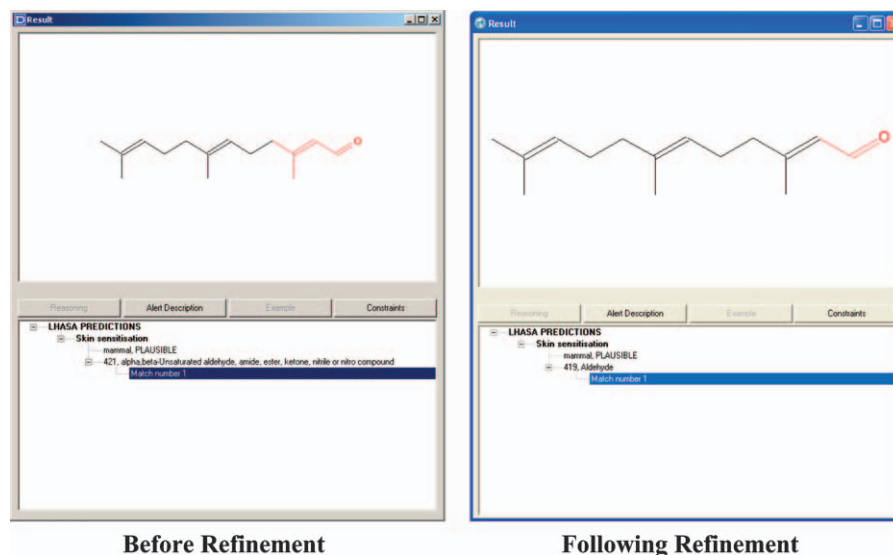


Fig. 3. DfW skin sensitization predictions for the skin sensitizer farnesal (10). Before refinement of the alerts describing the skin sensitization of aldehydes and α,β -unsaturated aldehydes to allow for the observation that β -disubstituted α,β -unsaturated aldehydes most probably react with skin proteins predominantly via Schiff base formation (DfW version 7.0.0), farnesal is predicted to be a plausible skin sensitizer in mammals on the basis of Michael addition to the α,β -unsaturated aldehyde group. Following refinement (DfW version 9.0.0), skin sensitization activity is associated with Schiff base formation of the aldehyde group.

in addition to comments, references, and examples that summarize the information used to derive, or subsequently modify, an alert.

Figure 4 shows the updated alert description for the DfW alert describing the skin sensitization of isothiazolinones in DfW version 9.0.0, which is present in 2-methylisothiazol-3-one. In this case, improvements to the comments and references in DfW version 7.0.0 were made using mechanistic information derived during the course of a Uni-

lever sponsored studentship (11). These studies showed that chlorinated isothiazolinones appear to react differently with amino acid model nucleophiles in comparison with non-chlorinated isothiazolinones and that this may account for the greater skin sensitization potency of the former. A search of the published literature furthermore identified a review, published since the creation of the original alert, summarizing available animal and human data relating to the skin sensitization

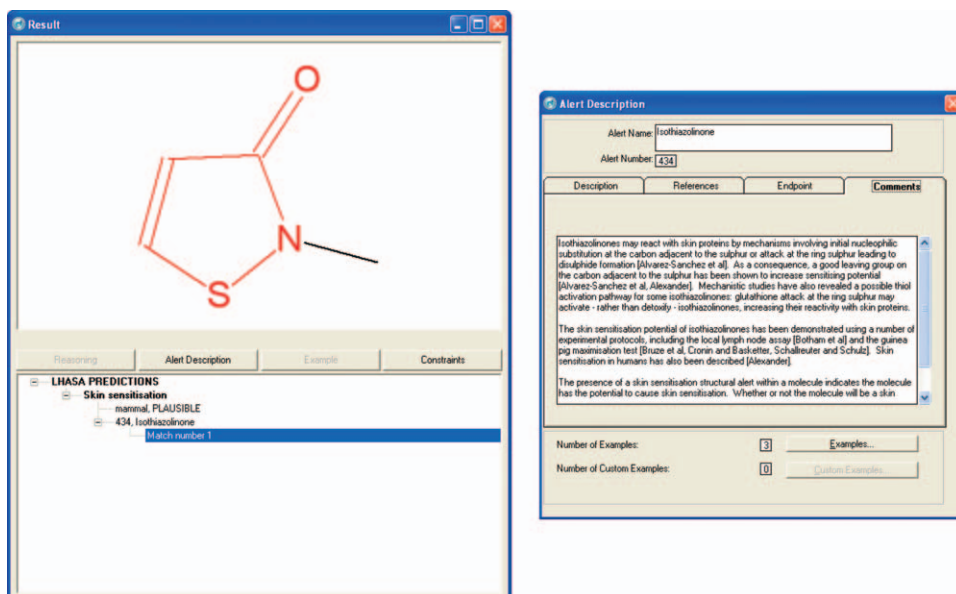


Fig. 4. The skin sensitizer 2-methylisothiazol-3-one (11) is predicted as a plausible skin sensitizer in mammals in DfW version 9.0.0. Supporting evidence for the basis of this prediction, including a summary of available toxicological and mechanistic data, is presented in the alert description window.

of isothiazolinones, the details of which were also added to the alert description (18).

Conclusions

Computer prediction models provide an important alternative approach for the assessment of skin sensitization potential. These models require continued development to take account of new experimental and theoretical study outcomes as they become available. In the current work, improvements to the DfW skin sensitization knowledge base have been achieved using results from recent Unilever collaborative skin sensitization projects and additional information derived from the published literature. The definitions of the toxicophores describing the skin sensitization of aldehydes and 1,2-diketones have been refined on the basis that aryl and diaryl-substituted structure types, respectively, are rarely found to exhibit significant activity. Furthermore, a clearer distinction has been made in terms of the potential for α,β -unsaturated aldehydes to act preferentially via a Schiff base rather than a Michael addition mechanism where steric factors disfavour the latter. Finally, more supporting evidence in the form of mechanistic and experimental data have been added to the alert describing the skin sensitization of isothiazolinones. In addition to better predictive performance, the benefits of these types of modifications include improved mechanistic insight and enhanced support for predictions. These are important considerations in relation to the understanding and regulatory acceptance of computer prediction models (19, 20).

Acknowledgements

The authors are grateful to Dr Stephen Gutsell for providing experimental results in advance of the completion of his PhD thesis.

References

1. Smith C K, Hotchkiss S A M. *Allergic Contact Dermatitis: Chemical and Metabolic Mechanisms*. London, Taylor and Francis, 2001.
2. Rietschel R L. Human and economic impact of allergic contact dermatitis and the role of patch testing. *J Am Acad Dermatol* 1995; 33: 812–815.
3. Kimber I, Cumberbatch M, Betts C J, Dearman R J. Dendritic cells and skin sensitisation hazard assessment. *Toxicol in Vitro* 2004; 18: 195–202.
4. Karol M H, Graham C, Barratt M D. Structure–activity relationship studies of hypersensitivity. *Comments Toxicol* 1999; 7: 59–77.
5. Judson P N, Marchant C A, Vessey J D. Using argumentation for absolute reasoning about the potential toxicity of chemicals. *J Chem Inf Comput Sci* 2003; 43: 1364–1370.
6. Barratt M D, Basketter D A, Chamberlain M, Admans G D, Langowski J J. An expert system rulebase for identifying contact allergens. *Toxicol in Vitro* 1994; 8: 1053–1060.

7. Barratt M D, Langowski J J. Validation and subsequent development of the Derek skin sensitization rulebase by analysis of the BgVV list of contact allergens. *J Chem Inf Comput Sci* 1999; 39: 294–298.
8. Barratt M D, Castell J V, Miranda M A, Langowski J J. Development of an expert system rulebase for the prospective identification of photoallergens. *J Photochem Photobiol B* 2000; 58: 54–61.
9. Patlewicz G, Basketter D A, Smith C K, Hotchkiss S A M, Roberts D W. Skin-sensitization structure–activity relationships for aldehydes. *Contact Dermatitis* 2001; 44: 331–336.
10. Patlewicz G Y, Wright Z M, Basketter D A, Pease C K, Lepoittevin J P, Giménez Arnau E. Structure–activity relationships for selected fragrance allergens. *Contact Dermatitis* 2002; 47: 219–226.
11. Álvarez-Sánchez R, Basketter D, Pease C, Lepoittevin J P. Studies of chemical selectivity of hapten, reactivity, and skin sensitization potency. 3. Synthesis and studies on the reactivity toward model nucleophiles of the ^{13}C -labeled skin sensitizers, 5-chloro-2-methylisothiazol-3-one (MCI) and 2-methylisothiazol-3-one (MI). *Chem Res Toxicol* 2003; 16: 627–636.
12. Roberts D W, York M, Basketter D A. Structure–activity in the murine local lymph node assay for skin sensitization: α , β -diketones. *Contact Dermatitis* 1999; 41: 14–17.
13. Gutsell S. Structure–activity relationships for the allergenic potential of diketones. PhD Thesis, University of Wales, Swansea; 2004.
14. Enslein K, Gombor V K, Blake B W, Maibach H I, Hostynek J J, Sigman C C, Bagheri D. A quantitative structure–toxicity relationships model for the dermal sensitization guinea pig maximization assay. *Food Chem Toxicol* 1997; 35: 1091–1098.
15. Bernard G, Giménez-Arnau E, Rastogi S C et al. Contact allergy to oak moss: search for sensitizing molecules using combined bioassay-guided chemical fractionation, GC-MS, and structure–activity relationship analysis. *Arch Dermatol Res* 2003; 295: 229–235.
16. Johansen J D, Andersen K E, Svedman C et al. Chloroatranol, an extremely potent allergen hidden in perfumes: a dose-response elicitation study. *Contact Dermatitis* 2003; 49: 180–184.
17. Patlewicz G Y, Basketter D A, Smith Pease C K et al. Further evaluation of quantitative structure–activity relationship models for the prediction of the skin sensitization potency of selected fragrance allergens. *Contact Dermatitis* 2004; 50: 91–97.
18. Alexander B R. An assessment of the comparative sensitization potential of some common isothiazolinones. *Contact Dermatitis* 2002; 46: 191–196.
19. Report from the expert group on (quantitative) structure–activity relationships [(Q)SARs] on the principles for the validation of (Q)SARs. OECD Series on Testing and Assessment Number 49. Paris, 2004.
20. Worth A P, Bassan A, Gallegos A et al. The characterisation of (quantitative) structure–activity relationships: preliminary guidance, European Chemicals Bureau EUR 21866 EN. Ispra, 2005.

Address:

Dr K Langton
Lhasa Limited
22-23 Blenheim Terrace
Woodhouse Lane
Leeds
LS2 9HD
UK
Tel: 44 (0)113 394 6042
Fax: 44 (0)113 394 6099
e-mail: kate.langton@lhasalimited.org