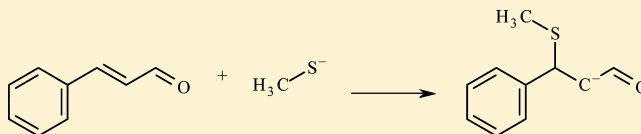


Predicting Skin Sensitization Potency for Michael Acceptors in the LLNA Using Quantum Mechanics Calculations

S. J. Enoch* and D. W. Roberts

School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, England L3 3AF

ABSTRACT: This study outlines the development of a series of quantitative mechanistic models enabling skin sensitization potency in the LLNA to be predicted for direct acting Michael acceptors. These models utilized several computational descriptors based on knowledge of the Michael addition reaction mechanism. The key descriptor was calculated using density functional theory and modeled the stability of the reaction intermediate. A second descriptor relating to the available surface area at the site of the reaction was also found to be important. Several poorly predicted compounds were identified, and in all cases, these could be rationalized mechanistically. The analysis of these compounds allowed a well-defined mechanistically driven applicability domain to be developed. The study showed that *in silico* quantitative mechanistic models, with a well-defined applicability domain, can be used to predict skin sensitization potency in the LLNA. The approach presented has the potential to be of use as part of a weight of evidence approach for predicting skin sensitization without the use of animals in risk assessment.



■ INTRODUCTION

Skin sensitization leading to allergic contact dermatitis (ACD) is the most common form of immunotoxicity that occurs when an individual is exposed to a sensitizing chemical. The resulting immune response causes a range of skin conditions from mild reddening through to painful blisters and wheals. Crucially, once an individual has been sensitized to a chemical he or she will remain sensitized for the remainder of his or her life. Given that humans are regularly exposed to a wide range of chemicals, it is important that skin sensitization is assessed thoroughly. The local lymph node assay (LLNA) is the favored animal test by which skin sensitization is assessed within the European Union. The LLNA offers a number of advantages in terms of animal welfare and the ability to assess skin sensitization potency. However, in order to meet the demands of the REACH legislation and the seventh amendment to the cosmetics directive, nonanimal alternatives to the LLNA are required.^{1,2} For example, at the time of writing 8,000 chemicals have been registered with ECHA due to REACH.³ Using the LLNA to assess all of these chemicals would use in excess of 100,000 animals and cost more than €50 million (assuming OECD guideline 429 was followed). This is clearly costly, time-consuming, and unethical in terms of animal usage. Thus, there has been much research into the development of computational methods for the assessment of skin sensitization.

Research has shown that the key step in the biochemical pathway that leads to skin sensitization is the formation of a covalent bond between a skin protein and a chemical.^{4–8} This has led to the definition of structural alerts that outline the mechanistic domains associated with covalent bond formation (acylation, Michael addition, Schiff base formation, S_NAr , S_N1 , and S_N2).^{9–12} These structural alerts have been used to directly predict skin sensitization potential (binary yes/no) and to form chemical categories suitable for data gap filling.^{13,14} In the case of the latter, and within a mechanistic domain, research has

shown the rate of covalent bond formation is a key factor in predicting skin sensitization potency.^{15–20} A prediction of skin sensitization potency is essential if computational methods are going to play a role in replacing the LLNA in risk assessment. The published methods rely on either experimentally determined rate constants or the use of Taft parameters to model the rate constant (Taft parameters are numerical values, determined experimentally, that can be used to model electronic effects).^{16–20} These models have been termed quantitative mechanistic models or QMMs as they rely on the use of mechanistically interpretable descriptors.^{8,21} These are typically hydrophobicity and either the experimentally determined rate constant or a descriptor(s) designed to predict it. In addition, many common functional groups relevant to the prediction of the LLNA do not have Taft parameters. For example, there are no parameters for ortho-substituents in aromatic ring systems. However, this research does demonstrate that, given the availability of suitable descriptors to model the rate of covalent bond formation, skin sensitization potency in the LLNA can be predicted using the QMM approach.

Therefore, the aim of this study was to identify computational descriptors related to the rate of covalent bond formation and thus develop a series of QMMs capable of predicting skin sensitization potency in the LLNA. This was undertaken using quantum chemical calculations to model the key mechanistic step in the Michael addition reaction.

■ COMPUTATIONAL METHODS

Data Set. Skin sensitization data for 33 chemicals previously reported as acting via Michael addition were extracted from the literature.^{22,23} Only chemicals containing a single site of nucleophilic attack were included in the data set. This excluded chemicals such as

Received: February 14, 2013

Published: April 9, 2013



Table 1. Summary of the Calculated Reactivity Parameters, Predicted Volatilities, and Experimentally Determined LLNA Data for 33 Chemicals Acting via Michael Addition^a

ID	Name	Structure	EC3 (%wt)	pEC3 (mol/l)	E _{ACT} (kcal/mol)	SAS (Å ²)	LogVP	Predicted pEC3			
								(1)	(2)	(3)	(4)
1	Ethyl acrylate		28.0	0.55	5.14	39.53	1.61	1.45	1.61	1.86	2.04
2	2-Hydroxyethyl acrylate		1.4	1.92	4.28	39.53	-0.85	1.47	1.66	1.91	2.08
3	<i>Trans</i> -2-decenal		2.5	1.79	4.90	12.91	-1.08	1.45	1.63	1.56	1.57
4	<i>Trans</i> -2-hexenal		5.5	1.25	6.24	12.91	0.71	1.43	1.55	1.49	1.49
5	1-(4-Methoxyphenyl)-1-penten-3-one		9.3	1.31	9.49	13.95	-2.73	1.37	1.36	1.33	1.33
6	Diethyl maleate		5.8	1.47	5.15	13.94	-0.72	1.45	1.61	1.56	1.57
7	α -Amyl cinnamic aldehyde		11.0	1.26	10.58	13.95	-3.47	1.35	1.30	1.27	1.27
8	α -Hexyl cinnamic aldehyde		11.0	1.29	10.64	13.95	-3.45	1.34	1.29	1.27	1.27
9	α -Butyl cinnamic aldehyde		11.0	1.23	10.51	13.95	-2.55	1.35	1.30	1.28	1.28
10	Benzylidene acetone		3.7	1.60	6.96	13.95	-2.00	1.41	1.51	1.46	1.47
11	α -Methyl cinnamic aldehyde		4.5	1.51	9.91	13.95	-1.59	1.36	1.33	1.31	1.31
12	α -Phenyl cinnamic aldehyde		2.5	1.92	0.44	13.95	-4.22	1.54	1.89	1.81	1.83
13	Cinnamic aldehyde		3.0	1.64	6.04	13.95	-1.46	1.43	1.56	1.51	1.52
14	Vinyl pyridine		1.6	1.82	12.01	38.73	0.50	1.32	1.21	1.49	1.64
15	Benzyl-cinnamate		18.4	1.11	11.93	13.51	-4.34	1.32	1.22	1.19	1.19
16	α -Damascone		3.3	1.76	3.77	15.36	-1.38	1.48	1.69	1.65	1.67
17	Methyl-nonynoate		2.5	1.83	5.36	24.36	-1.07	1.44	1.60	1.67	1.75
18	2,4-Heptadienal		4.0	1.44	6.52	14.49	-0.29	1.42	1.53	1.49	1.51
19	β -Phenyl-cinnamic-aldehyde		0.6	2.54	14.47	1.90	-4.22	1.27	1.07	0.92	0.84

Table 1. continued

ID	Name	Structure	EC3 (%wt)	pEC3 (mol/l)	E_{ACT} (kcal/mol)	SAS (Å ²)	LogVP	Predicted pEC3			
								(1)	(2)	(3)	(4)
20	Methyl-methacrylate		90.0	0.05	10.13	36.68	1.59	1.35	1.32	1.56	1.71
21	Methyl-acrylate		20.0	0.63	5.14	39.53	1.95	1.45	1.61	1.86	2.04
22	5-Methyl-2-phenyl-2-hexenal		4.4	1.63	4.25	8.62	-2.31	1.47	1.66	1.55	1.52
23	5,5-Dimethyl-3-methylene-dihydro-2(3H)-furanone		1.8	1.85	6.05	37.59	-0.76	1.43	1.56	1.79	1.95
24	r-Perillaldehyde		8.1	1.27	11.71	10.37	-1.32	1.32	1.23	1.17	1.14
25	Safranal		7.5	1.30	9.93	16.23	-0.77	1.36	1.33	1.33	1.35
26	Trans-2-methyl-2-butenal		NS	NS	6.32	16.02	1.28	1.43	1.54	1.52	1.54
27	α-Iso-methyl-ionone		21.8	0.98	9.76	4.85	-1.57	1.36	1.34	1.21	1.15
28	2-Ethyl-hexyl-acrylate		10.0	1.27	5.98	39.53	-0.71	1.43	1.56	1.82	1.99
29	r-Carvone		12.9	1.07	12.56	10.26	-0.86	1.31	1.18	1.12	1.10
30	Crotonyl thioglycerol		NS	NS	2.65	15.62	-5.33	1.50	1.76	1.71	1.74
31	Geranyl nitrile		NS	NS	Diss	3.31	-1.43	NC	NC	NC	NC
32	2,4-Hexadienal		3.5	1.44	6.04	14.49	0.15	1.43	1.56	1.52	1.53
33	Linalool aldehyde		9.5	1.25	6.34	9.99	-2.51	1.43	1.54	1.45	1.43

^aSAS = solvent accessible surface area, logVP = logarithm of the calculated vapour pressure, NS = nonsensitizer, Diss = dissociates, NC = not calculated.

pomaroze and benzoquinone. All skin sensitization data were converted to molar units prior to modeling.

Computational Chemistry Calculations. All calculations were carried out using the Gaussian 09 suite of software using density functional theory (DFT).²⁴ All structures were optimized using the hybrid B3LYP functional coupled with a triple- ζ basis set (6-311+G(d,p)). All structures were either drawn or converted from SMILES strings, using Avogadro V1.1.0 (freely available from <http://avogadro.openmolecules.net>). The solvent accessible surface area (SAS) available at the site of Michael addition was calculated using the Chimera software (freely available from <http://www.cgl.ucsf.edu/chimera/>). All vapor pressure calculations were carried out using the Antoine method in the MPBPVP module of EPI Suite (V4.0) (freely

available from <http://www.epa.gov/opptintr/exposure/pubs/episuitd.htm>).

The activation energy (E_{ACT}) for each chemical investigated was calculated as follows: (1) Optimization of the parent chemical structure with the resulting energy value being the ground state energy (E_{GS}). (2) Optimization of the negatively charged intermediate structure obtained by adding a negatively charged methyl thiol moiety to the parent chemical structure. The resulting energy is the intermediate energy (E_{INT}). (3) Optimization of the negatively charged methyl thiol moiety (E_{THIOL}). (4) $E_{ACT} = E_{INT} - (E_{GS} + E_{THIOL})$.

Statistical Analysis. All QMMs, statistics, and plots were developed using regression analysis in Minitab V16.

RESULTS AND DISCUSSION

The aim of this study was to utilize quantum mechanics calculations to predict skin sensitization potency in the LLNA for 33 chemicals previously assigned as being direct acting Michael acceptors.^{8,23} This involved calculating two descriptors based on the mechanism of the Michael addition reaction. The first of these descriptors models the rate of covalent bond formation by assessing the stability of the negatively charged intermediate in the Michael addition reaction using DFT calculations. The second descriptor assesses the accessible surface area at the site of nucleophilic attack in the ground state structures. The effect of volatility on the relationship between skin sensitization potency and the calculated chemical reactivity parameters was also investigated. All calculated data are summarized in Table 1.

Quantum Chemical Modeling of the Michael Addition Reaction. Density functional calculations were used to investigate the relative stability of the negatively charged species that has been proposed as the key intermediate on the potential energy surface of the Michael addition reaction (Figure 1).^{11,15} The stability of this species is related to the

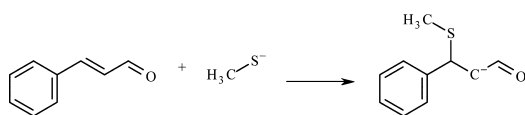


Figure 1. Proposed Michael addition mechanism leading to a negatively charged intermediate on the potential energy surface.

activation energy and thus the rate of the Michael addition reaction. Interestingly, this mechanism involving a negatively charged intermediate is in contrast to that suggested by previous quantum chemical calculations.²⁵ These calculations outlined a mechanism for Michael addition that proceeded via a four coordinate transition state in which the neutral methane thiol moiety added directly across the double bond. However, the presence, and relative stability of, the negatively charged intermediate located on the potential energy surface during the current study suggests this concerted mechanism to be less likely. The calculated energy of activation values for each of the chemicals in the data set are tabulated in Table 1 under the heading E_{ACT} .

Quantitative Mechanistic Modeling of the Michael Addition Reaction. The ability of the calculated energy of activation (E_{ACT}) to predict skin sensitization potency (pEC3) was investigated using the quantitative mechanistic modeling (QMM) approach. An initial QMM considering all of the data together showed a poor correlation between skin sensitization potency (pEC3) and the calculated energy of activation (E_{ACT}). The QMM is shown in eq 1, with the correlation between experimental and predicted pEC3 shown in Figure 2 (entitled correlation 1).

$$\text{pEC3} = 1.55 - 0.02E_{ACT} \quad (1)$$

$n = 30$, $r^2 = 0.02$, $r^2_{\text{adj}} = 0.00$, and $s = 0.47$.

Inspection of correlation 1 in Figure 2 shows methyl acrylate, ethyl acrylate, and methyl methacrylate (shown as lower filled squares) are predicted to be more potent skin sensitizers and are thus outliers to the general trend in the remaining data. Previous research has shown that these short chain acrylates and methacrylates are less potent in the LLNA than predicted from experimentally derived rate constants.¹⁷ This research

suggested that ethyl acrylate and ethylene glycol dimethacrylate were susceptible to oxygen initiated free radical polymerization resulting in the observed lower LLNA results. This hypothesis was supported by the fact that the LLNA value for 2-hydroxyethylacrylate was well predicted based on its rate constant. This was rationalized in terms of the increased solubility of this chemical due to the hydroxyl group that protects it from the oxygen initiated polymerization. It is also likely that the high volatility of these short chain compounds contributes to their reduced skin sensitizing ability. This can be seen in the significant reduction in the logarithm of the calculated vapor pressure of 2-hydroxyethylacrylate compared to methyl acrylate, ethyl acrylate, and methyl methacrylate (-0.85 vs 1.95 vs 1.61 vs 1.59 , respectively). In addition, β -phenyl cinnamic aldehyde is also a significantly more potent skin sensitizer than predicted from eq 1 (shown as the upper filled square in the plot entitled eq 1 in Figure 2). The low potency prediction of eq 1 can be easily rationalized in terms of the severe steric hindrance at the β -carbon (the available accessible surface area is reduced to $1.90 \text{ \AA}^2$ compared to that of cinnamic aldehyde with a value of $13.95 \text{ \AA}^2$). In addition, the geometry of the Michael addition intermediate involves the two benzene rings on the β -carbon coming into close contact with each other. This is due to the bond angle around the β -carbon going from around 120° to around 109° . The higher potency of this compound may be due to its reacting not by Michael addition but via Schiff base formation at the carbonyl group. It is possible to predict the skin sensitization potency in the LLNA for β -phenyl cinnamic aldehyde via a Schiff base mechanism, using a previously published QMM.^{26,27} This QMM leads to a predicted pEC3 value of 2.36, which is in good agreement with the experimental value of 2.54 (pEC3 calculated using the equation $\text{pEC3} = 1.12\Sigma\sigma^* + 0.42 \log P - 0.62$; where $\Sigma\sigma^* = 1.29$, $\log P = 3.65$). This analysis illustrates that separate QMMs can be used to predict the skin sensitization potency of chemicals with more than a single mechanism. The prediction from the QMM resulting in the higher skin sensitization potency value is the one of concern, and thus, the chemical should be assigned to this mechanistic domain. In the case of β -phenyl cinnamic aldehyde, this analysis suggests that Schiff base formation is the primary mechanism by which skin sensitization occurs. Removal of methyl acrylate, ethyl acrylate, methyl methacrylate, and β -phenyl cinnamic aldehyde, for the mechanistic reasons discussed above, results in an improved QMM (eq 2 and correlation 2 in Figure 2).

$$\text{pEC3} = 1.91 - 0.06E_{ACT} \quad (2)$$

$n = 26$, $r^2 = 0.43$, $r^2_{\text{adj}} = 0.40$, $s = 0.22$.

The correlation between the experimental and predicted pEC3 values shows vinyl pyridine to be a significant outlier to the remaining data (correlation 2 in Figure 2). This chemical has a relatively large calculated energy of activation that results in it being poorly modeled by the QMM shown in eq 2. However, the β -carbon atom of vinyl pyridine is virtually free from steric hindrance because it is substituted by two hydrogen atoms. The effect of bulky substituents at the reaction site upon the rate of a chemical reaction can be understood from the Arrhenius equation ($k = Ae^{-E_{ACT}/(RT)}$). In this equation, the pre-exponential factor, A , represents the frequency of collisions that could lead to a reaction, whereas the exponential term, $E_{ACT}/(RT)$, represents the fraction of colliding molecules that have sufficient energy for a reaction to occur. This means an increase in steric hindrance on going from the reactants to the transition

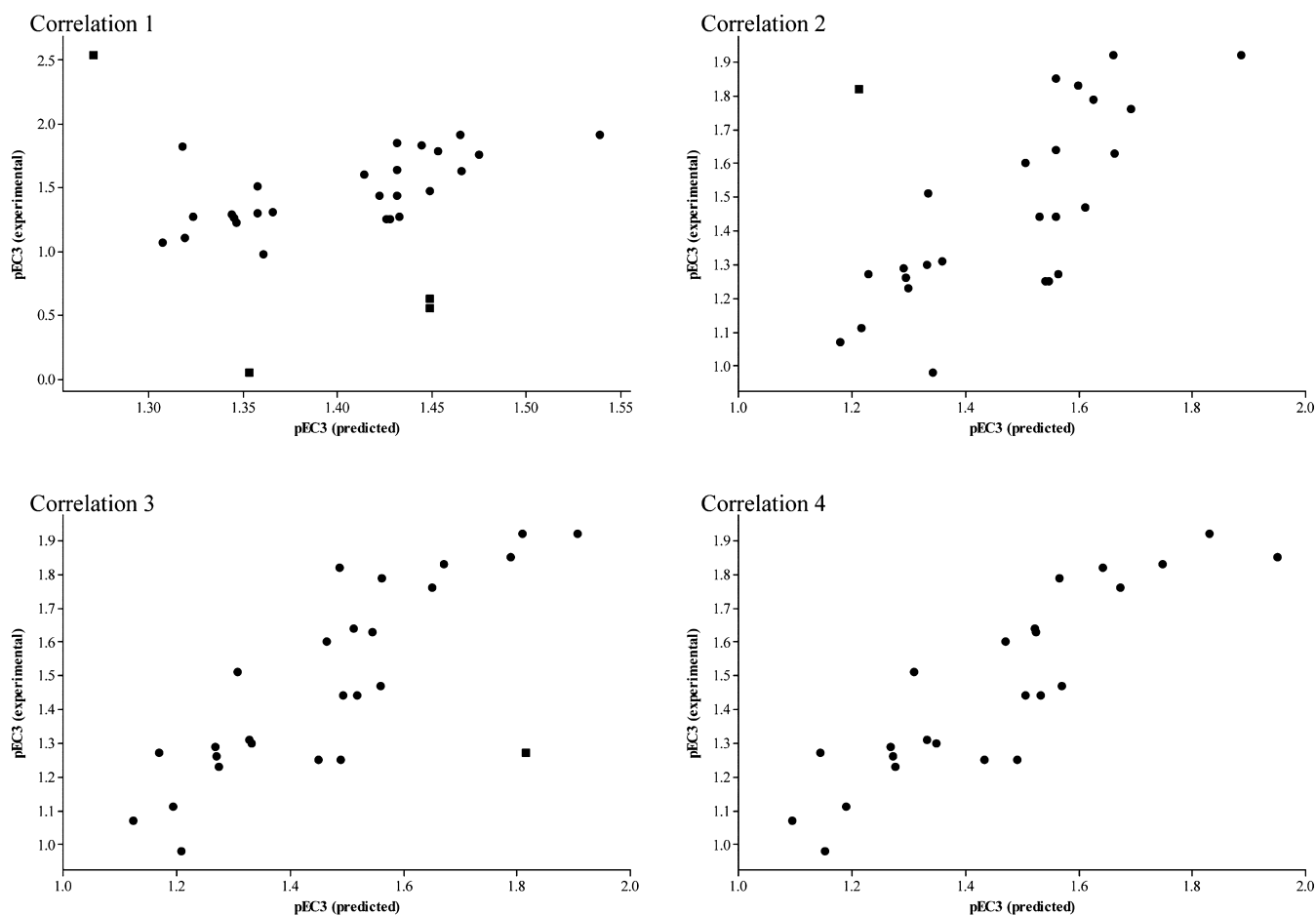


Figure 2. Correlation between experimental (y-axis) and predicted (x-axis) pEC3 values for the QMMs developed in the current study (filled squares are as follows: correlation 1, methyl acrylate, ethyl acrylate, methyl methacrylate, and β -phenyl cinnamic aldehyde; correlation 2, vinyl pyridine; correlation 3, 2-ethylhexyl acrylate).

state will increase E_{ACT} . The value of A can also be decreased by the effect of steric hindrance between the reactants that limit the range of collision approaches and rotational conformers that can lead to a reaction. The consequence of these two steric factors combined ultimately lead to a slower reaction rate, k . Vinyl pyridine has a virtual absence of steric hindrance at the β -carbon which, as discussed, results in an increased pre-exponential term compared to the other Michael acceptors (correlation 2, Figure 2). This effect is not accounted for by the calculated energy of activation, which models only the E_{ACT} term in the Arrhenius equation. Previous studies have shown that the effect of steric bulk at a reaction site can be modeled using the calculated solvent accessible surface area.²⁵ Including this calculated parameter resulted in an improved QMM in which vinyl pyridine was no longer poorly predicted (eq 3 and correlation 3 in Figure 2). This suggests that, in part at least, the calculated solvent accessible surface area term is modeling the pre-exponential term in the Arrhenius equation. Interestingly, this QMM has a single outlier, the longer chain acrylate 2-ethylhexyl acrylate that is predicted to be a more potent skin sensitizer than is observed experimentally (pEC3 1.82 vs 1.27, shown as a filled square in correlation 3, Figure 2). Inspection of the logarithm of the calculated vapor pressure value for 2-ethylhexyl acrylate shows it to be significantly less volatile than methyl acrylate, ethyl acrylate, and methyl methacrylate (-0.71 vs 1.95 vs 1.61 vs 1.59 , respectively). This suggests that for longer chain acrylates with low volatility, the reduction in skin

sensitizing potential (compared to what would be predicted from reactivity) can be attributed to the polymerization reaction alone. In terms of the QMM, the data show that the skin sensitization potential of acrylates and methacrylates is overpredicted and should be excluded from the model's applicability domain. Excluding these chemicals results in a significantly improved QMM for the remaining 25 Michael acceptors in the data set (eq 4 and correlation 4 in Figure 2).

$$\text{pEC3} = 1.67 - 0.05E_{ACT} + 0.01\text{SAS} \quad (3)$$

$$n = 26, r^2 = 0.60, r^2_{\text{adj}} = 0.56, \text{ and } s = 0.19.$$

$$\text{pEC3} = 1.60 - 0.06E_{ACT} + 0.02\text{SAS} \quad (4)$$

$$n = 25, r^2 = 0.79, r^2_{\text{adj}} = 0.78, \text{ and } s = 0.13.$$

Energy of Activation of Nonsensitizing Michael Acceptors. Crotonyl thioglycerol, geranyl nitrile, and *trans*-2-methyl-2-butenal are Michael acceptors that are reported as being nonsensitizers in the LLNA. The energy of activation and the solvent accessible surface area for these chemicals was also calculated (Table 1). These results showed both crotonyl thioglycerol and *trans*-2-methyl-2-butenal to be potentially reactive with relatively low energies of activation (2.35 and 6.32 kcal/mol, respectively). In contrast, a stable intermediate could not be isolated for geranyl nitrile, which was taken as evidence of a lack of Michael addition reactivity. This is in keeping with the absence of skin sensitizing ability for this compound. The lack of skin sensitizing ability for crotonyl thioglycerol can be

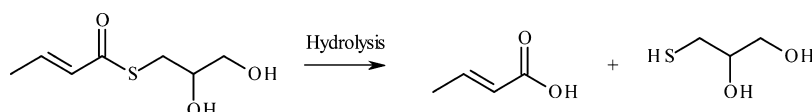


Figure 3. Potential hydrolysis of crotonyl thioglycerol into thioglycerol and crotonic acid.

potentially explained by it being rapidly hydrolyzed into thioglycerol and crotonic acid reducing its skin sensitization potential (Figure 3). This type of hydrolysis has been reported to occur with similar compounds via the action of the monoacylglycerol lipase enzyme, an enzyme that is widely expressed in lipid metabolizing tissue such as the skin.^{28–30} The discrepancy between the calculated energy of activation and absence of skin sensitization for *trans*-2-methyl-2-butenal can potentially be explained by the high volatility of such low weight compounds. Inspection of the logarithm of the calculated vapor pressure for *trans*-2-methyl-2-butenal shows it to be significantly higher than the chemicals that are well modeled, with a value closer to those for the methyl acrylate, ethyl acrylate, and methyl methacrylate (1.28 vs 1.95 vs 1.61 vs 1.59, respectively). As discussed, the skin sensitizing ability of these chemicals is lower than expected due to a combination of volatility and free radical polymerization. The data for these short chain chemicals suggest that volatile compounds are likely to be weaker sensitizers in the LLNA than would be expected based purely on their reactivity. These data suggest that chemicals with a logarithm of the calculated vapor pressure greater than 1 log unit should be considered as outside the applicability domain of reactivity based models for the prediction of skin sensitization potency. This physicochemical boundary applies to both *in silico* models as developed in the current study and to *in chemico* models such as those previously published.¹⁷

Applicability Domain of the Quantitative Mechanistic Model. There have been several publications in the literature relating to the nonanimal prediction of skin sensitization for direct acting electrophiles within the Michael addition mechanistic domain.^{13,15,17} Upon first inspection, it seems as if these publications contradict each other, as it has been previously suggested that subcategorization into smaller groupings is required for predictions of skin sensitization to be made using reactivity parameters.¹³ In contrast, other research makes no mention of the need for subcategorization within the Michael addition domain for direct acting electrophiles.^{15,17} These differences highlight an important aspect in the use of alternative methods, the applicability domain. It is important to realize that differing methods (*in chemico*, *in vitro*, and *in silico*) will have different applicability domains. The key aspect to using an alternative method (or combination of methods in a weight of evidence approach) is to ensure the chemical for which a prediction is being made falls within the applicability domain of the method being used. Thus, it is important that the applicability domain of the QMM detailed by eq 4 is clearly outlined in order for it to be of use in predictive toxicology. The domain can be defined in terms of the chemical space that has been explored during the development of the QMM described by eq 4. Therefore, chemicals that meet the following criteria can be considered as within the domain: direct acting Michael acceptors with a single reactive site; cyclic and acyclic alkenes and alkynes polarized by an aldehyde, ketone, ester, cyano, or aromatic ring moiety; in the case of polarized alkenes, the substituents at the α - and β -

carbon atoms can be any combination of hydrogen or carbon (sp^3 or sp^2 hybridized); in the case of polarized alkynes, the substituents at the β -carbon atom can be any combination of hydrogen or carbon (sp^3 or sp^2 hybridized); chemicals with a calculated vapor pressure less than or equal to 1 log unit (calculated using the Antoine method in the MPBPVP module of EPI Suite V4.0); and chemicals with a calculated SAS value greater than 2.00 Å² (calculated using the Chimera software).

Weight of Evidence Approach for the Prediction of Skin Sensitization. The data presented in this study outline how skin sensitization potency (as measured in the LLNA) can be predicted using computational methods within a well-defined applicability domain. However, it is important to realize such a method is unlikely to be used as a one-to-one replacement for the LLNA (or other *in vivo* assays) in regulatory toxicology. Instead, it is envisaged that it will be used as part of a weight of evidence scheme consisting of data from both experimental (*in chemico* and/or *in vitro*) and computational sources (predictions of potential and potency). Existing computational tools such as Derek Nexus and the OECD QSAR Toolbox enable skin sensitization potential to be predicted (either via the presence of a structural alert or category formation/read across). In contrast, the method outlined in the current study allows skin sensitization potency to be predicted for chemicals acting via Michael addition. Clearly, a computational prediction of skin sensitization potency supported by data from other *in silico*, *in chemico*, and *in vitro* studies is more likely to be accepted by a regulatory agency than a single piece of evidence (either computational or experimental).

CONCLUSIONS

This study has outlined how quantum chemical calculations can be used to predict skin sensitization potency in the LLNA for chemicals acting via Michael addition. The results showed that the stability of a negatively charged intermediate on the potential energy surface is the rate determining step in the reaction. The study also highlights how mechanistically driven modeling can lead to a quantitative mechanistic model with good predictability and a well-defined applicability domain. In terms of predicting the LLNA for direct acting Michael acceptors, the data showed that volatile chemicals and those able to readily polymerize are outside the applicability domain of the model. *In silico* quantitative mechanistic models have the potential to be of use in risk assessment as part of a weight of evidence approach for the nonanimal prediction of skin sensitization.

AUTHOR INFORMATION

Corresponding Author

*Tel: + 44 151 231 2164. Fax: + 44 151 231 2170. E-mail: s.j.enoch@lpmu.ac.uk.

Funding

The research leading to these results has received funding from the European Community's Seventh Framework Program

(FP7/2007-2013) COSMOS Project under grant agreement number 266835 and from Cosmetics Europe.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

ACD, allergic contact dermatitis; ECHA, European Chemicals Agency; E_{ACT} , energy of activation (calculated); E_{INT} , energy of the intermediate (calculated); E_{GS} , energy of the ground state (calculated); E_{THIO} , energy of thiol (calculated); DFT, density functional theory; LLNA, local lymph node assay; OECD, Organisation for Economic Cooperation and Development; QMM, quantitative mechanistic model; QSAR, quantitative structure–activity relationship; REACH, registration, evaluation, authorization and restriction of chemicals; SAS, solvent accessible surface area; SMILES, simplified molecular input line entry system; S_N1 , unimolecular aliphatic nucleophilic substitution; S_N2 , bimolecular aliphatic nucleophilic substitution; S_NAr , bimolecular aromatic nucleophilic substitution

REFERENCES

- (1) EC (2006) Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. *Off. J. Eur. Union*, L 396/1 of 30.12.2006.
- (2) EC (2003) Commission of the European Communities. 2003. Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. *Off. J. Eur. Union*, L 66/26–L 33/35 of 11.3.2003.
- (3) Data available at <http://echa.europa.eu/information-on-chemicals/registered-substances/identified-substances-for-registration-in-2013> (accessed Apr 4, 2013).
- (4) Schultz, T. W. (2010) Adverse Outcome Pathways: A Way of Linking Chemical Structure to *in Vivo* Toxicological Hazards, in *In Silico Toxicology: Principles and Applications* (Cronin, M. T. D., and Madden, J. C., Eds.), Royal Society of Chemistry, Cambridge, UK.
- (5) Aptula, A. O., Patlewicz, G., and Roberts, D. W. (2005) Skin sensitization: Reaction mechanistic applicability domains for structure-activity relationships. *Chem. Res. Toxicol.* 18, 1420–1426.
- (6) Aptula, A. O., Patlewicz, G., Roberts, D. W., and Schultz, T. W. (2006) Non-enzymatic glutathione reactivity and *in vitro* toxicity: A non-animal approach to skin sensitization. *Toxicol. in Vitro* 20, 239–247.
- (7) Aptula, A. O., and Roberts, D. W. (2006) Mechanistic applicability domains for nonanimal-based prediction of toxicological end points: General principles and application to reactive toxicity. *Chem. Res. Toxicol.* 19, 1097–1105.
- (8) Roberts, D. W., Patlewicz, G., Kern, P. S., Gerberick, F., Kimber, I., Dearman, R. J., Ryan, C. A., Basketter, D. A., and Aptula, A. O. (2007) Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. *Chem. Res. Toxicol.* 20, 1019–1030.
- (9) Enoch, S. J., Cronin, M. T. D., and Schultz, T. W. (2012) The definition of the applicability domain relevant to skin sensitization for the aromatic nucleophilic substitution electrophilic mechanism. *SAR QSAR Environ. Res.* 23, 649–663.
- (10) Enoch, S. J., Cronin, M. T. D., and Schultz, T. W. (2013) The definition of the toxicologically relevant applicability domain for the S_NAr reaction for substituted pyridines and pyrimidines. *SAR QSAR Environ. Res.*,
- (11) Enoch, S. J., Ellison, C. M., Schultz, T. W., and Cronin, M. T. D. (2011) A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity. *Crit. Rev. Toxicol.* 41, 783–802.
- (12) Enoch, S. J., Madden, J. C., and Cronin, M. T. D. (2008) Identification of mechanisms of toxic action for skin sensitization using a SMARTS pattern based approach. *SAR QSAR Environ. Res.* 19, 555–578.
- (13) Schultz, T. W., Rogers, K., and Aptula, A. O. (2009) Read-across to rank skin sensitization potential: subcategories for the Michael acceptor domain. *Contact Dermatitis* 60, 21–31.
- (14) Aptula, N., Roberts, D. W., Schultz, T. W., and Pease, C. (2007) Reactivity assays for non-animal based prediction of skin sensitization potential. *Toxicology* 231, 117–118.
- (15) Enoch, S. J., Cronin, M. T. D., Schultz, T. W., and Madden, J. C. (2008) Quantitative and mechanistic read across for predicting the skin sensitization potential of alkenes acting via Michael addition. *Chem. Res. Toxicol.* 21, 513–520.
- (16) Roberts, D. W., Aptula, A. O., and Patlewicz, G. Y. (2011) Chemistry-based risk assessment for skin sensitization: quantitative mechanistic modelling for the S_NAr domain. *Chem. Res. Toxicol.* 24, 1003–1011.
- (17) Roberts, D. W., and Natsch, A. (2009) High throughput kinetic profiling approach for covalent binding to peptides: Application to skin sensitization potency of Michael acceptor electrophiles. *Chem. Res. Toxicol.* 22, 592–603.
- (18) Natsch, A., and Gfeller, H. (2008) LC-MS-based characterisation of the peptide reactivity of chemicals to improve the *in vitro* prediction of the skin sensitization potential. *Toxicol. Sci.* 106, 464–478.
- (19) Patlewicz, G., Roberts, D. W., and Walker, J. D. (2003) QSARs for the skin sensitization potential of aldehydes and related compounds. *QSAR Comb. Sci.* 22, 196–203.
- (20) Patlewicz, G. Y., Basketter, D. A., Pease, C. K. S., Wilson, K., Wright, Z. M., Roberts, D. W., Bernard, G., Arnau, E. G., and Lepoittevin, J. P. (2004) Further evaluation of quantitative structure-activity relationship models for the prediction of the skin sensitization potency of selected fragrance allergens. *Contact Dermatitis* 50, 91–97.
- (21) Roberts, D. W., Aptula, A. O., Cronin, M. T. D., Hulzebos, E., and Patlewicz, G. (2007) Global (Q)SARs for skin sensitization - assessment against OECD principles. *SAR QSAR Environ. Res.* 18, 343–365.
- (22) Gerberick, G. F., Ryan, C. A., Kern, P. S., Schlatter, H., Dearman, R. J., Kimber, I., Patlewicz, G. Y., and Basketter, D. A. (2005) Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods. *Dermatitis* 16, 157–202.
- (23) Kern, P. S., Gerberick, G. F., Ryan, C. A., Kimber, I., Aptula, A., and Basketter, D. A. (2010) Local lymph node data for the evaluation of skin sensitization alternatives: A second compilation. *Dermatitis* 21, 8–32.
- (24) Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G. A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H. P., Izmaylov, A. F., Bloino, J., Zheng, G., Sonnenberg, J. L., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery, J. A., Peralta, J. E., Ogliaro, F., Bearpark, M., Heyd, J. J., Brothers, E., Kudin, K. N., Staroverov, V. N., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J. C., Iyengar, S. S., Tomasi, J., Cossi, M., Rega, N., Millam, J. M., Klene, M., Knox, J. E., Cross, J. B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R. E., Yazyev, O., Austin, A. J., Cammi, R., Pomelli, C., Ochterski, J. W., Martin, R. L., Morokuma, K., Zakrzewski, V. G., Voth, G. A., Salvador, P., Dannenberg, J. J., Dapprich, S., Daniels, A. D., Farkas, O., Foresmann, J. B., Ortiz, J. V., Cioslowski, J., and Fox, D. J. (2009) *Gaussian 09*, revision A.1, Gaussian Inc., Wallingford, CT.
- (25) Schwobel, J. A. H., Madden, J. C., and Cronin, M. T. D. (2010) Examination of Michael addition reactivity towards glutathione by transition-state calculations. *SAR QSAR Environ. Res.* 21, 693–710.

- (26) Roberts, D. W., Aptula, A. O., and Patlewicz, G. (2006) Mechanistic applicability domains for non-animal based prediction of toxicological endpoints. QSAR analysis of the Schiff base applicability domain for skin sensitization. *Chem. Res. Toxicol.* 19, 1228–1233.
- (27) Hansch, C., and Leo, A. (1979) *Substituent Constants for Correlation Analysis in Chemistry and Biology*, John Wiley & Sons, Inc, New York.
- (28) King, A. R., Lodola, A., Carmi, C., Fu, J., Mor, M., and Piomelli, D. (2009) A critical cysteine residue in monoacylglycerol lipase is targetted by a new class of isothiazoline-based enzyme inhibitors. *Br. J. Pharmacol.* 157, 974–983.
- (29) Feingold, K. R. (2009) The outer frontier: the importance of lipid metabolism in the skin. *J. Lipid Res.* 50, S417–S422.
- (30) Ulloa, N. M., and Deutsch, D. G. (2010) Assessment of a spectrophotometric assay for monoacylglycerol lipase activity. *Am. Assoc. Pharm. Sci.* 12, 197–201.