Toxicology Mechanisms and Methods, 2011; 21(2): 86–92

informa
healthcare

RESEARCH ARTICLE

Hazard identification of strong dermal sensitizers

Janet C. Gould, and Stephan Taylor

Pharmaceutical Development, Bristol-Myers Squibb Co., New Brunswick, NJ, USA

Abstract

Dermal reactions are the most frequently reported chemical health-related occupational hazard. Identifying dermal sensitizers is important for improving workplace safety. This paper takes a close look at the physico-chemical properties and results from the Local Lymph Node Assay (LLNA) to better understand and predict potent dermal sensitizers. The LLNA was used to identify 28 pharmaceutical agents or chemical intermediates as potent dermal sensitizers, EC3 < 1%. Certain parameters were examined to determine if there was any predictability to identify potent dermal sensitizers. These included a computer structure activity analysis using Derek® for Windows, molecular weight (Mw), calculated log P, and the log-linear extrapolation approach for estimating the potency. With Derek® for Windows, 13 compounds were identified as negative and 15 as positive for structural alerts, the most common being haloalkanes, and hydrazines. Additional mechanisms of reactivity were postulated for the remaining compounds. The examination of the Mw showed that all molecules had Mw < 550 Da. For 21 compounds, the interpolated vs extrapolated methods for determining the EC3 value were compared. For eight of the 21 compounds, the extrapolated EC3 was in the correct order of magnitude, eight were incorrect (five were too high and three were too low) and five could not be calculated. The use of a tiered approach including examination of the structural and physico-chemical properties and the LLNA to identify potent dermal sensitizers is integral in the selection of effective safe handling guidance to protect from sensitization hazards.

Keywords: *Local lymph node assay; structure activity relationship; dermal sensitization; haloalkane; hydrazine*

Introduction

Dermal sensitization is of concern for individuals who work with chemicals; as it is the most common occupational health-related adverse response with an estimated cost exceeding one billion dollars per year (NIOSH 2009). In 2003, 43,400 recordable skin diseases were reported at a rate of 4.9 injuries per 10,000 employees (OSHA 2005). In the UK in 2008 and 2009, 1573 (72%) of reported cases of occupational skin disease were contact dermatitis (COSHH 2009). Thus, it is important to identify chemicals that may contribute to occupational dermal reactions.

Dermal sensitization is a complex biological response. Key considerations for sensitization to occur include the dose per unit area of skin, the ability of a chemical to penetrate the skin, and the chemical's reactivity with biological molecules (i.e. proteins) to form a hapten. Understanding these events will aid in predicting dermal sensitization. Since the approval of the OECD Guideline 429, 'Skin Sensitization: Local Lymph Node Assay for Identification of Dermal Sensitization', industrial toxicologists at Bristol-Myers Squibb Co. have used the assay to predict dermal sensitization. The assay has the additional benefit of providing a dose response for risk assessment (Basketter et al. 1992; 1999; Hilton et al. 1998). If the sensitization potency is known, appropriate handling precautions can be taken to significantly reduce the risk of sensitization reactions in the workplace.

The drug synthesis process may take many steps in order to build the drug molecule from chemical intermediates that are generally reactive by nature. Thus, it is not surprising that ~ 70% of compounds tested in the Local Lymph Node Assay (LLNA) were positive, and, of those, ~ 15% were strong sensitizers, causing a response at a concentration of less than 1%. In order to better predict potent dermal sensitizers, this paper presents a retrospective look at molecular weight, calculated partition coefficient, structure–activity relationships, and the use of the LLNA. In addition, the extrapolation equation proposed by Gerberick et al. (2007) was examined to see if it could replace the need for an additional LLNA study at lower concentrations.

Address for Correspondence: Janet C. Gould, Bristol-Myers Squibb Co., 1 Squibb Dr, New Brunswick, NJ, 08903, USA. Tel: 732-227-7366. Email: janet.gould@bms.com

ISSN 1537-6516 print/ISSN 1537-6524 online
DOI: 10.3109/15376516.2010.484622

Methods

Chemicals

Pharmaceutical intermediates and drug candidates, designated 'a' through 'bb', were synthesized and documented according to Bristol-Myers Squibb Co internal procedures.

Physico-chemical parameters

The molecular weight in daltons (Da) and sensitization potential (EC3) were compared using Microsoft Excel©. The calculated partition coefficient (clogP) was calculated using the BioByte Sybyl 7.2 calculator (http://www.biobyte.com/) and then compared to sensitization potential (EC3) using Microsoft Excel©.

Local lymph node assay

All studies were conducted according to Good Laboratory Practices, approved by an animal ethics committee; and followed OECD Guideline 429, Skin Sensitization: Local Lymph Node Assay at different contract research organizations. Briefly, 25 µl of increasing concentrations of test substance or vehicle was applied to the five CBA/Ca or CBA/J mice per group for 3 consecutive days. Vehicles included olive oil/acetone, dimethyl sulfoxide, and dimethyl formamide. Concentration selection was according to OECD guidelines, using the highest concentration possible in the concentration series, 50%, 25%, 10%, 5%, 2.5%, 1%, 0.5% w/w. On day 6 of study, [3H-methyl] thymidine was injected into the tail vein. Five hours later the draining auricular lymph nodes from each ear were excised and single cell suspensions of lymph node cells were prepared for determination of radioactivity of individual animals or pooled lymph nodes. A Stimulation Index (SI) was calculated by dividing the mean disintegrations per minute (DPM) per mouse within each test substance group by the mean DPM per mouse for the vehicle control group. A positive response is considered when the SI is ≥ 3-fold the control. The effect concentration that results in this 3-fold stimulation is called the EC3 and provides a measure of potency. If an EC3 could not be determined because the EC3 was lower than the lowest concentration tested in the initial study, a follow-up study was conducted. Dosing generally ranged in three log scale concentrations of 1%, 0.1%, and 0.01% in order to bracket Bristol Myers Squibb dermal sensitization categories (Table 1). For certain potent dermal sensitizing compounds, only one study was conducted and an interpolated and extrapolated equation could not be compared.

Table 1. Dermal sensitization hazard categories and surface limits used at Bristol-Myers Squibb Co., which are based on LLNA EC3 values and the dose per unit area (ECETOC 2003; Api et al. 2008; Kimber et al. 2008).

Dermal sensitization category	EC3 %	Exposure control band	Surface wipe test method developed	Surface wipe test limit
Weak	> 1%	2–3	No	Not applicable
Moderate	0.1–1%	3	Yes	100 µg/100 cm^2
Potent	0.01–0.1%	4	Yes	10 µg/100 cm^2
Extremely potent	< 0.01%	5	Yes	1 µg/100 cm^2

Calculation of EC3

An EC3 was calculated by two methods and compared: (1) EC3 log-linear extrapolation calculation (Gerberick et al. 2007; Ryan et al. 2007):

$$\mathrm{EC3} = 2 \wedge \left(\log2\left(c\right) + \left(3 - d\right) / \left(b - d\right) * \left(\log2\left(a\right) - \log2\left(c\right)\right)\right) \quad (1)$$

where a is the second lowest concentration giving an SI of > 3; b is the actual SI at a, c is the lowest concentration giving an SI > 3, and d is the actual SI at c. Gerberick et al. (2007) suggest criteria for using the equation as c being near the EC3 and there being a dose response. The present authors here have interpreted an SI near the EC3 as being twice an SI of 3 (SI = 6). (2) Interpolated EC3, standard method for EC3 calculation (Kimber and Basketter 1997; Basketter et al. 1999):

$$\mathrm{EC3} = c + \left[\left(3 - d\right) / \left(b - d\right)\right]\left(a - c\right) \quad (2)$$

where a is the lowest concentration giving an SI of > 3; b is the actual SI at a; c is the highest concentration failing to produce an SI 3, and d is the actual SI at c.

For several compounds, only one study was conducted. In these cases, the extrapolated and interpolated results are calculated from the same study.

Structure activity relationship

All compounds identified with an EC3 less than 1% were examined by DEREK for Windows® version 10.0.2-2007 software for structure activity relationships with respect to dermal sensitization. Structural analysis from a chemical reactivity perspective was also examined by the authors.

Results

The results from the Local Lymph Node Assay conducted on 28 potent compounds are presented in Table 2. These compounds were examined for parameters indicative of dermal penetration, molecular weight, and calculated partition coefficient (cLogP), to see if they can be used to aid in identifying dermal sensitizers of most concern for the workplace. Table 2 displays the molecular weight and cLogP. A relationship between molecular weight of the chemicals and their EC3 was examined (Figure 1a). The range of molecular weights was 150–510 Da. Plotting the EC3 value by molecular weight did not reveal any correlation in this range. In addition, a relationship between calculated partition coefficient of the chemicals and their EC3 was examined (Figure 1b). The range of cLogP was –0.51–4.93. There was no correlation between clog P and EC3.

The structure activity relationship for dermal sensitization was examined using DEREK for Windows® version 10.0.2-2007. The results and structural alerts are displayed in Table 2. The analysis identified 15 as negative and 13 as positive for structural alerts. Table 3 displays the structural moieties and the frequency, the most common were 'hydrazine or precursor' or haloalkane. A proposed mechanism of chemical reaction of the compound with protein structures was assessed for each compound and provided in Table 2.

Table 2. The physico-chemical parameters, computer, and chemical structure–activity relationship reactivity analysis of compounds identified as potent dermal sensitizers.

Compound	Calculated* EC3	SAR‡ result	SAR alert	Mechanism of action	Molecular weight (Da)	clogP
a	0.0008**	negative		The lactone hydrolysis mechanism proceeds through a carbonium ion, which can also result in alkylation. Carbonium ion stabilized by delocalization. Carbonium ion stabilization expected to be greater than Compound 'b'. No epoxide moiety.	489	4.44
b	0.005	negative		The lactone hydrolysis mechanism proceeds through a carbonium ion, which can also result in alkylation. Carbonium ion stabilization expected to be less than Compound 'a'. No epoxide moiety.	491	4.93
c	0.006**	positive	Phenol or precursor; Hydrazine or precursor	Alpha halo ketone Iminoyl chloride Hydrazone (Hydrazine precursor) Probably hydrolyzed to hydrazine Structurally similar to 'g'	227	2.06
d	0.0079	positive	epoxide	The lactone hydrolysis mechanism proceeds through a carbonium ion, which can also result in alkylation. Carbonium ion stabilization expected to be equivalent to Compound 'b'. Also contains epoxide moiety.	506	3.08
e	0.0104	positive	epoxide	The lactone hydrolysis mechanism proceeds through a carbonium ion, which can also result in alkylation. Carbonium ion stabilization expected to be equivalent to Compound 'b'. Also contains epoxide moiety.	493	2.7
f	0.0127	negative		Alkylation by Bis Nucleophilic Heteroaromatic substitution	188	1.94
g	0.0182	positive	Phenol or precursor Hydrazone (Hydrazine precursor)	Iminoyl chlorideHydrazine Hydrazone (Hydrazine precursor) Probably hydrolyzed to hydrazine Structurally similar to 'c'	256	3.02
h	0.0244	negative		N-tosyl aminoacid amide	344	1.53
i	0.0335	negative		Alkylation by Bis Nucleophilic Heteroaromatic substitution	263	1.48
j	0.0382	positive	haloalkane	Alkylation by alpha haloketone	297	2.87
k	0.0400	negative		Alkylation by Bis Nucleophilic Heteroaromatic substitution	263	1.48
l	0.0494	negative		Strained ring bicyclic	440	0.14
m	0.0665	negative		Alkylation by Nucleophilic Heteroaromatic substitution	487	2.73
n	0.073	positive	haloalkane	Alkylation by alpha haloketone	291	2.18
o	0.077	positive	haloalkane	Alkylation by alpha haloketone	396	3.47
p	0.138**	negative	hydrazine precursor	HydrazideSuspected slow hydrolysis to hydrazine	232	1.76
q	0.162	positive	alpha,beta-Unsaturated aldehyde,	Alkylation by Michael Addition	242	4.04
r	0.186	negative		Alkylation by Electron deficient Nucleophilic Heteroaromatic substitution	232	2.19
s	0.2336	negative		Alkylation by Nucleophilic Heteroaromatic substitution	267	2.59
t	0.2793	negative		O-acyl hydroxylamines	182	0.55
u	0.3114	positive	hydrazine	Hydrazine	247	1.65
v	0.313**	positive	alpha,beta-Unsaturated aldehyde,	Alkylation by Michael adduct	274	3.96
w	0.313**	positive	Hydrazone Hydrazine precursor	Hydrazine	274	2.14
x	0.3218	negative		Alkylation by Nucleophilic Heteroaromatic substitution	151	1.11
y	0.3302	positive	HaloalkaneHydrazone (Hydrazine precursor)	Alkylation by alpha haloimine or cleaved to a Hydrazine	271	2.34
z	0.4737	negative		Strained ring bicyclic	455	−0.02
aa	0.6845	positive	Acid anhydride or analog	AcylationCantharidin-like anhydride	303	−0.51
bb	0.8143	negative		No rationalization for activity	322	3.18

‡ Structure activity relationship analysis using DEREK for Windows® version 10.0.2-2007.
* The standard interpolated equation (2) was used for the EC3% unless otherwise specified.
** EC3 calculated by using the extrapolation equation (1).

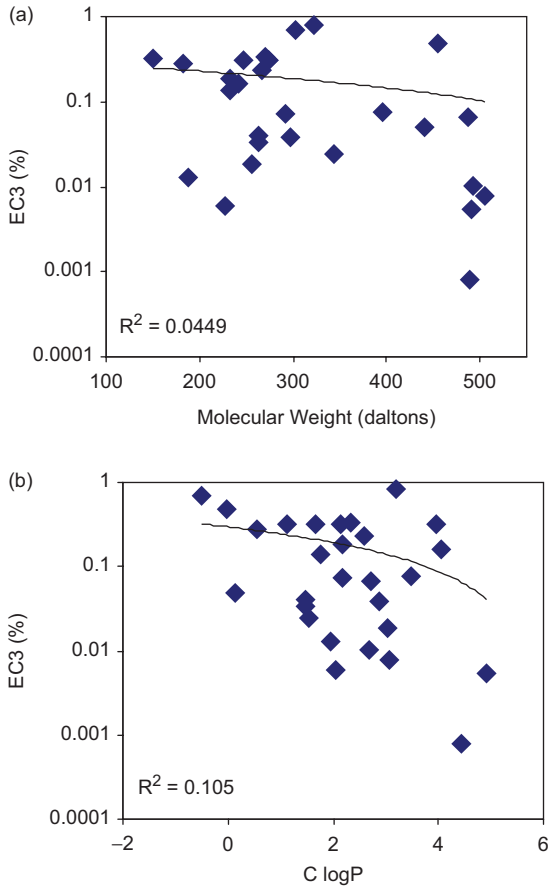


Figure 1. (a) A plot of the EC3 value by molecular weight did not reveal any correlation in $R^2 = 0.0449$. (b) A plot of the EC3 value by calculated partition coefficient did not reveal any correlation in $R^2 = 0.105$.

Table 4 displays the compounds and their LLNA results grouped by the ability of the log-linear extrapolation equation to predict the correct Dermal Sensitization Category used at Bristol-Myers Squibb Co. (Table 1). Unless otherwise noted, two assays were conducted when the first assay did not identify an EC3. The SI index at each concentration tested is shown with both the interpolated and extrapolated EC3 values. An additional seven compounds were identified as dermal sensitizers with an EC3 < 1%; however, the data did not allow for a comparison of an interpolated to an extrapolated EC3 value (Table 5).

Only four of 21 compounds had data sets appropriate to use the extrapolation equation; however, for the results of these four compounds, the extrapolated and interpolated values were derived from the same assay. The comparison of the interpolated and extrapolated EC3 results with respect to hazard categorization identified 41% (9/21) of the compounds had EC3 values within the same Dermal Sensitization Category (Table 4). The data for 27% (6/21) of the compounds would not allow calculation; 23% (5/21) predicted a too lenient hazard category; and 9 % (2/21) predicted a too conservative hazard category. For the seven compounds in Table 5, interpolated EC3 values identified an EC3 values < 1% for two compounds. Although the other five compounds are likely to have EC3 values < 1%, it is difficult to know for certain, since the SI at the lowest dose tested was not near the EC3, and a follow-up study was not conducted to confirm.

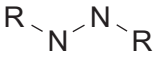
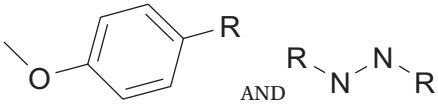
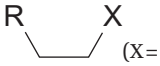
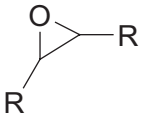
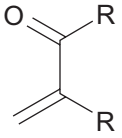
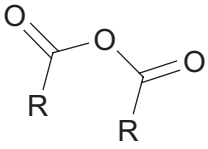
Discussion

Numerous proprietary compounds are tested by Bristol-Myers Squibb Co to identify dermal sensitizers, which then allows for adequate worker protection. Of ~ 300 compounds tested in the LLNA over the past 10 years by Bristol-Myers Squibb Co., 28 have been identified as dermal sensitizers with an EC3 less than 1%. The LLNA is very useful in understanding potency. Unlike previous methods, the Guinea Pig Maximization and Buehler assays did not give a quantitative value for potency. The potency information allows for clear guidance on containment, handling, personal protective equipment, and industrial hygiene monitoring. For example, a compound with an EC3 at 0.05% would be considered a potent dermal sensitizer for hazard communication purposes and be placed in Exposure Control Band 4 which prescribes handling and personal protective equipment requirements. In addition, a surface wipe method would be developed to a limit of 10 μg/100 cm^2. In the pharmaceutical manufacturing environment, dermal sensitization reactions generally do not arise unless the chemical has an EC3 of less than 0.1% (personal observation). In addition, sensitization responses do not appear to occur if the environment in which the compound is handled is controlled to an air concentration of less than 10 μg/m^3 and wipe sampling is performed to ensure cleanliness.

For a compound to cause skin reactions, it must cross the dermal barrier. The potent sensitizers examined had molecular weights of less than 510 Da The calculated partition coefficients (clogP), ranging from –0.50 to 4.93, indicated primarily hydrophobic properties. Together, these parameters fit within recognized physico-chemical properties that correlate inversely with dermal penetration (Moss et al. 2002; Babu et al. 2004; Brand et al. 2004). It was noted that TOPKAT software, which is available for structure activity relationship analysis, requires a molecular weight to be less than 300 Da to alert for dermal sensitization (Fedorowicz et al. 2005). This would mean TOPKAT would have missed nine of the compounds presented here based on molecular weight alone. DEREK uses a modified version of the Potts and Guy equation: Log Kp (cm/h) = –2.72 + 0.71 Log P – 0.0061 Mw (Potts and Guy 1992); which results in a negative prediction for skin sensitization if the Log Kp value is below –5.

Dermal penetration does not correlate with potency of a dermal sensitizer with an EC3 < 1%. The ability of a chemical to react with a protein to form a hapten may be the more critical step. The structures with positive LLNA responses were examined for chemical moieties that have potential to react with proteins or other endogenous compounds. For the compounds tested, DEREK for Windows® analysis alone identified less than half of these compounds as dermal sensitizers. The combination of the software and expert analysis identified specific reactive moieties that function

Table 3. Structures identified as having potential for causing dermal sensitization by DEREK for Windows® version 10.0.2.-2007.

General structure*	DEREK structure description	Potent compounds with structure
	Hydrazine or precursor	3
	Phenol or precursorANDHydrazine or precursor	2
 (X = F, Cl, Br, or I)	Haloalkane	3
	Epoxide	2
	alpha,beta-Unsaturated aldehyde	2
	Acid anhydride or analogue	1

* The structures presented represent the general structure as depicted by the description and observed in the Bristol-Myers Squibb Co. chemicals tested. These structures do not necessarily equal exact DEREK alerting substructures.

mostly as alkylating agents (halo-heteroaromatics and alpha haloketones, and Michael acceptors), or acylating agents (anhydrides). The other groups can function as either amide cleaving agents (hydrazinolysis) or coupling with carbonyls (hydrazines and hydrazine precursors; hydrazides and hydrazones and O-acyl hydroxylamines). These can cleave proteins at peptide linkages or glycoprotein at sugar linkages. For example, protein deglycation with hydrazine analogs could result in the liberation of the protein with concomitant formation of glycoprotein or glycated hydrazone adducts (Kobayashi et al. 1993), which can possibly function as a hapten.

It is interesting to note that five epothilone analogs were analyzed and four were among the most potent sensitizers presented. Two of the epothilones contained an epoxide moiety and two of the potent sensitizing epothilones did not, suggesting that the epoxide moiety is not the causal factor. Lactones (esters) present in the epothilone structure do not trigger structural alerts in DEREK for Windows® version 10.0.2-2007; however, evidence from literature suggests that the hydrolysis of epothilone lactone does not proceed via the normal carbonyl attack, but by C-O bond breakage to form an allylic stabilized carbonium ion (Jumaa et al. 2004). The LLNA results also confirm this by showing that compounds with different heterocycles conjugated to the allylic system are more potent sensitizers if that heterocycle is better able to stabilize the carbonium ion. The analog that failed to illicit a response was a silyl protected intermediate (results not reported). The significantly increased hydrophobicity is suspected to diminish the activity.

In order to understand the potential to use the extrapolation equation provided by Gerberick et al. to replace additional follow-up animal testing, the equation was applied indiscriminately to all 21 data sets. With the data presented here, the equation was only within the correct Dermal Sensitization Category 41% of the time (Figure 2). Further caution should be used with interpretation of this accuracy, since extrapolated and interpolated values were derived from the same assay which does not represent the data from an assay when the EC3 calculation will require extrapolation. Dose–response and nearness to EC3 were considered important criteria for using the log-linear extrapolation equation. Of the 21 data sets, only two fit the criteria recommended by Gerberick et al. for use of the equation. The data presented here reinforces that it is not appropriate to use the extrapolation equation unless a dose–response is observed and the lowest concentration with an SI greater than the EC3 is near the EC3. An additional LLNA assay with lower concentrations should be considered to understand the potency of dermal sensitizers if the criteria are not met for using the log-linear extrapolation equation.

Dermal sensitization is of concern in the work place. A tiered approach including an examination of first the physico-chemical parameters, structural analysis by both SAR programs, and expert judgment, and then the design and results from the Local Lymph Node Assay can be utilized to identify and understand dermal sensitizers of the greatest concern. Although SAR software programs such as DEREK for Windows® version 10.0.2-2007 are useful for identifying dermal sensitizers, not all structural moieties

Table 4. The LLNA results and calculation of extrapolated and interpolated EC3 from 21 potent dermal sensitizers. The SI at each concentration tested is provided.

Compound		Experiment 2 c	Experiment 2 d	Experiment 2 a	Experiment 2 b	Interpolated EC3 Equation (2)	Experiment 1 c	Experiment 1 d	Experiment 1 a	Experiment 1 b		Extrapolated EC3 Equation (1)
Extrapolated EC3 and experimental EC3 are within the same dermal sensitization hazard category.												
b‡	% conc.		0.001	0.01		0.005	0.01	0.1	0.5			0.008
	SI		1.5	4.6			4.6	19.2	35.8			
d‡	% conc.		0.001	0.01	0.1	0.008	0.01	0.1				0.009
	SI		1.7	3.4	9.5		3.4	9.5				
e‡	% conc.		0.01	0.1	1	0.01	0.1	1			*	0.03
	SI		3	8.1	17.9		8.1	17.9				
h‡	% conc.		0.01	0.1		0.024	0.25	0.5			*	0.064
	SI		1.3	11.9			29.9	43.6				
i	% conc.		0.01	0.1	1	0.034	10	25	50		*	0.09
	SI		1.3	7.8	32.7		39.5	46.6	62.1			
k‡	% conc.		0.01	0.1	1	0.04	0.1	1				0.059
	SI		1.4	6.2	20.2		6.2	20.2				
n‡	% conc.		0.01	0.1	1	0.073	0.1	1				0.079
	SI		1	3.9	12.5		3.9	12.5				
t	% conc.	0.0	0.1	1		0.279	5	10	25		*	0.105
	SI	2.5	2.5	5			38.6	44.9	47.9			
x‡	% conc.		0.1	1		0.322	1	5			*	0.643
	SI		1.1	8.8			8.8	29.7				
Data do not allow use of extrapolation equation												
f	% conc.		0.01	0.05	0.1	0.01	1	2.5	5		*	—
	SI		1.3	26.5	33.2		69.5	49.6	48.1			
j	% conc.	0.0025	0.025	0.25		0.04	0.25	2.5	25		*	—
	SI	1.1	2.3	13.9			14.3	18	25.1			
l	% conc.		0.03	0.25	2.5	0.05	2.5	5	10		*	—
	SI		1.7	13.7	17.1		6.6	5.5	4.2			
r	% conc.	0.01	0.1	1		0.19	10	25	50		*	—
	SI	1.1	1.9	13.4			20.3	18.9	17.9			
s	% conc.	0.01	0.1	1		0.23	2.5	5	10		*	—
	SI	1.0	2.1	8.1			3.75	3.6	4.3			
aa	% conc.	0.01	0.1	1		0.69	5	10	25		*	—
	SI	1.0	1.1	4			17.5	13	17.1			
Extrapolated EC3 under-estimates experimental EC3												
q	% conc.	0.01	0.1	1		0.16	2.5	5	10		*	0.04
	SI	1.0	2.5	9.4			15.9	18.1	22.5			
y	% conc.	0.01	0.1	1		0.33	10	25	50		*	0.05
	SI	0.9	0.8	9.4			24.7	28.5	44.8			
Extrapolated EC3 over-estimated the experimental EC3												
g	% conc.		0.01	0.1	1	0.02	5	10	25		*	0.77
	SI		1.2	21	38.3		54.6	73.7	94.4			
o	% conc.		0.01	0.1	1	0.08	2.5	5	10		*	1.21
	SI		1.2	3.6	5.7		9.8	16.3	18.6			
z	% conc.		0.5	1	2.5	0.47	5	10	25		*	3.28
	SI		3.2	7	4.1		9.1	19.1	14.4			
bb‡	% conc.		0.25	2.5	25	0.81	2.5	25			*	1.65
	SI		1.4	7.7	33.5		7.7	33.5				

* Data do not meet the criteria for using the extrapolation equation described by Gerberick et al.
‡ Interpolated and extrapolated EC3 values calculated from the same assay.

have been identified. However, certain structural alerts such as hydrazines, haloalkanes, epothilones, and halosubsituted electron deficient aromatics should be considered potent dermal sensitizers unless proven otherwise. In addition, caution should be used when applying the Gerberick extrapolation equation to predict the potency of potent dermal sensitizers, and a follow-up study should be considered. Determining an accurate potency is an important hazard identification step to support the development of appropriate worker protection from sensitization hazards.

Table 5. The LLNA results and calculation of extrapolated or interpolated EC3 from seven potent dermal sensitizers.

Compound		LLNA result			EC3
a	% conc.	0.001	0.01	0.1	0.0008 **
	SI	4.1	14.3	40.1	
c	% conc.	0.01	0.1	1	0.006 **
	SI	8.4	32.2	37	
m	% conc.	0.001	0.01	0.1	0.067*
	SI	1.2	1.3	4	
p	% conc.	2.5	5	10	0.14 **
	SI	7.6	8.7	11.1	
v	% conc.	5	10	25	0.313 **
	SI	15	18	19.8	
w	% conc.	5	10	25	0.313 **
	SI	4.6	5	8.5	
u	% conc.	0.1	1	10	0.314*
	SI	2.0	6.2	toxic	

* The standard interpolated equation (2) was used for the EC3%.
** EC3 calculated by using the extrapolation equation (1).

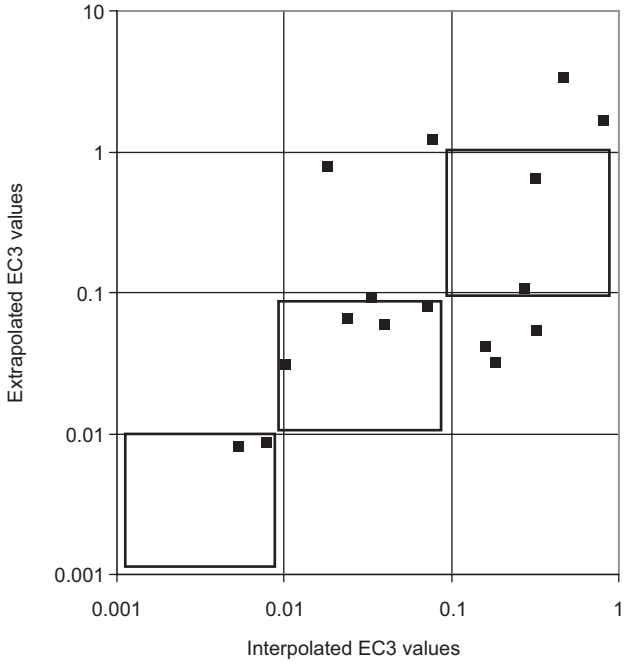


Figure 2. A comparison of EC3 values derived by extrapolation vs interpolation for compounds with an interpolated EC3 less than 1%. Data points within the highlighted boxes represent extrapolated and interpolated values falling into the same hazard category.

Acknowledgements

We thank David Brandwene, Dr Robert Feldman, and Dr Caren Villano for their in-depth review and comments on the manuscript. We also thank Dr Wendy Luo and Nicholas Roden for their comments.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Api AM, Basketter DA, Cadby PA, Cano MF, Ellis G, Gerberick GF, Griem P, McNamee PM, Ryan CA, Safford R. 2008. Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients. Regul Toxicol Pharmacol 52:3–23.

Babu RJ, Chatterjee A, Ahaghotu E, Singh M. 2004. Percutaneous absorption and skin irritation upon low-level prolonged dermal exposure to nonane, dodecane and tetradecane in hairless rats. Toxicol Ind Health 20:109–118.

Basketter DA, Lea LJ, Dickens A, Briggs D, Pate I, Dearman RJ, Kimber I. 1999. A comparison of statistical approaches to the derivation of EC3 values from Local Lymph Node Assay dose responses. J Appl Toxicol 19:261–266.

Basketter DA, Roberts DW, Cronin M, Scholes EW. 1992. The value of the Local Lymph Node Assay in quantitative structure-activity investigations. Contact Dermat 27:137–142.

Brand RM, Charron AR, Dutton L, Gavlik TL, Mueller C, Hamel FG, Chakkalakal D, Donohue TM Jr. 2004. Effects of chronic alcohol consumption on dermal penetration of pesticides in rats. J Toxicol Environ Health 67:153–161.

COSHH. 2009. Dermatitis and other skin disorders. Available online at: http://www.hse.gov.uk/statistics/causdis/dermatitis/index.htm (Website accessed December 31, 2009)

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals). 2003. Contact sensitisation: Classification according to potency, a commentary. Technical Report No. 43, Brussels, Belgium: ECETOC.

Fedorowicz A, Singh H, Soderholm S, Demchuk E. 2005. Structure-activity models for contact sensitization. Chem Res Toxicol 18:954–969.

Gerberick GF, Ryan CA, Dearman RJ, Kimber I. 2007. Local Lymph Node Assay (LLNA) for detection of sensitization capacity of chemicals. Methods 41:54–60.

Hilton J, Dearman RJ, Harvey P, Evans P, Basketter DA, Kimber I. 1998. Estimation of relative skin sensitizing potency using the Local Lymph Node Assay: a comparison of formaldehyde with glutaraldehyde. Am J Contact Dermat 9:29–33.

Jumaa M. Carlson B, Chimilio L, Silchenko S, Stella VJ. 2004. Kinetics and mechanism of degradation of epothilone-D: an experimental anticancer agent. J Pharm Sci 93:2953–2961.

Kimber I, Basketter DA. 1997. Contact sensitization: a new approach to risk assessment. Human Ecol Risk Assess 3:385–395.

Kimber I, Dearman RJ, Basketter DA, Ryan CA, Gerberick GF, McNamee PM, Lalko J, Api AM. 2008. Dose metrics in the acquisition of skin sensitization: thresholds and importance of dose per unit area. Regul Toxicol Pharmacol 52:39–45.

Kobayashi K, Yoshimoto K, Hirauchi K, Uchida K. 1993. Deglycation of glycated proteins with hydrazine analogs. Life Sci 53:291–295.

Moss GP, Dearden JC, Patel H, Cronin MT. 2002. Quantitative structure-permeability relationships (QSPRs) for percutaneous absorption. Toxicol In Vitro 16:299–317.

NIOSH. 2009. NIOSH.Safety and Health Topic: Skin exposures & effects. National Institute of Occupational Safety and Health. Education and Information Division. Available online at: http://www.cdc.gov/niosh.topics/skin/ (Website accessed December 31, 2009)

OSHA. 2005. Health and Safety Page, Dermal Exposure. Available online at: http://www.osha.gov/SLTC/dermalexposure/index.html (Website accessed December 31, 2009)

Potts RO, Guy RH. 1992. Predicting skin permeability. Pharm Res. 9:663–669.

Ryan CA, Chaney JG, Gerberick GF, Kern PS, Dearman RJ, Kimber I, Basketter DA. 2007. Extrapolating local lymph node assay EC3 values to estimate relative sensitizing potency Cutan Ocul Toxicol 26:135–145.