



Chemical applicability domain of the Local Lymph Node Assay (LLNA) for skin sensitisation potency. Part 3. Apparent discrepancies between LLNA and GPMT sensitisation potential: False positives or differences in sensitivity?[☆]

David W. Roberts^{a,*}, Terry W. Schultz^b, Anne Marie Api^c

^a School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, L3 3AF, United Kingdom

^b The University of Tennessee, College of Veterinary, 2407 River Drive, Knoxville, TN, 37996, USA

^c Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, 07677, USA

ARTICLE INFO

Article history:

Received 18 June 2016

Received in revised form

25 July 2016

Accepted 26 July 2016

Available online 29 July 2016

Keywords:

Unsaturated fatty acids

Surfactants

Autoxidation

ABSTRACT

The Local Lymph Node Assay (LLNA) is the gold standard regulatory toxicology test for skin sensitisation along with the guinea pig maximisation test (GPMT). Compared with the GPMT, LLNA uses fewer animals, it is quantitative, and it gives a numerical prediction of potency. However several concerns have been raised with this assay, mainly related to false positives and false negatives. Over the years, many authors, including the developers of the assay, have presented cases where there have been discrepancies between the GPMT and LLNA results. Several theories have been put forward for these discrepancies, the main one being the “over-sensitivity” of the GPMT. This paper analyses the data from a systematic study, published in three papers from 2008 to 2011, covering several classes of chemicals, in particular unsaturated fatty acids, sugar surfactants and ethoxylated alcohols, with many cases of chemicals testing positive in the LLNA being negative in the GPMT. Based on consideration of reaction chemistry and structural alerts, it is concluded that these discrepancies are not LLNA false positives, but can be rationalised in terms of the different protocols of the assays.

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1. Introduction

Skin sensitisation is a human health endpoint which has been extensively studied. The chemical and biological pathway driving the induction and elicitation of allergic contact dermatitis is relatively well understood (OECD, 2012). Currently, in a regulatory context only animal tests, the guinea pig maximisation test (GPMT) (OECD test guideline 406) and the murine local lymph node assay (LLNA) (OECD test guideline 429) are accepted. In the pursuit of non-animal alternative methods for skin sensitisation, other approaches are currently being explored. One such approach is the integration and interpretation of non-standard data (i.e., in

chemico, *in vitro* and *in silico* information) so as to correctly predict skin sensitisation potential (Patlewicz et al., 2014; Jaworska, 2016; Strickland et al., 2016; Nukada et al., 2013; Takenouchi et al., 2015; Van der Veen et al., 2014). It is recognised that the molecular initiating event in skin sensitisation is the covalent modification of cutaneous protein by reaction with the allergenic chemical or a chemical derived from it by metabolic or abiotic activation (Roberts et al., 2008). One approach proposed for modeling of skin sensitisation is the application of chemical reaction mechanistic domains (Aptula and Roberts, 2006), based on the concept that different quantitative relationships between skin sensitisation potency and chemical properties should apply for each reaction mechanism. The advantage here is that this is the concept that underpins the understanding of the chemical basis of skin sensitisation itself (OECD, 2012; 2014). These principles have been evaluated and assessed with respect to numerous different skin sensitisation-related datasets covering both guinea pig and LLNA data (summarised in Roberts et al., 2007; Roberts and Aptula, 2008; Roberts et al., 2016).

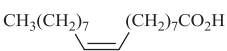
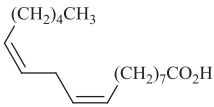
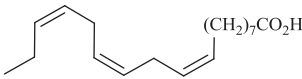
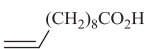
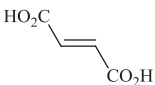
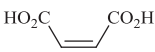
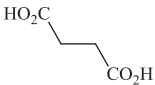
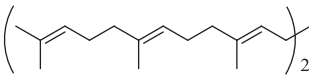
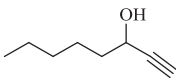
The development of the LLNA and its becoming the first choice

[☆] Part 2. Roberts D.W., Api A.M., Aptula A.O. (2016). Chemical applicability domain of the Local Lymph Node Assay (LLNA) for skin sensitisation potency. Part 2. The Biological Variability of the Murine Local Lymph Node Assay (LLNA) for Skin Sensitisation. This journal, <http://dx.doi.org/10.1016/j.yrtph.2016.07.013>.

* Corresponding author.

E-mail address: D.W.Roberts@ljmu.ac.uk (D.W. Roberts).

Table 1
Chemicals investigated by Kreiling et al., 2008.

Name	Structure	LLNA EC3, %	GPMT
Oleic acid		10.5	NS
Linoleic acid		14.0	inconclusive
Linolenic acid		9.5	NS
Undecylenic acid		19.0	Sensitiser
Fumaric acid		NS	NS
Maleic acid		2.7	NS
Succinic acid		NS	NS
Squalene		6.7	NS
Oct-1-yn-3-ol		3.6	NS

method within Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) has raised the issue of false positive and false negative results (Basketter et al., 1998, 2006, 2009a, 2009b).

This issue has become highlighted by efforts to develop non-animal methods for the identification of skin sensitisers. Essentially, the question has become: what should be the gold standard for skin sensitisation, i.e. what animal data should be used for

benchmarking new non-animal approaches? For example, if a chemical is known to give a false positive in the LLNA, then a negative prediction for that chemical from a non-animal method should not be treated as an incorrect prediction, and vice versa. In this context, recent attention has focused primarily on the LLNA; however, for many years prior to its introduction, the GPMT and the occluded patch test of Buehler were the methods of choice (Basketter and Kimber, 2010).

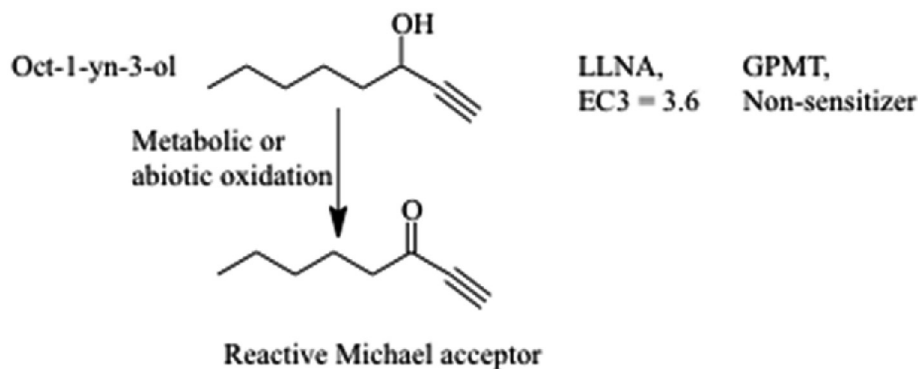


Fig. 1. Oct-1-yn-3-ol.

Table 2
GSH reactivity for 1-octyn-3-ol analogues.

Name	CAS	RC50 (mM)	Highest conc. tested	% Purity
1-Pentyn-3-ol	4187-86-4	1.5	10	98
1-Hexyn-3-ol	105-31-7	2.3	10	97
1-Heptyn-3-ol	7383-19-9	1.1	5	97

One issue clouding the correlation of *in vivo* skin sensitisation results is how the protocols for the different *in vivo* tests impact the results, especially as related to the mechanistic basis for the activation of compounds that are not directly protein-reactive. We hypothesise that it is this lack of understanding that has resulted in questioning the suitability of the LLNA for assessing sensitisation potential of certain types of compounds, and the call for the GPMT to remain available as an accepted test method for skin sensitisation hazard identification.

The major activation processes known in skin sensitisation are oxidative, and these are expected to apply to data from the LLNA and the GPMT. However, confirmation of this expectation is required. Moreover, there are other activation processes that also need to be considered – hydrolysis, dehydrohalogenation, sulphation etc.

At this point it is appropriate to consider the various causes of disagreement between LLNA results and findings or experience in guinea pigs and humans.

Firstly, there may be cases of genuinely false positives – an SI value > 3 is achieved in the LLNA but the biological mechanism is not sensitisation. Conversely there may be cases of genuine false negatives where for some reason, possibly related to differences in skin metabolism between species, the chemical is unable to sensitise mice but can sensitise guinea pigs and/or humans.

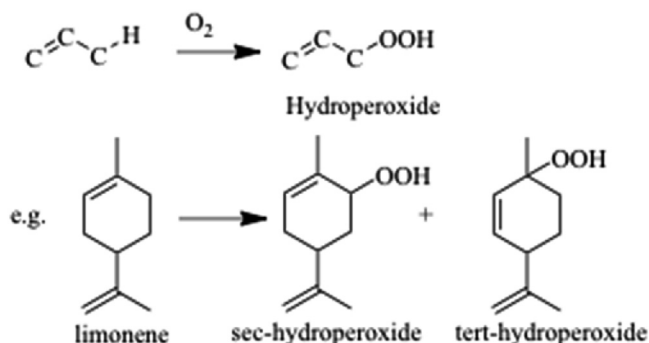


Fig. 2. Structural alert for sec- and tert-hydroperoxides by autoxidation.

Secondly, it should be borne in mind that the boundary between sensitisers and non-sensitisers is fuzzy. There are chemicals that are completely incapable of sensitising and there are chemicals that have enough potency to product positive results in any test. But there are others whose ability to sensitise is marginal (e.g. due to low but not zero chemical reactivity), and these may be classed as non-sensitising in one assay (e.g. failing to produce an SI > 3 at any concentration) and as weakly sensitising in another assay or on the basis of clinical evidence (e.g. a low but not zero incidence of reports of human sensitisation). Conversely some marginal sensitisers might fail to register positives in guinea pig assays and not have any reports of human sensitisation (particularly when human exposure is not extensive) yet be classed as sensitisers in the LLNA.

Thirdly, chemicals can give positive results due to impurities they contain. If human exposure is mainly to the “commercial grade” material whereas animal testing is with higher purity “laboratory grade” material, there may be incidences of human

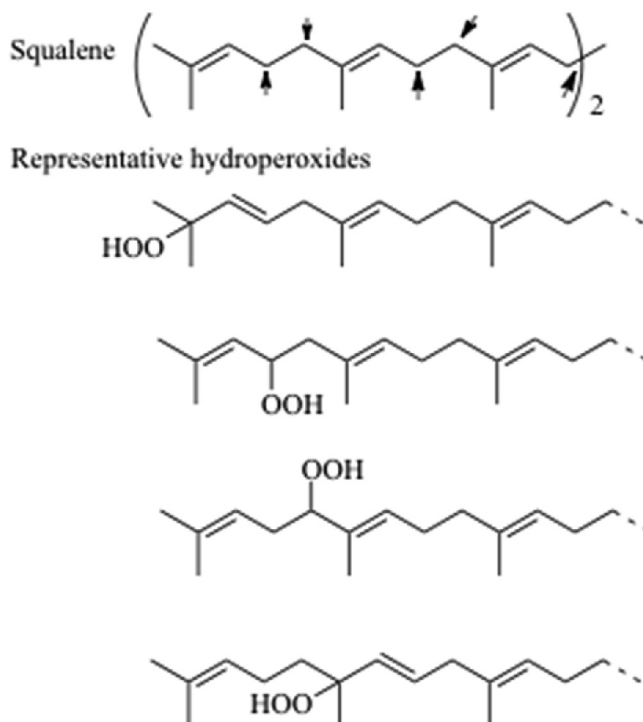


Fig. 3. Squalene, and some representative autoxidation-derived sec- and tert-hydroperoxides.

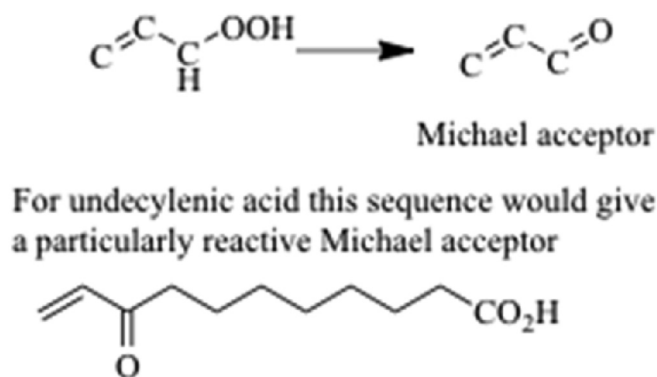


Fig. 4. Dehydration of sec-hydroperoxides.

sensitisation that, in comparison with negative results in the animal assays, can be interpreted as false negatives in the latter. Conversely, a laboratory synthesised chemical can sometimes contain more of a sensitising contaminant than when it is produced on production scale, and a positive animal result with the former material may be misinterpreted as a false positive.

Recently there have been a number of studies comparing and contrasting the results in the LLNA with results in the GPMT for several groups of compounds that are not directly reactive (Kreiling et al., 2008; Garcia et al., 2010; Ball et al., 2011). The differences observed have been attributed to the LLNA producing false positives for these types of chemicals, i.e. the stimulation index values observed resulting from processes other than sensitisation. It is the aim of this study to explore a simpler hypothesis: that the LLNA results are not false positives but rather, as a result of the differences in protocols between the LLNA and GPMT, the LLNA offers a greater opportunity for these non-directly reactive compounds to express their potential to induce sensitisation. We will discuss the chemicals investigated in these three papers in chronological order.

1.1. Group 1. chemicals investigated by Kreiling et al. (2008)

Table 1 shows the nine chemicals examined by Kreiling et al. (2008) in both the LLNA and the GPMT. Their aim was to address the hypothesis that chemicals with carbon-carbon double bonds may result in a higher number of unspecific (false positive) results in the LLNA compared to the GPMT. Specifically, Kreiling et al. (2008) reported that in the LLNA oleic acid, linoleic acid, linolenic acid, undecylenic acid, maleic acid, squalene and 1-octyn-3-ol gave clear positive results (i.e., stimulation index (SI) ≥ 3) while fumaric acid and succinic acid gave clearly negative results. In contrast, results in the GPMT revealed reproducible skin reactions indicating an allergic response in only a few animals, for four test substances. Based on the GPMT results, only undecylenic acid would have to be classified a skin sensitizer. While the results for linoleic acid were

inconclusive, the other substances would not require labelling.

We begin our analyses of these findings with the beta-unsaturated alcohol 1-octyn-3-ol. This β -ethynyleneic ($C\equiv C$) alcohol is a secondary propargylic alcohol with a terminal β -ethynyleneic group; as such it is considered a pro-electrophile (Enoch et al., 2008). 1-Octyn-3-ol is readily metabolised via alcohol dehydrogenase (Alston et al., 1979) or abiotically oxidised into the electrophilic α,β -unsaturated analogue 1-octyn-3-one, a highly reactive Michael acceptor (Schultz et al., 1994) (Fig. 1).

While 1-octyn-3-ol has not been tested for *in chemico* reactivity, the C5, C6 and C7 analogues have been tested in the GSH RC₅₀ assay (unpublished data) (Table 2). In this assay glutathione (GSH) is incubated with the test chemical at 3 or more concentrations and the extent of reaction of the GSH thiol group is determined. The RC₅₀ value (concentration of test chemical giving 50% reaction of the GSH thiol group under the assay conditions) is estimated from the resulting dose–response (Yarborough and Schultz (2007)).

The mean RC₅₀ value for these 1-alkyn-3-ols is 1.63 ± 1.00 mM. These results support the premise that abiotic oxidation of 1-octyn-3-ol contributes significantly to its sensitisation potency in the LLNA. The open application and thin layer of test material in the LLNA protocol facilitates autooxidation during the assay. Thus, 1-octyn-3-ol is converted to the α,β -unsaturated ketone, 1-octyn-3-one. In contrast, in occluded test protocols (i.e., GPMT and Human Repeat Insult Patch Test, HRIPT) the exposure to oxygen is lower than in the LLNA, limiting the extent of oxidation. Interestingly, comparisons of abiotic reactivity between parent β -unsaturated alcohols and their corresponding oxidised analogues, α,β -unsaturated ketones (Schultz and Yarborough, 2004) reveal the apparent GSH reactivity of the alcohol to be 50 to 100 times less than that of the corresponding ketone, suggesting that under the GSH assay conditions no more than 1–2% of the alcohol becomes oxidised.

Of the seven unsaturated acids examined five are positive and two are negative in the LLNA. Four of the positives: oleic, linoleic, linolenic and undecylenic acids, have secondary allylic hydrogen atoms, a pre-hapten alert for the potential to form allergenic hydroperoxides by autooxidation (Fig. 2).

Similarly, squalene, another positive chemical in the LLNA, has both sec- and tert-hydroperoxide alerts (Fig. 3).

Secondary (but not tertiary) allylic hydroperoxides can react further by elimination of water to give α,β -unsaturated ketones: these can react as Michael acceptors, but in many cases are less potent allergens than the hydroperoxides. This probably applies for oleic, linoleic and linolenic acids, but in the case of undecylenic acid, the ketone derived from the secondary hydroperoxide is vinylic, i.e. with the sub-structure $CH_2=CHCO-$, which is an alert for high Michael reactivity and likely high allergenic potency (Fig. 4).

This is consistent with undecylenic acid being the only compound in Table 1 that is clearly positive not only in the LLNA but also in the GPMT. All sec- and tert-hydroperoxides that have been tested are strong/moderate in the LLNA (Johansson et al., 2010). However,

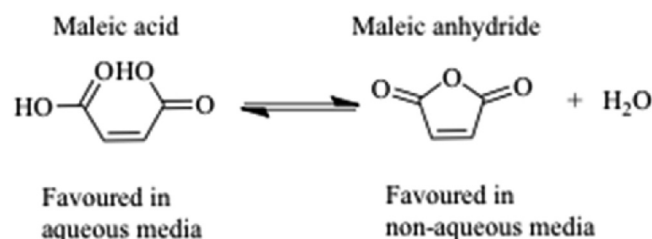


Fig. 5. Maleic acid and maleic anhydride.

Table 3

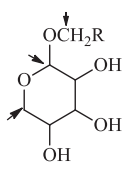
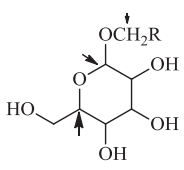
Low molecular weight acids. LLNA data taken from the NICEATM database unless otherwise indicated.

Acid	pKa	Vehicle	LLNA EC3 (%)
Maleic	1.90	DMSO	2.7 ^a , 7.0
Oxalic (3 entries)	1.25	DMF	2.1 ^b , 6.3, 15.0
Squaric	1.50	DMSO	7.1
Fumaric	3.03	DMSO	NS
Succinic	4.20	DMSO	NS
Lactic	3.86	DMSO	NS

^a From Kreiling et al. (2008).

^b See Roberts et al. (2016) for calculation of this EC3 value.

Table 4
Sugar surfactants investigated by Garcia et al., 2010.

General structure xylosides	General structure glucosides	LLNA EC3, %	GPMT
			
RCH ₂ =C8–10	RCH ₂ =C8–10	9.8; NS	NS
	RCH ₂ =C10–14	NS	NS
	RCH ₂ =C12–18A	NS	NS
	RCH ₂ =C12–18 B	8.4	NS
	RCH ₂ =C14	5.9	NS
	RCH ₂ =C16–18	NS	NS
	RCH ₂ =C18 branched	0.43	NS
	RCH ₂ = > C18	NS	NS
RCH ₂ = > C18 branched		3.8	NS

pre-haptens with the sec- or tert-allylic hydrogen alert (in this case unsaturated fatty acids) are not necessarily allergenic. Rather, they can be transformed with sufficient exposure to oxygen to an allergenic hydroperoxide derivative (Fig. 2). Again, the open application and thin layer of test material in the LLNA protocol facilitates autoxidation during the assay. In contrast, in occluded test protocols (i.e., GPMT and HRIPT) the marked reduction in exposure to oxygen sharply limits the extent of oxidation and formation of definitive allergen. The non-sensitiser results for fumaric and succinic acids in the LLNA are consistent with the above rationale as neither have sec- or tert-hydroperoxides alerts.

The positive LLNA result with maleic acid may seem at first surprising. Maleic acid is non-electrophilic (i.e. not directly protein reactive) and there are no autoxidation alerts. The result can be rationalised as follows. In aqueous solution, maleic acid is in equilibrium with maleic anhydride + water (Fig. 5).

Maleic anhydride, 2,5-furandione, is highly reactive as a Michael acceptor towards thiols (Dmuchovsky et al., 1964) and as an acyl transfer agent (Schwöbel et al., 2011; Rodriguez-Sanchez et al., 2013). In aqueous systems the equilibrium favours the acid, while in non-aqueous systems the equilibrium favours the anhydride. Again, consideration of the test protocols can rationalise the different results between the LLNA and the GPMT. Specifically, the LLNA vehicle was DMSO (i.e., non-aqueous), while the GPMT vehicle was aqueous saline. Thus, the mice would have been exposed more than the guinea pigs to the highly reactive maleic anhydride. Maleic anhydride itself has an LLNA EC3 value of 0.16% (Natsch et al., 2013), and comparing this with the observed EC3 of ca. 3% (estimated from the SI value of 6.7 at 10% by the rLLNA method (Roberts, 2015) for maleic acid we can calculate that about 5% of the maleic acid acts as the anhydride under the LLNA conditions used. We note that the maleic acid/anhydride dynamic equilibrium will probably cease to be significant after the test material has been applied to the skin, since at skin pH the maleic acid will be predominantly in its ionised forms. The important issue is the position of the acid/anhydride equilibrium when the test material is ready to be applied.

An alternative interpretation of the positive LLNA result for maleic acid is suggested by consideration of LLNA results for other low molecular weight acids that show no reactivity alerts or alerts for hydroperoxide formation by autoxidation. Table 3 shows LLNA data (NICEATM, 2015) and pKa values (first ionisation for dibasic acids) for these acids.

The three acids that are positive in the LLNA are all relatively strong acids, with pKa values below 2, whereas the ones that are

negative are all relatively weak with pKa values above 3. This may indicate that the positive results in the LLNA are the result of high acidity – possibly these acids are borderline corrosive and can cause significant hydrolysis of skin tissue.

1.2. Group 2. sugar surfactants investigated by Garcia et al. (2010)

Table 4 shows the 10 compounds investigated in this study, together with the LLNA, GPMT and HRIPT findings. Eight of the compounds are glucosides and two are xylosides. The aim of the Garcia et al. (2010) study was to address the hypothesis that non-ionic sugar lipid surfactants may result in a higher number of un-specific (false positive) results in the LLNA compared to the GPMT and HRIPT. Garcia et al. (2010) report that of the ten surfactants tested in the LLNA, five demonstrated $SI \geq 3$ (i.e., classified as sensitizers). Three of five positive reactions were concomitant with signs of skin irritation. In the GPMT, all ten surfactants were classified as non-sensitisers. In the HRIPT, no skin reactions suggestive of sensitisation were observed. It was concluded that the results are indicative of the LLNA overestimating sensitisation potentials for this category of chemicals (Garcia et al., 2010). Furthermore it was proposed that the overestimation of sensitisation may, in part, be due to irritant effects generated by the surfactants. We note however that the chemical structures for all of these compounds show three structural alerts for ether autoxidation with formation of hydroperoxides. The reaction sites are indicated, in the structural formulae shown in Table 4, by arrow heads. Hydroperoxides derived from ethers have been shown to be potent allergens (Bodin et al., 2003).

Pre-haptens with the ether-hydroperoxide alert are not necessarily allergenic. Rather, they can be transformed with sufficient exposure to oxygen to an allergenic derivative (Fig. 6).

Again, the open application and thin layer of test material in the LLNA protocol facilitates autoxidation during the assay. In contrast, in occluded test protocols (i.e., GPMT and HRIPT) the marked reduction to exposure to oxygen sharply limits the extent of oxidation and formation of definitive allergen. Hence the absence of sensitisation in the GPMT and HRIPT findings, and the observation of sensitisation for half of the compounds tested in the LLNA, can be rationalised in terms of autoxidation to allergenic hydroperoxides occurring to different extents.

1.3. Group 3. surfactants investigated by Ball et al. (2011)

Table 5 shows 8 surfactants investigated by Ball et al. (2011).



Fig. 6. Ether hydroperoxide alert.

Table 5
Surfactants investigated by Ball et al., 2011.

Name	LLNA EC3, %	GPMT
SLS	9.6	NS
C16EO2	2.2	NS
C14EO4	2.8	NS
C12EO6	7.4	NS
Isononyl glucoside	12.1	NS
Thioglucopyranoside	8.3	NS
Sodium nonanesulphonate	NS	NS
Decylphenol ethoxylate	2.9	NS

Two of these are anionic surfactants (SLS and sodium nonanesulphonate), four are ethoxylated alcohols (including one ethoxylated phenol) and two are non-ionic sugar surfactants (isononyl glucoside and thioglucopyranoside, neither of which were investigated in the Garcia et al. (2010) study summarised in Table 4).

Ball et al. (2011) conducted a battery of *in vivo* and *in vitro* tests for these eight surfactants. In general, the surfactants were negative in the GPMT, KeratinoSens™ (Emter et al., 2010, 2013) and the human Cell Line Activation Test or h-CLAT (Sakaguchi et al., 2007) and none formed covalent adducts in a peptide reactivity assay (Natsch and Gfeller, 2008) that detects covalent binding to a cysteine thiol unit in a synthetic peptide. In contrast, all but one were positive in the LLNA. Most were rated as being irritants by the EpiSkin assay with the additional endpoint, IL1- α . The weight of evidence based on this comprehensive testing could be interpreted to mean that, with one exception, these surfactants are non-sensitising skin irritants and the LLNA results are false positives. However we do not consider that this would be a safe conclusion, since there is a simpler explanation. Only one of the eight compounds tested has no alerts – this is sodium nonanesulphonate, which is the only one giving a non-sensitiser result in the LLNA. Of the remaining seven, six of them have alerts for ether (in one case also thioether) autoxidation giving allergenic hydroperoxides (Fig. 5). Again, the open application and thin layer of test material in the LLNA protocol facilitates autoxidation during this assay relative to the GPMT and HRIPT. None of these six compounds are directly electrophilic, nor do they have alerts for activation, other than by autoxidation,¹ to electrophilic species, so they would not be expected to give positive results in the peptide binding assay or the h-CLAT and KeratinoSens™ assays.

The only chemical in Table 5 whose positive LLNA result cannot be explained in terms of autoxidation is SLS, sodium lauryl sulphate. SLS consistently gives positive LLNA results close to EC3 = 10%, but is widely considered to be a false positive in the LLNA (Basketter et al., 2009a). It does have an alert for reactivity as an S_N2 electrophile, but its reactivity is low (for example its reaction with dimethylamine to manufacture long chain amines requires temperatures of about 150 °C) (French patent 1346673 (1963)). We cannot completely exclude the possibility that it is able to be metabolically activated in the mouse, to a greater extent than in

humans and guinea pigs – in which case SLS would be a true positive in the LLNA (i.e. the animals are genuinely sensitised).

Ball et al. (2011) note these findings confirming that the LLNA tends to overestimate the sensitisation potential of surfactants. Therefore, results obtained from LLNAs should not automatically be considered as the gold standard for the development of new non-animal alternative test methods; rather it is necessary to carefully evaluate the applicability domains of test methods in order to develop reliable non-animal alternative testing strategies for sensitisation testing.

2. Conclusions

With the possible exception of SLS, all of the chemicals found by Kreiling et al. (2008), Garcia et al. (2010) and Ball et al. (2011) to be positive in the LLNA but negative in the GPMT can be rationalised without the need to invoke the LLNA cell proliferation arising from any source other than sensitisation. In other words, there are alerts to justify the conclusion that the animals tested in the LLNA are genuinely sensitised. In most cases the sensitisation can be explained by a pre-hapten mechanism in which the test compound gives rise to an allergenic hydroperoxide. The exceptional case is maleic acid, where it is proposed that maleic anhydride may be the real sensitiser.

We need however to consider to what extent the positives in the LLNA may be false positives in terms of predicted potency in humans, i.e. is the LLNA over-sensitive for the types of materials discussed here? To address this issue we start by considering oleic acid, linoleic acid and linolenic acid. All of these three acids, in the form of their sodium or potassium salts, are found at substantial levels (oleic in the 10s of %) in soaps and detergents. We note that at physiological pH carboxylic acids are predominantly ionised, so from the skin's perspective oleic acid and sodium oleate deliver the same molecular species. Clothing washed in soaps or detergents is often hung outside in sunlight to dry, which would give ideal conditions for autoxidation of any susceptible materials absorbed onto the surface of the fabric, then worn for extended periods in contact with skin. Yet to our knowledge, contact allergy to soap is rarely, if at all, reported. A study of skin effects associated with wearing fabrics washed with commercial laundry detergents (Rodriguez et al., 1994) showed no evidence of any sensitisation effects. We further note that oleic acid, linoleic acid and linolenic acid in the form of their mixed triglyceride esters are major components of many animal and vegetable oils used both domestically and industrially, yet again as far as we know there are few if any reports of contact allergy to such oils. Our conclusion is therefore that although oleic, linoleic and linolenic acids can sensitise mice, they have no significant sensitisation potential in humans. Similar arguments apply to the non-ionic surfactants discussed here.

An important difference between the hydroperoxides derived from fatty acids and non-ionic surfactants on the one hand and hydroperoxides derived from terpenes such as limonene and linolool, is that the former are surface active and more hydrophobic than the terpene hydroperoxides. Also, in all cases the hydroperoxides derived from fatty acids and non-ionic surfactants are open chain and secondary, whereas the terpene hydroperoxides are tertiary or, when secondary, are cyclic. This leads us to two hypotheses, which are not mutually exclusive:

1. Possibly, being open chain and freely able to adopt conformations conducive to elimination, the secondary hydroperoxides derived from fatty acids and non-ionic surfactants may be shorter lived than the terpene-derived secondary hydroperoxides, decomposing to a significant extent by loss of water to form α,β -unsaturated ketones. These ketones would be expected to

¹ Here we distinguish between autoxidation, referring to reactions of carbon-hydrogen bonds with molecular oxygen and involving formation of peroxides and hydroperoxides, from the oxidation pathways by which chemicals such as hydroquinone and paraphenylenediamine are activated (Aptula et al., 2009).

sensitise as Michael acceptors, but would be expected to be less potent than the hydroperoxides.

- Because of their hydrophobicity and surface activity, the hydroperoxides derived from fatty acids and nonionic surfactants are more liable to partition strongly into the stratum corneum lipids, where they can be deactivated by reaction with unsaturated components of these stratum corneum lipids before they can reach the epidermal proteins that they would need to react with if they were to sensitise. We note that the mouse ear is relatively deficient in stratum corneum lipids, making the target epidermal proteins more accessible to hydrophobic/surface active hydroperoxides in the LLNA than under conditions of human exposure.

The above hypotheses have not yet been tested experimentally and therefore provide an explanation of the facts that is at present only provisional. However, the key findings are:

The differences between LLNA and guinea pig potency found by Kreiling et al. (2008), Garcia et al. (2010) and Ball et al. (2011) can be rationalised in terms of structural alerts and do not require a non-specific proliferation explanation.

The evidence indicates that the fatty acids and non-ionic surfactants discussed here, although able to sensitise mice, do not appear to have any significant human sensitisation potential. These differences, and contrast with terpene pre-haptens, are probably rooted in their physical chemistry and reaction chemistry. There is clearly scope to develop a better experimentally based understanding of structure-activity relationships for pre-haptens.

Conflict of interest

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

Acknowledgements

The authors thank Cefic LRI for financial support of this study (B14-RIFM: Skin Sensitisation – Chemical Applicability Domain of the Local Lymph Node Assay (LLNA)).

Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.yrtph.2016.07.018>.

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