Linalool
(CAS No. 78-70-6)

Position and comments of the REACH lead registrant

DSM Nutritional Products AG

on the proposed harmonized classification and labelling of linalool as a skin sensitizer category 1a

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1. Overall Summary and Conclusion

Since January 2012, linalool is on the registry of "Harmonized Classification and Labelling intentions" on the ECHA webpage. The endpoint considered in this intention is skin sensitization. The respective CLH report was expected to be submitted by 01-November-2012. The public consultation started on 24-June-2014 based on the CLH report ((2014) dated 28-May-2014).

The REACH lead registrant DSM Nutritional Products AG, takes the opportunity to present in detail scientific argumentation for our conclusion that classification and labelling of linalool as skin sensitizer category 1a is not justified.

Therefore, we compile in this document the following data on linalool:

(i) Information already presented in the DSM REACH Dossier submitted in 2010 (REACH Dossier (2010), of which not all data was taken into account in the CLH report (2014): state of the art skin penetration data (Green et al. 2007) and/or information on the absence of skin sensitization in human volunteers (Harrison and Spey 2005, Belsito et al. 2008).

(ii) Additional information on mechanistic investigation on skin sensitization of linalool in laboratory animal (Khan & Dearman 2010). These investigation clearly show that linalool is not a skin sensitizer in laboratory animal. This information was only available after submission of the DSM REACH Dossier in 2010 but this information was submitted to the evaluating Member State Sweden in September 2012.

(iii) New information in peer reviewed scientific literature.

Thus, our conclusions presented here are based on those data already included in the REACH Dossier (2010) together with data newly available.

Substance identity

This document and the substance identity presented in the DSM REACH Dossier (2010) refers to linalool with a purity between $\geq$ 96.7 and $\leq$ 98.2 % (w/w). Linalool is stabilized with an antioxidant (additive), alpha-tocopherol, in a concentration range of $\geq$ 0.02 to $\leq$ 0.03 % (w/w) which is part of the substance identity according to the definition of a substance in Regulation (EC) No 1907/2006 Article 3(1) and Regulation (EC) No 1272/2008 Article 2(7)).

It is noticed that non-relevant data were used in the CLH report (2014) throughout the document: For example data on lavender oil containing linalool, artificially produced mixtures such as "oxidized" linalool or data on linalool hydroperoxides were used to justify the proposed classification and labelling as skin sensitizer category 1a. All these substances / mixtures fail to meet the specifications of the substance identity for linalool as placed on the market. This is not in accordance with Regulation (EC) No. 1272/2008 Article 8 (6) which states "*Tests that are carried out for the purposes of this Regulation shall be carried out on the substance or on the mixture in the form(s) or physical state(s) in which the substance or mixture is placed on the market and in which it can reasonably be expected to be used*." Thus, for the purpose of discussing the skin sensitization potential of linalool only such information should be used which specifically addresses the substance linalool as given in the substance identity.

Reactivity of linalool upon air exposure

It is known that substances with allylic structural elements which are also present in linalool are prone to oxidation. Sköld et al. (2004) showed that pure non-stabilized linalool is

degraded and that linalool hydroperoxides and degradation products thereof are formed: after 10 weeks 30% of the initial linalool were degraded.

The conditions used in these investigations are not realistic and do not reflect normal production and use conditions. Adequate protection by antioxidants or a combination of antioxidants and further stabilizers such as UV-filters and/or chelating agents prevent such oxidative degeneration both in the pure substance and in personal care products (DSM 2014, Kern et al. 2014). The substance identity of linalool therefore specifies an antioxidant for stabilization (see also section 2).

In the CLH report (2014) it is argued (based on a paper by Karlberg et al. (1994)) that the use of antioxidants does not adequately protect against oxidation. However, the Karlberg paper studied limonene and its degradation but not linalool. We do not agree that such argumentation is adequate and refer to newer data which specifically addresses the question of potential oxidative degradation of linalool in the presence or absence of antioxidant both as pure substance or in personal care formulations (DSM 2014, Kern et al. 2014). These documents show that linalool is effectively protected from oxidation even under prolonged and accelerated storage conditions.

Toxicokinetics and metabolism upon dermal exposure

According to our interpretation of the available data, the key study on dermal penetration of linalool is the study of Green et al. (2007) in which ^{14}C-radiolabelled linalool was used and which was conducted in compliance with existing OECD guidelines on the conduct of an in vitro dermal penetration study (OECD 428 (2004)). This study was available in our REACH Dossier (2010) but not taken into account in the CLH report (2014). In contrast, published papers were used: The studies of Cal (2006a) or Cal & Sznitowska (2003) do not comply with the OECD 428 guideline (2004) because not all required samples were investigated. For details please refer to section 4.

Once applied to the skin, linalool quickly evaporates (see section 4.1, Green et al. 2007) from skin with only 7% of applied dose remaining after 1h. The available data on dermal absorption of linalool into the viable skin (epidermis and dermis) show that only a minor amount of the applied substance is absorbed being about 4% of the applied substance under non-occluded conditions within 24h.

We are in addition surprised about the use of data on other substances in the CLH report (2014). For example Cal et al. (2001) addressed limonene, diterpene, terpinolene, and eucalyptol, Cal (2006b) studied lavender oil, and Kitahara et al. (1993) did investigations with several terpenes but not with linalool. It is our opinion that such data should not be used when evaluating linalool.

Up to date we found no information whether any form of oxidized linalool once applied dermally is systemically available and/or whether oxidized linalool does penetrate through skin. The only information is that forms of oxidized linalool can induce skin sensitization upon dermal application (e.g. Sköld et al. 2002, 2004, Bezard et al. 1997). In addition, we have no evidence that any form of oxidized linalool can be formed by metabolism in the skin. Any conclusion that this occurs is highly speculative. We consider it therefore not justified to take such discussion into consideration.

Skin sensitization

Linalool was tested extensively with regard to skin sensitization potential in laboratory animal. Most of the data available show no sensitizing potential of linalool: Studies in guinea pigs (Sköld et al. 2002) showed no skin sensitization potential (negative result) for linalool. Some results of local lymph node assays (LLNA) may indicate weak sensitizing potential (Sköld et al. 2004, Basketter et al. 2002).

The concentrations being positive in the LLNA were always equal to 50% or higher (see Table 15 on page 32) and showed the typical behavior of a false positive result due to skin irritant properties as suggested by Basketter et al. (1998). A mechanistic investigation according to Gerberick et al. (2002) and Betts et al. (2007) was performed to investigate whether the positive responses in the LLNA were true or false positives. The results of this mechanistic LLNA performed showed that the positive responses seen in the standard LLNAs can be considered the result of skin irritant properties (Khan & Dearman 2010).

In addition, the purity of linalool was important in the LLNA studies: Linalool having been purified prior to the LLNA experiment produced lower responses than the non-purified quality (see Table 15 on page 32, Basketter et al. 2002). In further studies, it was clearly shown that the linalool autoxidation products hydroperoxides and some of their subsequent degradation products (epoxides and α,β-unsaturated aldehydes as well as mixtures thereof) were skin sensitizing (positive) in both the LLNA and the Freund's Complete Adjuvant Test (FCAT) (Sköld et al. 2002, Sköld et al. 2004, Bezard et al. 1997). For details please refer to Table 16 on page 33 and to Table 17 on page 34.

Overall, there are several reasons for the positive responses to linalool in the LLNA: (i) irritation properties of linalool at the higher concentrations, (ii) oxidation of the substance resulting in strongly sensitizing degradation products, (iii) a combination of (i) and (ii), and/or (iv) the use of a test item not complying with the relevant specifications.

Predictive human studies, amongst others a Human Repeated Insult Patch Test (HRIPT) on 135 healthy volunteers, also showed no skin sensitization potential (Harrison & Spey 2005). In the reported case studies (Schubert 2006; Schaller and Korting 1995), some patients showed positive patch test results with linalool; however, it is not clear whether the exposure of the individuals was only to pure linalool.

We are not aware on general population studies in terms of frequency of positive patch tests in the normal population. The CLH report (2014) gives a figure of 2%. However, this figure refers to oxidized linalool and not to linalool itself (see also sections 7.6 and 7.7).

In diagnostic human patch tests there were only very few positive reactions in patients (see Table 19 on page 40). Overall only 28 patients out of 12132 consecutive patients reacted positively. Likewise, the SCCS (2012) also concluded that there are less than 100 positive patch tests reported on the basis of the same database as presented here. Even in selected patients only 8 out of 461 showed positive reactions.

Conclusion

Mixtures of linalool with other substances such as essential oils or artificially aged linalool as well as isolated other substances such as linalool hydroperoxide do not comply with the substance identity and consequently such information is not relevant for linalool as placed on the market.

In this document we have shown that linalool as specified in the substance identity does not autoxidize under normal production and use conditions. Antioxidants successfully prevent the oxidative degeneration.

Thus, we do not agree with the proposal of a harmonized classification and labelling of linalool as a skin sensitizer (CLH report (2014)) which is based on the argumentation that it may be potentially oxidized to known skin sensitizers whereas this oxidation step is unlikely to occur under normal production and use conditions.

The dermal absorption of linalool is low because the majority of a substance on the skin evaporates. Only about 4% of the applied linalool penetrates into the deeper skin layers. We are not aware of information on the metabolism of linalool in skin or on the extent of dermal

absorption of linalool hydroperoxide or its degradation products. Thus, it should not be speculated about it.

We have shown that linalool is not a skin sensitizer in laboratory animals. Mechanistic investigations show that certain studies in mice (i.e. LLNAs) were false positive due to the skin irritant properties of linalool and/or the use of a test substance not being compliant with the test substance identity.

In terms of human data, studies in healthy volunteers showed not skin sensitizing properties and the overall frequency of patients reacting towards linalool is remarkably low also in consideration of its extensive use.

Based on the overall weight of evidence it is concluded that classification and labelling of linalool as skin sensitizer according to Guidance on the Application of the CLP Criteria of Regulation (EC) No 1272/2008 (November 2013) is not justified.

2. Substance identity

Linalool (CAS 78-70-6) is a naturally-occurring terpene alcohol found in plants with many commercial applications, the majority of which are based on its pleasant scent (floral, with a touch of spiciness). Linalool is also used as a chemical intermediate.

Table 1 Identity of linalool (as placed on the market)

Purity:	**Concentration range**
Linalool	≥ 96.7 — ≤ 98.2 % (w/w)
Impurities:	
Linalool structurally related impurities	
Additive for stabilization	
dl-alpha-tocopherol (EC no.: 233-466-0)	≥ 0.02 — ≤ 0.03 % (w/w)

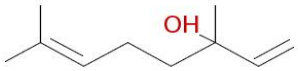


Figure 1 Chemical structure of linalool

More details on the composition are not included in this document for confidentiality reasons. These details are of course available upon request in a separate document.

3. Oxidation of linalool as pure substance and of formulated linalool

Linalool can autoxidize upon exposure to air thereby forming allylic hydroperoxides (Sköld et al. 2004). These hydroperoxides are thought to either degrade as described by Bezard et al. 1997 (see also Figure 4 on page 26) or Kern et al. 2014 and/or to react with protein/amino acids by radical processes (Kao et al. 2014).

In this section we summarize the existing data on the formation of hydroperoxides under different conditions being

(i) artificial aging of pure linalool (purity > 97%, not stabilized by an antioxidant) as described e.g. by Sköld et al. (2004).

(ii) In addition, we provide results of experiments on pure substance (stabilized by an antioxidant) upon exposure to air (DSM 2014).

(iii) Finally, we make reference to stability experiments performed with formulated linalool in typical consumer products which show no oxidative degeneration (Kern et al. 2014).

3.1. Oxidation of pure, non-stabilized linalool

Under accelerated oxidation conditions as described in Table 2 below, pure linalool (most likely in the absence of antioxidants) degrades to 80% of the initial concentration within 10 weeks, after 45 weeks only 30% linalool is left. In two cases it is clear that the pure linalool was distilled prior to the experiment (Christensson et al. 2010 and 2012). Thus, any antioxidant which may have been present was removed by this process.

Concurrently, the content of hydroperoxides (major hydroperoxide 7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol (usually about 80% of the hydroperoxide content) and minor hydroperoxide 6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-ol) increases up to about 19% at the maximum.

Table 2 Overview on occurrence of autoxidation product linalool hydroperoxides using pure but non-stabilized linalool

Purity	Supplier	Conditions to air exposure	Duration	Content linalool at end	Content hydroperoxide	Reference
97%[5]	Sigma-Aldrich	see Sköld et al. 2004[4]	25 weeks	61%	14.6% of major hydroperoxide[1]	Christensson et al. 2012
97%	Sigma-Aldrich	see Nilsson et al. 1996[4]	45 weeks	30%	19% linalool hydroperoxides	Christensson et al. 2009
97%[5]	Sigma-Aldrich	see Karlberg et al. 1992[4]	45 weeks	30%	19% linalool hydroperoxides[2]	Christensson et al. 2010
97%	Lancester and Sigma-Aldrich	see Karlberg et al. 1992[4]	45 weeks	30%	16% linalool hydroperoxides[3]	Matura et al. 2005
97%	Lancester	see Karlberg et al. 1994[4]	10 weeks	80%	n.i.	Sköld et al. 2002
97%	Lancester and Sigma-Aldrich	see Karlberg et al. 1992[4]	10 weeks	75%	4% hydroperoxides	Sköld et al. 2004
			45 weeks	30%	15% hydroperoxides	

[1] 7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol
[2] 15% 7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol and 4% 6-hydroperoxy-3,7-dimethylocta-1,7-diene-3-ol
[3] 83% of the hydroperoxides were 7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol
[4] exposure to air in an Erlenmeyer flask at room temperature for a given time, the substance is stirred 2-4 times a day for 1 h
[5] purified by distillation before use

n.i. not indicated

3.2. Oxidation of antioxidant protected linalool

To study the potential oxidation of linalool upon exposure to air linalool either in the presence or absence of antioxidant was stirred continuously in open glass bottles for up to 23 or 28 days under aerobic conditions at room temperature (20°C) or at 40°C. The amount of peroxides were measured at regular intervals (DSM 2014). For the measurement a semi-quantitative colorimetric method was used and the results are expressed as mg H_2O_2/L. For comparison to the published results described in section 3.1 and 3.3, we additionally expressed the results in µg/g and %.

The results of these experiments show that indeed linalool not protected by antioxidant formed peroxides whereas the stabilized linalool was not oxidized. In Table 3 we show the results of the experiment with stabilized and non-stabilized linalool upon exposure at ambient temperature.

The experiments show that formation of peroxides is effectively inhibited in stabilized linalool samples. The experiment indicates that oxidation to hydroperoxides in non-stabilized linalool appears to be a slow process. It is also remarkable that in DSM experiments after 23 days, the concentration of linalool hydroperoxides was only 0.019-0.06% (Table 3 bottom) in comparison to hydroperoxide levels of 4% after 10 weeks in experiments reported by Sköld et al. (2004) (Table 2).

Table 3 **Concentration of linalool hydroperoxide in linalool samples with or without dl-alpha-tocopherol incubated for 23 days at ambient temperature.**

Date	Time	days of incubation	Linalool (0 mg/kg Tocopherol)			Linalool (>200 mg/kg Tocopherol)		
			H_2O_2 (mg/L)[2]	linalool hydroperoxide (µg /g linalool)[1]	Linalool hydroperoxide (%)	H_2O_2 (mg/L)[2]	linalool hydroperoxide (µg /g linalool)[1]	Linalool hydroperoxide (%)
05/06/2013	15:00	Start	2	12.8	0.0013	0	0	0
06/06/2013	15:10	1	2	12.8	0.0013	0	0	0
07/06/2013	14:35	2	5	32	0.0032	0	0	0
08/06/2013	11:30	3	5	32	0.0032	0	0	0
09/06/2013	12:00	4	5	32	0.0032	< 0.5	<3.2	<0.00032
10/06/2013	15:10	5	5-10	32-64	0.0032-0.0064	< 0.5	<3.2	<0.00032
11/06/2013	14:00	6	10	64	0.0064	< 0.5	<3.2	<0.00032
12/06/2013	14:00	7	10-25	64-160	0.0064-0.016	< 0.5	<3.2	<0.00032
13/06/2013	14:40	8	10-30	64-192	0.0064-0.019	< 0.5	<3.2	<0.00032
14/06/2013	15:30	9	10-30	64-192	0.0064-0.019	< 0.5	<3.2	<0.00032
15/06/2013	15:30	10	10-30	64-192	0.0064-0.019	< 0.5	<3.2	<0.00032
16/06/2013	13:00	11	10-30	64-192	0.0064-0.019	< 0.5	<3.2	<0.00032
17/06/2013	13:35	12	10-30	64-192	0.0064-0.019	< 0.5	<3.2	<0.00032
18/06/2013	13:40	13	30	192	0.019	< 0.5	<3.2	<0.00032
19/06/2013	14:00	14	30	192	0.019	< 0.5	<3.2	<0.00032
21/06/2013	14:10	16	30	192	0.019	< 0.5	<3.2	<0.00032

Date	Time	days of incubation	Linalool (0 mg/kg Tocopherol)			Linalool (>200 mg/kg Tocopherol)		
			H_2O_2 (mg/L)[2]	linalool hydroperoxide (µg /g linalool)[1]	Linalool hydroperoxide (%)	H_2O_2 (mg/L)[2]	linalool hydroperoxide (µg /g linalool)[1]	Linalool hydroperoxide (%)
23/06/2013	13:50	18	30	192	0.019	< 0.5	<3.2	<0.00032
25/06/2013	13:40	20	30	192	0.019	0.5	3.2	0.00032
28/06/2013	13:15	23	30-100	192-640	0.019-0.064	0.5	3.2	0.00032

[1] the concentration of hydroperoxides present in linalool expressed in mg H_2O_2/L was converted into µg linalool hydroperoxide / g linalool using the factor of 6.4 as explained below:
x mg H_2O_2 / L = x (186.25 / 34) mg linalool hydroperoxide / L with 186.25 and 34 being the molecular weights of linalool hydroperoxide and H_2O_2, respectively
x L linalool = 860 g linalool with the density of linalool being 0.86 g/cm^3

[2] the semi-quantitative colourimetric method gives ranges of H_2O_2 concentrations

3.3. Oxidation of formulated linalool under normal in-use conditions

The accelerated oxidation conditions without any stabilizer as described in Table 2 are not reflective of the normal storage/handling conditions of formulated personal care products and therefore Kern et al. 2014 performed additional experiments with more realistic conditions thereby also applying the stability testing at higher temperature usually used in personal care industry. Several formulations were prepared amongst others a hydroalcoholic fragrance which was stored for 45°C in a half-empty flask (for two and nine months) which was opened every 14 days for 1 minute. The authors used two different linalool sources: synthetic linalool and natural linalool. In addition, they analyzed old products recalled from consumers.

A 2 months storage at 45°C reflects the storage of a product for 8 months at room temperature (stability study). The results indicate that linalool is stable when formulated into typical hydroalcoholic products either in the presence or absence of a stabilizer (see Table 4). In addition, the content of the hydroperoxide degradation products was not increasing with time. Thus, there was no evidence for the oxidation of linalool to the hydroperoxides in the final hydroalcoholic products.

Low levels of hydroperoxides (max. 83 µg/g formulation corresponding to 0.0083%) were mainly detected in products formulated from the natural linalool source.

The aged re-called 39 fragrance samples had hydroperoxide contents in a similar range as observed in the stability study.

Thus, there was no indication of degradation of formulated linalool under normal use conditions.

Table 4 **Linalool hydroperoxide content (µg/g formulation, mean +/- SD)) in hydroalcoholic fragrances (containing initially 10% linalool) after a storage period of two and nine months at 45°C (taken from Kern et al. 2014)**

Personal care product type	Nominal Linalool (Linalool origin)	Stabilizer	Content linalool at end	Content hydroperoxides[a]	Content linalool at end	Content hydroperoxides[b]
			2 months storage		9 months storage	
Hydroalcoholic fragrance, ½ full flask, opened every 14 days for 1 minute	10% (synthetic[3])	yes[1]	97330+/-1666	<LOD	110553+/-2499	18+/-0.4
		no	104931+/-2552	<LOD	113100+/-5102	15+/-0.2
	10% (natural[4])	yes[1]	105429+/-7797	64+/-3	105780+/-9042	83+/-4
		no	110298+/-545	74+/-1	107732+/-5033	83+/-4

1 0.05% of the antioxidant tert-butyl-hydroxytoluene (BHT), 0.05% of the chelating agent ethylenediaminetetraacetate (EDTA), and 0.2% of the UV-filtering formulation Covabsorb (ethylhexyl methoxycinnamate / butyl methoxydibenzoylmethane / ethylhexyl salicylate).

3 racemic mixture of 50% (-)-linalool and 50% (+)-linalool

4 98/2 isomeric excess of (-)-linalool

5 linalool was formulated at 10%

a analytical method has an LOQ of 50 µg/g

b analytical method has an LOD of 0.5 µg/g

3.4. Exposure consideration of linalool hydroperoxides to consumer

Overall, the available data indicate that exposure of consumers to linalool hydroperoxides is low due to the low concentrations found in consumer products (Kern et al. 2014). Consumer products and even old products re-called from consumers were below 20 µg linalool hydroperoxides /g.

4. Dermal penetration

One aspect when discussing skin sensitization is the question whether or not the substance can penetrate through the skin. Regulatory agencies provide clear guidance how such studies should be conducted and interpreted (OECD 428 (2004), SCCS 2010). Thereby the gold standard is the use of ^{14}C-radiolabelled material.

For either in vitro or in vivo dermal penetration studies, all guidelines and guidance documents require a recovery of the applied test substance of 100 +/- 15%. Of course this high recovery is difficult to obtain when testing highly volatile substances. In the case of linalool, Green et al. 2007 found that linalool very rapidly evaporates with less than 7% recovered 1 h after application. After 24h, 3% were recovered indicating that approximately 97% of the applied dose had been evaporated within 24h.

Table 5 gives an overview on existing studies on dermal penetration of linalool. Importantly, the most robust study (Green et al. 2007) is a non-published study being part of the REACH Dossier submitted in 2010. However, this study was not considered in the CLH report (2014). This study is summarized in more detail in section 4.1.

All other literature on dermal penetration of linalool lacks information on the overall recovery of the material mainly because not all samples were analyzed: for example often the amount of the test substance in the washing solution or the remaining material in the penetration chamber were neglected (Cal & Sznitowska 2003, Cal 2006a). Nevertheless in Table 5, an attempt is made to calculate the extent of dermal penetration from the available data thereby using the respective guidance of the SCCS (2010). Specifically, the a mount of the test substance in the stratum corneum is NOT considered systemically available.

Other experiments were done with lavender oil and other essential oils (Cal 2006) or used other substances of the terpene family only (Cal et al. 2001, Kitahara et al. 1993) but provide no information on linalool. The essential oils contain high amounts of linalool, however, these preparations are distinct from the substance definition (see section 2) and are therefore not relevant.

The results from the dermal absorption studies show that the majority of the applied linalool will evaporate quickly within 1h thereby limiting the systemic bioavailability under non-occluded conditions to about 4% of the applied dose (Green et al. 2007).

Table 5 Summary of in vitro dermal penetration studies performed with either 14C-labelled linalool or non-radiolabelled linalool

	Concentration		Application time	Recovery (%)	Amount systemically available[3] (%)	Reference
In vitro human skin 5 µL of the preparation/cm^2 (=201 µg linalool/cm^2) 14C-linalool	4% in 70/30 ethanol/water	Occluded	24h	36.3+/-2.9[1]	14.4 +/- 1.2	Green et al. 2007 (in REACH Dossier 2010)
		Non-occluded		8.01 +/- 0.61[1]	3.57+/-0.5	
In vitro human skin 500 mg linalool/ 0.65 cm^2 (=769.2 mg/cm^2) Unlabeled linalool	linalool >95% from Fluka	Occluded	1 h	0.12[2]	0.11	Cal & Sznitowska 2003 (in REACH Dossier 2010)
			2 h	0.16[2]	0.14	
			4 h	0.23[2]	0.17	
In vitro human skin 100 mg preparation/cm^2 (=5 mg linalool/cm^2) Unlabeled linalool	5% (w/w) in grape seed oil	Occluded	1h	0.2[2]	0.09	Cal 2006a
			4h	3.1[2]	1.5	
	5% (w/w) in hydrogel		1h	4.8[2]	1.8	
			4h	7.7[2]	3.9	
	5% (w/w) in oil-in-water emulsion		1h	1.1[2]	0.5	
			4h	2.2[2]	1.1	

[1] within 1 h, 93% of the applied substance evaporates; within 24 h only 3% remain
[2] amount in skin wash was not investigated; linalool was not found in the acceptor fluid
[3] Amount of applied substance in epidermis, dermis, and receptor fluid; the amount in the stratum corneum is not considered to penetrate through skin (SCCS 2010)

4.1. Individual Studies on Dermal Penetration of Linalool

Green DM, Walters KA, Jones C (2007) In vitro human skin penetration of fragrance material linalool under both in-use and occluded conditions from an ethanol/water vehicle, An-eX Analytical Services, Report No. R03/13a/05, dated 03-October-2007

This study was designed to determine the in vitro human skin penetration rate and distribution of the fragrance material linalool following application in 70% ethanol solution, under occluded and non-occluded conditions, at a concentration of 4%. For the experiments ^{14}C-labelled linalool was used. Twelve active dosed diffusion cells were prepared (from 7 donors) for each application condition. Epidermal membranes were used and their integrity was assessed by measuring the permeation of tritiated water over a period of 1h. Permeation of linalool from a 5 µL/cm^2 dose of a 4% (w/v) solution in 70/30 (v/v) ethanol/water was then measured over 24h using a pH7.4 PBS receptor phase. For cells in the occluded group, a greased glass coverslip was applied to the donor chamber immediately after dosing. After 24h, the epidermal membranes were wiped, tape stripped (10 times) and the radioactivity content of the wipes, strips and remaining epidermis was determined. The filter paper skin supports were extracted and the diffusion cell donor chambers (and coverslip for occluded cells) wiped to remove sealing grease and washed. Analysis of the samples allowed mass balance to be performed. Potential evaporative loss of linalool was estimated by measuring the loss from polytetrafluoroethylene sheets under the same unoccluded experimental conditions.

Overall recoveries of the applied linalool at 24h were low at 8.01 +/- 0.61 and 36.3 +/- 2.9% under unoccluded and occluded conditions, respectively, due to evaporative loss. The assessment of evaporation under unoccluded conditions was rapid with less than 7% recovered one hour after application. By 24h, recovery was 3% of the applied dose. Following 24h exposure, 3.57 +/- 0.5 and 14.4 +/- 1.2% of the applied dose had penetrated for the unoccluded and occluded groups, respectively. Occluded conditions not only reduce loss of volatile application vehicle and the test compounds but also increased greatly skin hydration, and these factors clearly caused a significant increase in the penetration of linalool (see Table 6).

Table 6 **Summary of the ^{14}C-linalool in vitro skin penetration experiment (taken from Green et al. 2007)**

	Unoccluded conditions		Occluded conditions	
Compartment	**linalool (µg/cm^2)**	**linalool (% applied dose)**	**linalool (µg/cm^2)**	**linalool (% applied dose)**
Wipe	5.41 ± 0.31	2.67 ± 0.15	6.32 ± 0.53	3.14 ± 0.26
Donor chamber	2.16 ± 0.26	1.07 ± 0.13	36.7 ± 5.2	18.2 ± 2.6
Strip 1	0.399 ± 0.074	0.198 ± 0.037	0.317 ± 0.67	0.158 ± 0.033
Strips 2-3	0.553 ± 0.128	0.274 ± 0.064	0.312 ± 0.059	0.155 ± 0.029
Strips 4-6	0.295 ± 0.047	0.146 ± 0.024	0.227 ± 0.037	0.113 ± 0.019
Strips 7-10	0.172 ± 0.028	0.085 ± 0.014	0.161 ± 0.028	0.080 ± 0.014
Epidermis	0.650 ± 0.129	0.321 ± 0.064	1.02 ± 0.15	0.506 ± 0.073
Filter paper	0.609 ± 0.055	0.301 ± 0.027	2.39 ± 0.20	1.19 ± 0.10
Permeated	5.95 ± 0.91	2.95 ± 0.46	25.6 ± 2.2	12.7 ± 1.1
Overall recovery	16.2 ± 1.2	8.01 ± 0.61	73.0 ± 5.8	36.3 ± 2.9

Cal K, Sznitowska M (2003) Cutaneous absorption and elimination of three acyclic terpenes—in vitro studies, Journal of Controlled Release 93: 369– 376

Frozen human skin from 1 donor was used in flow-through diffusion cells. The diffusion are was 0.65 cm^2. Following the 24h-equilibrium, 500 mg linalool (equivalent to 769.2 mg/cm^2) was applied onto the skin under occluded conditions. After 1, 2, or 4h, the skin was washed. The stratum corneum was removed by tape stripping. The strips, the remaining skin (dermis and epidermis), and the receptor medium were analyzed for linalool. The experiments were run in quadruplicates.

The measured linalool concentrations are summarized in Table 7. Linalool was not detected in the receptor fluid. The overall recovery of linalool in these experiments were low, not only because the washing solution was not analyzed. Nevertheless, based on the comparison of applied linalool with the measured concentration of linalool in the epidermis, dermis, and receptor fluid, it was calculated that less than 0.2% of the applied radioactivity were systemically available.

Table 7 **Absorption of linalool (µg/cm^2) into human skin layers (mean +/-SD, n=4) (taken from Cal & Sznitowska M (2003)): 769.2 mg/cm^2 were applied on the skin.**

Skin layer*	Time		
	1 h	2 h	4 h
Linalool			
SC I	34.3 ± 8.8	65.9 ± 36.5	242.6 ± 36.4
SC II	22.4 ± 3.5	22.4 ± 4.7	144.3 ± 13.2
SC III	21.6 ± 3.7	20.8 ± 6.1	92.0 ± 12.9
SC total	78.3 ± 4.1	109.1 ± 5.6	478.9 ± 18.8
ED	827.0 ± 66.5	1083.5 ± 106.1	1343.0 ± 127.1
Skin total	905.3 ± 64.7	1192.6 ± 104.1	1821.9 ± 135.8

*SC–stratum corneum, ED–epidermis and dermis, SC I–III–fractions of the stratum corneum layers.

Cal K (2006a) How does the type of vehicle influence the in vitro skin absorption and elimination kinetics of terpenes?, Arch Dermatol Res 297: 311–315

In a further experiment, the systemic bioavailability of linalool when applied in formulations was investigated: Linalool was formulated at 5% (w/w) into three types of vehicles: grape seed oil, hydrogel, and oil-in-water emulsion. 100 mg/cm^2 were applied to the flow-through diffusion chamber which was occluded. After 1 and 4 h, the skin was washed and the stratum corneum was removed by tape stripping. The tape strips, the epidermis and dermis as well as the receptor fluid were analyzed for linalool. Again, the remaining samples were not analyzed.

Recovery was low: maximally 8% of the applied substance were found. The maximum amount of linalool being systemically available was 4% of the applied linalool (see Figure 2).

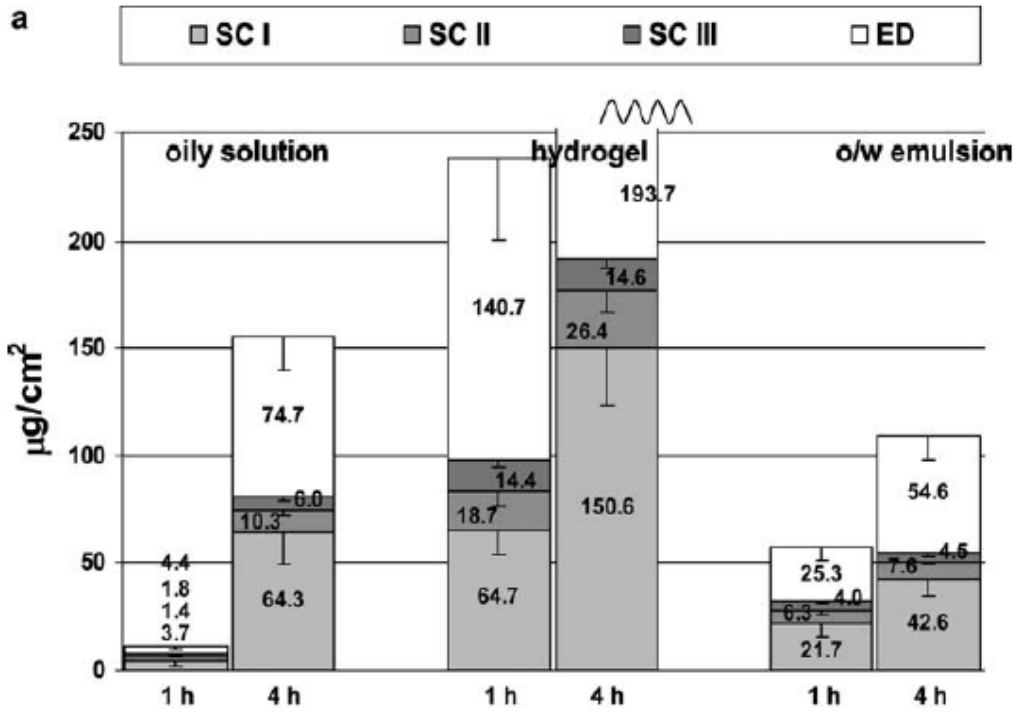


Figure 2 **Absorption of linalool in different vehicles into human skin layers (mean +/-SD, N=4), SC: Stratum corneum, ED epidermis and dermis (taken from Cal 2006a)**

4.2. Dermal penetration of linalool hydroperoxides

Information on the skin penetration of oxidized linalool mixtures, linalool hydroperoxides, and/or their degradation products was not identified.

5. Metabolism of linalool in skin

Information on the metabolism of linalool in skin preparations or metabolism investigation after application of linalool to skin (in vitro or in vivo) was not identified. The metabolism of linalool upon oral administration is described in detail in the REACH Dossier (2010).

In one publication (Meesters et al. 2007), the metabolism of linalool by recombinant enzymes CYP2D6 and CYP2C19 was investigated. (R/S)-furanoid-linalool oxide, (R/S)-pyranoid-linalool oxide and (cis/trans)-8-hydroxylinalool were found as metabolites (see Figure 3). The identity of the first two metabolites was confirmed by reference substances, the latter one was confirmed by calculation of Kovacs Indices (KI) and interpretation of the EI fragmentation pattern. Dihydrolinalool was also identified, but is an impurity in linalool.

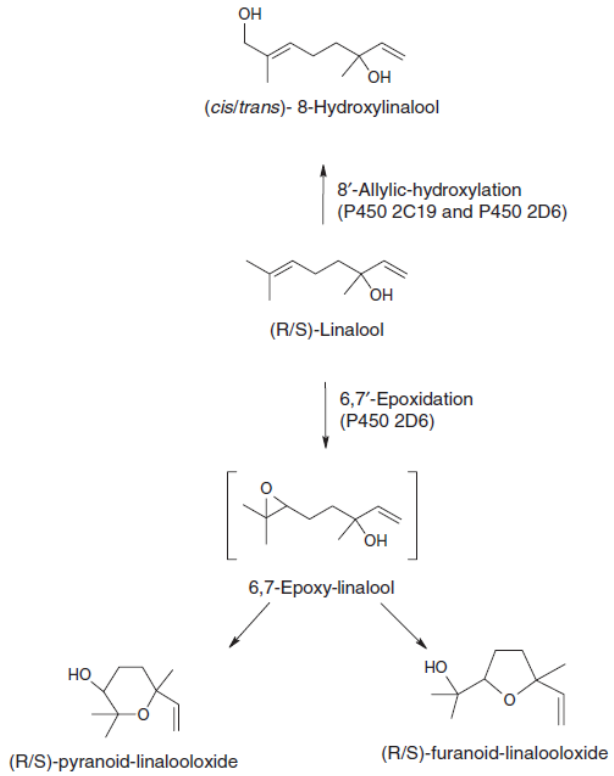


Figure 3 **Postulated enzymatic reaction of linalool to the furonoid and pyranoid linalool degradation products (taken from Meesters et al. 2007)**

The furanoid and pyranoid metabolites are discussed as degradation products of linalool hydroperoxides (Kern et al. 2014). Their presence could show indirectly that epoxides may have been formed.

However, these very early artificial experiments with recombinant enzymes only show the possibility for a formation. Up to now, the presence/formation of linalool epoxides was not confirmed under more realistic conditions being reflective of the actual exposure situation.

6. Skin Sensitization

In this section we summarized the published data on skin sensitization. We take into account data considered already in the REACH Dossier (2010) and additional data being available only after submission.

6.1. Individual Animal Studies

Basketter DA, Wright ZM, Colson NR, Patlewicz GY, Smith Pease CK (2002) Investigation on the skin sensitizing activity of linalool, Contact Dermatitis 47:161-164.

Linalool (97% pure obtained from Aldrich) was analyzed for impurities and found to contain dihydrolinalool (1.92%), linalool oxide (0.66%), 3-hexenyl butyrate (0.18%), epoxylinalool (0.14%), and 3,7-dimethyl-1,7-octadiene-3,6-diol (0.10%). Re-distilled / purified linalool (98.6%) did not contain the before mentioned impurities (below the respective LOD) except dihydrolinalool (1.4%). Both the non-purified linalool and the purified linalool were separately tested in a local lymph node assay. Groups of 4 CBA/Ca mice (7–12 weeks of age, Harlan Olac, UK) were treated with 25 µL of each test material at concentrations of 25, 50, or 100%, or with an equal volume of the vehicle (4:1 v/v acetone/olive oil) alone on the dorsum of both ears. Treatment was performed once daily for 3 consecutive days. Five days following the initiation of exposure, all mice were injected via the tail vein with 250 µL of phosphate-buffered saline (PBS) containing 20 µCi of tritiated thymidine. Mice were sacrificed 5 h later and the draining lymph nodes excised and pooled for each experimental group. A single cell suspension of lymph node cells was prepared by mechanical disaggregation. The lymph node cell suspension was washed twice in an excess of PBS and then precipitated with 5% trichloroacetic acid (TCA) at 4°C for 18 h. Pellets were resuspended in TCA and the incorporation of tritiated thymidine was measured by β-scintillation counting.

Prior to purification, linalool elicited a positive lymphocyte (sensitization) response with Stimulation indices (SI) of 2.6, 4.8, and 8.3 at 25, 50, and 100% concentration, respectively (see also Ryan et al. 2000 reporting identical results). Thus, non-purified linalool was a weak skin sensitizer under the conditions of the study with an EC3[1] value of 30%.

Table 8 **Comparison of LLNA results of non-purified linalool and purified linalool**
(copied from Basketter et al. 2002)

Sample	^{3}HTdR incorporated per lymph node (DPM/node)				
	0%	25%	50%	100%	EC3 value
Original linalool	361	919	1718	2988	30%
Purified linalool	366	771	1043	1777	55%

The purified linalool had SI of 2.1, 2.8, and 4.9 at 25, 50, and 100% concentration, respectively. Purified linalool had an EC3 value of 55%. Thus, a purification step to eliminate most impurities was shown to significantly reduce the positive response in the LLNA.

[1] EC3 Estimated Concentration needed to produce an SI of 3. An SI $\geq$3 is indicative for a positive response (induction of skin sensitization).

Sköld M, Börje A, Matura M, Karlberg AT (2002) Studies on the autoxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide, Contact Dermatitis 46:267-272.

Linalool (97% pure from Lancaster synthesis Ltd) was tested in the Freund's Complete Adjuvant Test (FCAT) and its modified version with closed challenge. Another linalool sample was stirred in a flask (the top was covered with para film) at room temperature for 1h, 4 times a day for in total 10 weeks. This oxidised linalool was used in the skin sensitization experiment 2. The 10-week air-exposure of linalool reduced the linalool content to about 80%. The resulting complex mixture contained several alcohols and hydroperoxides of which one was identified as 7-hydroperoxy-3,7-dimethyl-octa-1,5-diene-3-ol.

The sensitization experiments were performed in female Dunkin-Hartley albino guinea pigs (average weight 300–400 g) obtained from HB Lidköpings Kaninfarm (Lidköping, Sweden) and Bio Jet service (Uppsala, Sweden). The animals were housed in Macrolon cages, kept on a guinea pig standard diet and water *ad libitum*. Two experiments were carried out. The concentrations used at challenge were chosen on the basis of pretests on FCA-treated animals, where all the concentrations were shown to be non-irritating.

Experiment 1. For induction, the test animals (*n*=14) received intradermal injections (0.1 mL) of linalool (5.1% (w/w)) on the upper back on days 0, 6 and 9. The test substance was dissolved in an FCA[2]/water (1/1) emulsion for the 1st injection and in an FIA[3]/water (1/1) emulsion for the 2nd and 3rd injections. The animals in the control group (*n*=14) were injected with only the FCA/water and the FIA/water emulsions, respectively. Challenge testing was performed on D19. The test material, linalool 5.1% in petrolatum and a vehicle control (petrolatum) were applied on shaved flanks for 24 h and reactions were assessed at 48 h and 72 h after the start of exposure. The minimum criterion for a positive reaction was a confluent erythema.

No reactions to linalool were found in the exposed animals or in the controls. Pure linalool did not sensitize the animals.

Experiment 2. The experiment was performed according to the procedure described above. Two groups of 15 animals each were used. Induction was performed on days 0, 5 and 9. The exposed group was induced with oxidized linalool (5.1% w/ w) and the control group was treated with the FCA(FIA)/water emulsions. Challenge testing was performed on D20. Oxidized linalool was tested at concentrations of 1.0% and 5.1%. On D47 the animals were re-challenged with oxidized linalool at concentrations 2.6% and 10.3%. They were also challenged on D47 with a sample of non-purified linalool (5.1%) that had been stored in the refrigerator during the study. At both challenge and re-challenge, a vehicle control with petrolatum was applied on all animals.

[2] Freund's Complete Adjuvant

[3] Freund's Incomplete Adjuvant

The animals induced with the oxidised linalool showed sensitization responses at concentrations of 2.6, 5.1, and 10.3% but not at 1.0% (Table 9).

Table 9 **Results from the FCAT on oxidized linalool** (copied from Sköld et al. 2002)

	No. of animals with positive reactions[a]							
	1st challenge				Rechalleng			
	Exposed[b] ($n=15$)		Controls ($n=15$)		Exposed[b] ($n=15$)		Controls ($n=15$)	
Test material	48 h	72 h	48 h[1]	72 h[2]	48 h[3]	72 h[4]	48 h[5]	72 h[6]
Oxidized linalool								
10.3%	nt	nt	nt	nt	13[d]	13[d]	4	5
5.1%	8[c]	7[d]	0	0	nt	nt	nt	nt
2.6%	nt	nt	nt	nt	4[e]	5[e]	0	0
1.0%	1	0	0	0	nt	nt	nt	nt
Non-oxidized linalool[f]								
5.1%	nt	nt	nt	nt	3	3	0	0
pet.	0	0	0	0	0	0	0	0

[a]No. of animals with a confluent erythema 48 and 72 h after application of the test material.
[b]Induction: 5.1% of oxidized linalool in FCA(FIA)/H_2O emulsion intradermally.
[c]Significantly different from controls, $p<0.001$.
[d]Significantly different from controls, $p<0.01$.
[e]Significantly different from controls, $p<0.05$.
[f]97% as stated by the producer. Not further purified.
nt = not tested.

Sköld M, Börje A, Harambasic E, Karlberg AT (2004) Contact Allergens Formed on Air Exposure of Linalool. Identification and Quantification of Primary and Secondary Oxidation Products and the Effect on Skin Sensitisation, Chemical Research in Toxicology 17:1697-1705.

Linalool (redistilled the day prior to start of the LLNA), linalool air oxidised for 10 and 45 weeks, 2 linalool hydroperoxides as a mixture of 7-hydroperoxy-3,7-dimethyl-octa-1,5-diene-3-ol and 6-hydroperoxy-3,7-dimethyl-octa-1,7-diene-3-ol (ratio 5/3), linalool aldehyde (6-hydroxy-2,6-dimethylocta-2,7-dienal), and the corresponding linalool alcohol were tested in the LLNA.

Female mice (9 weeks of age) in groups of four were treated by topical application on the dorsum of both ears with 25 µL of test material or with a vehicle control. Treatments were performed daily for 3 consecutive days (days 0, 1, and 2). On day 5, following the start of treatment, all mice were injected intravenously via the tail vein with 20 µCi of [methyl-^{3}H]thymidine in 250 µL of phosphate buffered saline (PBS). After 5 h, the mice were sacrificed, the draining lymph nodes were excised and pooled for each group, and single-cell suspensions of lymph node cells in PBS were prepared. Cell suspensions were washed twice with PBS, precipitated with 5% TCA, and left in the refrigerator overnight. The samples were then centrifuged, re-suspended in 1 mL 5% TCA, and transferred to 10 mL of scintillation cocktail. Thymidine incorporation was measured by β-scintillation counting. For treatment of the ears of the mice, the test materials were dissolved in acetone:olive oil (4:1 v/v). All solutions were prepared freshly for every application, except for the aldehyde and the alcohol solutions, which were prepared on day 0 and stored in the refrigerator during the study. The test concentrations are given in weight/volume %.

The hydroperoxide mixture was shown to be a strong allergen with an EC3 value of 1.6. The aldehyde was shown to be a weaker allergen when compared to the hydroperoxides with an EC3 value of 9.5 (Table 10 and Table 17 on page 34). The alcohol was shown to be a non-sensitizer. The sample of linalool air-exposed for 10 weeks gave a clear positive result (EC3 of 9.4), and the sample of linalool that was air-exposed for 45 weeks showed an even stronger sensitizing capacity (EC3 of 4.8, see Table 10 and Table 16 on page 33). Pure linalool tested at 25% concentration revealed an SI of 1.9, 50% concentration an SI value of 3.2, while the 100% concentration revealed an SI value of 3.0.

Table 10 **LLNA responses for pure linalool and oxidization products** (copied from Sköld et al. 2004)

test material/concentration	DPM/lymphnode	SI	EC3
pure linalool (Sigma Aldrich Chemie)			46.2
control	430.0		
25%	813.3	1.9	
50%	1376.3	3.2	
100%	1301.1	3.0	
air-exposed linalool, 10 weeks			9.4
control	346.2		
5%	489.5	1.4	
10%	1110.2	3.2	
25%	4406.0	12.7	
air-exposed linalool, 45 weeks			4.8
control	542.1		
2.5%	839.9	1.6	
10%	3465.7	6.4	
25%	6311.1	11.6	
linalool hydroperoxides **4** and **9**			1.6
control	498.1		
0.5%	665.7	1.3	
2.5%	2123.7	4.3	
7.5%	3551.8	7.1	
linalool aldehyde **6**			9.5
control	545.6		
1%	658.5	1.2	
5%	1095.5	2.0	
15%	2299.3	4.2	
linalool alcohol **5**			
control	551.6		
1%	574.4	1.0	
10%	721.4	1.3	
30%	701.3	1.3	

In absence of a dose-response relationship pure linalool was considered not to be a skin sensitizer. The oxidation of linalool leads to skin sensitizing products; i.e., hydroperoxides, epoxides and aldehydes as was demonstrated in the study of Bezard et al. 1997, summarized next.

Bezard M, Karlberg AT, Montelius J, Lepoittevin JP (1997) Skin Sensitization to linalyl Hydroperoxides: Support for Radical Intermediate, Chem Res Toxicol 10: 987-993

LLNA: Female mice (CBA/Ca strain, 6-10 weeks old), in groups of four, received 25 µL of the test chemicals dissolved in dimethylformamide (DMF) on the dorsum of both ears for 3 consecutive days. Test solutions were made fresh each day and applied within 30 min. Linalool hydroperoxide (1), Linalool epoxides (2), (3), and (4), and their furan (5) and pyran (6) degradation products were tested in three different concentrations: 1%, 3%, and 9% (w/w). The chemical structures and their related numbers are displayed in Figure 4. Control mice were treated with an equal volume of DMF alone. Five days after the first treatment, all mice were injected intravenously through the tail vein with [^{3}H]thymidine After 5 h, the mice were sacrificed, the draining auricular lymph nodes were excised and pooled for each group, and a single-cell suspension of lymph node cells was prepared. After washing and precipitation with trichloroacetic acid, thymidine incorporation was determined by *β*-scintillation counting.

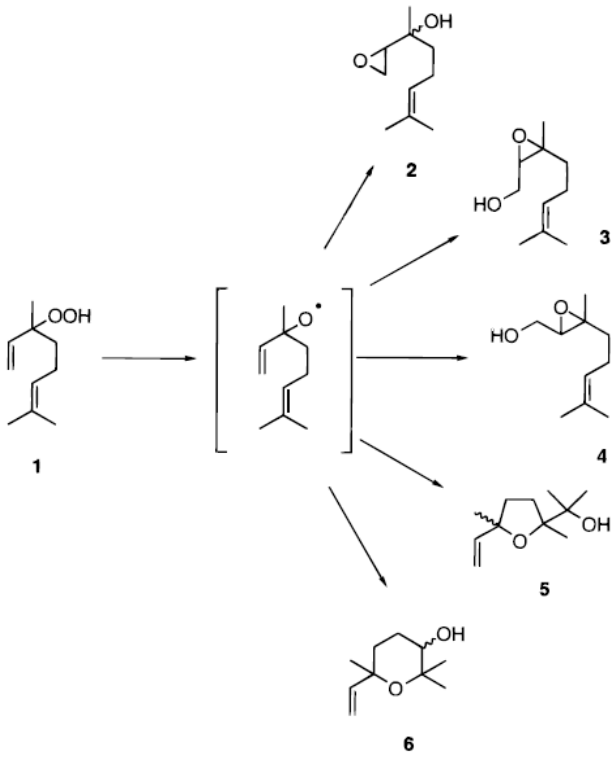


Figure 4 **Chemical structures of compounds used in the LLNA and FCAT** (copied from Bezard et al. 1997)

FCAT: The experiments were performed in outbred female albino Dunkin-Hartley guinea pigs. For induction the animals received intradermal injections of 0.1 mL of the test substance in FCA/water emulsion in the upper back on days 0, 6, and 10. The controls received FCA/water emulsion only. Challenge testing was performed on day 21. The test material (15 µL of each dose, vehicle not specified) was applied on the shaved flanks for 24 h, using Finn chambers. The reactions were assessed at 48 and 72 h after application. The minimum criterion for a positive reaction was a confluent erythema. The animals were randomized into five groups. Group A (*n* = 10) was induced with Linalool hyperperoxide (1), group B, C, and D (*n* = 9 or 10) with Linalool epoxide (2), (3), and (4), respectively. The fifth group E (*n*= 9) was a sham-treated control group. Challenge testing was performed in all

groups with all substances at the same time following a blinded randomized application scheme. Concentrations were 0.3, 1, and 3% for substance (1), 1 and 3% for substance (2), and 3% for substances (3) and (4). The minimum irritating concentration for induction was determined to be 4% as determined by testing epoxide (4) at 10, 3.3, and 1.1% in olive oil. The maximum non-irritating concentration for irritation was 3.3% in olive oil as determined by testing epoxides (2) and (4) at 9, 3.3, 1.1, and 0.33% concentrations in olive oil. A vehicle control with olive oil was applied on all animals.

The linalool hydroperoxide (1) and the linalool epoxide (2) were positive in the LLNA. The SI's for linalool hydroperoxide were of 2.2, 13.8, and 16.9 at 1, 3, and 9% (w/w) concentration, respectively; for linalool epoxide (2) the SI's were 1.4, 1.8, and 3.2 at 1, 3, and 9% respectively. The other 2 epoxides (3) and (4) as well as the degradation products (5) and (6) revealed SI's all being below 3 and were thus considered negative in the LLNA (Table 11).

Table 11 **Responses to synthesized linalool oxidation products in the LLNA** (copied from Bezard et al. 1997)

chemical	concn (w/w, %)	[^{3}H]thymidine incorpn (dpm/node)	stimulation index
hydroperoxide **1**	control	215	
	1.0	467	2.2
	3.0	2976	**13.8**
	9.0	3641	**16.9**
epoxide **2**	control	215	
	1.0	296	1.4
	3.0	385	1.8
	9.0	699	**3.2**
epoxide **3**	control	341	
	1.0	298	0.9
	3.0	476	1.4
	9.0	451	1.3
epoxide **4**	control	341	
	1.0	365	1.1
	3.0	348	1.0
	9.0	368	1.1
furan **5**	control	132	
	1.0	139	1.1
	3.0	189	1.4
	9.0	279	2.1
pyran **6**	control	132	
	1.0	177	1.3
	3.0	222	1.7
	9.0	190	1.4

In the FCAT, linalool hydroperoxide (1) and the linalool epoxides (2) and (4) were positive whereas epoxide (3) was negative. With 10/10 positive animals at a challenge concentration of 3.3% and 6/10 at a challenge concentration of 1.1% substance (1) should be considered as a strong sensitizer. The same conclusion also applies to epoxide (2) with 9/9 positive animals at a challenge concentration of 3% and 7/9 at a challenge concentration of 1.1%. Epoxide (4) testing revealed 5/10 positive animals at a challenge concentration of 3%, and this epoxide should be considered as a mild sensitizer. At the concentration tested no irritation was seen to any of the derivatives and no sensitizing activity was detected for epoxide (3) (Table 12).

Table 12 **Responses to synthesized linalool oxidation products in the FCAT** (copied from Bezard et al. 1997)

		challenge material (% in olive oil)							
		hydroperoxide **1**			epoxide **2**		epoxide **3**	epoxide **4**	
guinea pigs	time (h)	3	1	0.3	3	1	3	3	olive oil
exposed groups:[b]									
hydroperoxide **1**	48	10	6	0	0	0	0	0	0
group A ($n = 10$)	72	10	7	1	0	0	0	0	0
p exp/co[c]		<0.001	<0.01	NS[d]					
epoxide **2**	48	0	0	0	9	7	0	0	0
group B ($n = 9$)	72	3	0	0	7	9	0	0	0
p exp/co	48				<0.001	<0.01			
p exp/co	72	NS			<0.01	<0.001			
epoxide **3**	48	0	0	0	1	0	0	0	0
group C ($n = 10$)	72	1	0	0	1	0	2	1	0
p exp/co		NS			NS		NS	NS	
epoxide **4**	48	2	0	0	2	4	0	3	0
group D ($n = 10$)	72	6	0	0	5	4	1	5	0
p exp/co	48	NS			NS	0.054		NS	
p exp/co	72	<0.01			<0.05	0.054	NS	<0.05	
control group	48	0	0	0	1	0	0	0	0
$n = 9$	72	0	0	0	0	0	0	0	0

[a] The number of positive animals at 48 and 72 h after challenge is given. [b] All inductions were performed with 4% solutions (0.23 M) in an FCA/water emulsion. [c] According to Fischer's exact test. [d] NS = not significant.

Cross-challenge experiments showed no cross-sensitization between linalool hydroperoxide (1) and epoxide (2). Some statistically significant reactions were observed in animals sensitized to epoxide (4) when cross-challenged with either hydroperoxide (1) (6/10 at 72 h) and epoxide (2) (5/10 at 72 h).

It has been reported that the LLNA can produce “false” positives when tested with skin irritant substances. Indeed, false positive results in the LLNA are described for some skin irritants like sodium lauryl sulfate (Basketter et al. 1998) or benzalkonium chloride (Gerberick et al. 1992); however, a false positive response is not seen for all skin irritants. In Table 13, linalool skin irritation data are summarized. Linalool at concentrations of $\geq$ 30% is described to irritate the skin. Therefore, it was argued in the REACH Dossier (2010) that the positive responses seen in the LLNA by Basketter et al. 2002 and Sköld et al. 2004 might be due to the skin irritant properties of linalool as SI values ≥3 were only observed at high concentrations ($\geq$ 50%).

Table 13 **Summary of Skin Irritation Data** (modified from Belsito et al. 2008)

Test substance	Species	Irritant con-centration	Non-irritating con-centration	Vehicle	Reference
Linalool	Human	n.t.	12.7%[2]	Petrolatum	Harrison & Spey 2005
Linalool	Human	n.t.	20%[2]	Petrolatum or unguentum hydrophilicum	Belsito et al. 2008
Linalool	Human	n.t.	2%[2]	unguentum hydrophilicum	Belsito et al. 2008
Linalool	human	n.t.	0.4%[2]	EtOH, non irritant cream	Belsito et al. 2008
Linalool	Human	32%[2]	n.i.	Acetone	Belsito et al. 2008
Linalool	Human	n.t.	4%[2]	Petrolatum	Belsito et al. 2008
Linalool	Human	n.t.	0.5%[2]	Base cream or EtOH	Belsito et al. 2008
Linalool	Human	n.t.	40%[2]	Petrolatum	Christensson et al. 2009
Linalool	Rabbit	100%	n.t.	n.a.	ECETOC 1995
Linalool	Rabbit	100%	5%	DEP	Belsito et al. 2008
Linalool	Rabbit	100%	n.i.	n.a.	Belsito et al. 2008
Linalool	Rabbit	100%, 30%	10, 3%	Peanut oil	Belsito et al. 2008
Linalool	In vitro EpiDerm	100%	n.a.	n.a.	Kandarova et al. 2009
Oxidized linalool[1]	Human	>10%	5%	Petrolatum	Christensson et al. 2009
Oxidized linalool	Human	4-11% (w/w)	2%	Petrolatum	Christensson et al. 2010
Linalool hydro-peroxides	Human	1%		Petrolatum	Audrain et al. 2014
Linalool hydro-peroxides	Human	1%		Petrolatum	Christensson et al. 2012

n.t. not tested
n.i. not indicated
n.a. not applicable
EtOH ethanol
DEP diethyl phthalate

[1] oxidized linalool contained 30% linalool and 19% linalool hydroperoxide
[2] the only or highest concentration tested

Gerberick et al. 2002 developed a test system to distinguish “false” positive responses in the LLNA due to skin irritation from real positive responses due to sensitization. Using this

protocol true skin sensitizers and skin irritants can be distinguished by determination of cell surface markers of lymph node cells. After application of true skin sensitizers the percentage of B lymphocytes within the draining lymph node expressing the marker B220 (i.e. B220+ cells) is higher as compared to the vehicle control whereas the percentage of B220+ cells is not changed for skin irritants (Gerberick et al. 2002, Betts et al. 2007). Linalool was tested using this protocol after submission of the REACH Dossier in 2010 (see Khan & Dearman 2010 summarized next).

Khan S, Dearman RJ (2010) Linalool: B220 Assay Research Report, The University of Manchester, Oxford Road, Manchester M13 9PL, UK. (unpublished report dated 16-October 2010 provided by RIFM (Research Institute for Fragrance Materials , U.S.A)) .

Young adult female CBA/Ca mice (4 to 5 per group) were allocated to different groups: acetone vehicle control (vehicle for the positive controls), acetone/olive oil vehicle control (vehicle for linalool 50% group), 2,4-dinitrochlorobenzene positive control group (skin sensitizer), benzalkonium chloride positive control group (skin irritant), as well as linalool without antioxidant (100% and 50%) or linalool with antioxidant i.e. alpha-tocopherol (100% and 50% linalool) groups. Linalool had a purity of 99.9%. The peroxide content of linalool without antioxidant was 2.77 mM, of linalool with antioxidant 0.78 mM. The alpha-tocopherol level was 0.05%. Animals were treated daily for 3 consecutive days on the dorsum of both ears. Erythema and oedema were scored each day prior to topical exposure and on day 6. In addition, the ear weight was determined. On day 6, the draining lymph nodes were excised and pooled per treatment group. Single cell suspensions were prepared and the total cellularity (total number of viable lymph node cells) per lymph node was determined using trypan blue method. The lymph node cells were phenotyped using anti-B220 antibody and subsequent flow cytometer analysis. The ratio of the number of B220+ cells in the treatment groups vs in the vehicle group was determined.

No visible skin reactions were evident in any of the groups, except the benzalkonium chloride group. Ear weights – on the whole - were not significantly increased when compared to control groups. Body weight development was normal. The total number of viable lymph node cells was increased for both positive control groups as compared to the respective vehicle control group. Exposure to linalool (both in the presence and absence of antioxidant) also resulted in dose-dependent increase in total viable lymph node cell numbers as compared to the respective vehicle control group. The lymph node cells phenotyping showed the expected increase in B220+ cells for the positive control 2,4-dinitrochlorobenzene (DNCB) group as compared to the respective vehicle control. However, no such increase was seen for the skin irritant benzalkonium chloride (BZC) or in any of the 4 linalool groups (Table 14).

Table 14 **Mechanistic study results for linalool** (copied from Khan & Dearman 2010)

(A) linalool plus antioxidant

	Cells/node(x10^6)		% B220$^+$ cells		Test:Vehicle ratio	
Treatment	Chemical Treated	Vehicle Treated	Chemical Treated	Vehicle Treated	Cell/node	% B220$^+$ cells
Allergen DNCB (0.25% in acetone)	26.3	4.3	20.9	4.8	6.1	4.4
Irritant BZC (2% in acetone)	7.9	4.3	6.7	4.8	1.8	1.4
100% Linalool	12.3	4.0	6.7	5.7	3.1	1.2
50% Linalool in AOO	6.5	4.0	5.4	5.7	1.6	0.9

(B) linalool without antioxidant

	Cells/node(x10^6)		% B220 $^{+VE}$ cells		Test:Vehicle ratio	
Treatment	Chemical Treated	Vehicle Treated	Chemical Treated	Vehicle Treated	Cell/node	% B220 $^{+VE}$ cells
Allergen DNCB (0.25% in acetone)	17.9	3.3	17.4	6.9	5.4	2.5
Irritant BZC (2% in acetone)	13.7	3.3	8.3	6.9	4.2	1.2
100% Linalool (FG)	7.8	3.8	5.3	5.6	2.1	0.9
50% Linalool (FG) in AOO	4.7	3.8	5.7	5.6	1.2	1.0

In conclusion, the data from this study indicate that the positive responses recorded for linalool in the standard LLNA (where sensitization activity is measured as a function of lymphocyte proliferation) are likely due to the irritant properties of the material. This together with the fact that proliferation is induced only by relatively high doses of linalool further supports the conclusion that linalool is not a skin sensitizer in the LLNA; rather it displays characteristics seen with other skin irritants used in the LLNA.

To summarize the available data, the results of the studies by Basketter et al. 2002, Sköld et al. 2002 and 2004, Khan & Dearman 2010 were tabulated. Table 15 summarizes results of the LLNA, the FCAT, and the mechanistic study using linalool either purified or non-purified, Table 16 shows the results of the mixture called oxidized linalool, and Table 17 summarizes data on synthesized linalool oxidation products.

Table 15 Linalool: Results in LLNA and FCAT

Test type	Test substance	Supplier	Purity	Concentration	Result (SI or number of positives[3])	EC3	Reference
LLNA	Linalool as supplied[1]	Aldrich	97%	0 % (AOO[4])		30%	Basketter et al. 2002; Ryan et al. 2000
				25 % (w/v)	2.5		
				50 % (w/v)	4.8		
				100 % (w/w)	8.3		
FCAT (exp. 1)	Linalool as supplied	Lancester	97%	Intradermal induction and challenge: 5.1% (w/w)	0/14	n.a.	Sköld et al. 2002
LLNA	Linalool purified (redistillation)[2]	Aldrich	98.6%	0 % (AOO[4])		55%	Basketter et al. 2002
				25 % (w/v)	2.1		
				50 % (w/v)	2.9		
				100 % (w/w)	4.9		
LLNA	Linalool purified (redistillation)	Lancester or Sigma-Aldrich	97% prior to re-distillation[5]	0 % (AOO[4])		46%	Sköld et al. 2004
				25 % (w/v)	1.9		
				50 % (w/v)	3.2		
				100 % (w/w)	3.0		
Mechanistic LLNA	Linalool without antioxidant[6]	BASF	99.9%	0 % (AOO[4])		n.a.	Khan & Dearman 2010
				50 % (w/v)	Dose-dependently increased cellularity but no increase in B220+ cells		
				100% (w/w)			
Mechanistic LLNA	Linalool with antioxidant (0.05% alpha-tocopherol)[7]	BASF	99.9%	0 % (AOO[4])		n.a.	Khan & Dearman 2010
				50 % (w/v)	Dose-dependent increase in cellularity but no increase in B220+ cells		
				100% (w/w)			

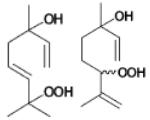
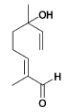
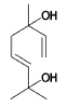
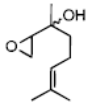
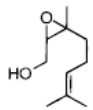
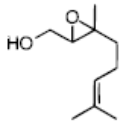
1 1.92% dihydrolinalool, 0.66% linalool oxide , 0.18% 3-hexenyl butyrate, 0.14% epoxy linalool, 0.1% 3,7-dimethyl-1,7-octadiene-3,6-diol
2 1.4% dihydrolinalool
3 number of positive animals / number of animals tested
4 vehicle: acetone /olive oil (4/1)
5 purity not indicated after purification
6 2.77 mM Peroxide content
7 0.78 mM Peroxide content

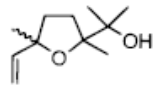
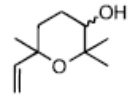
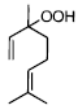
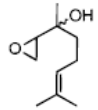
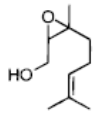
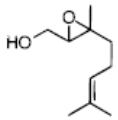
Table 16 Oxidized Linalool: Results in LLNA and FCAT

Test type	Test substance	Purity	Concentration	Result (SI or number of positives[2])	EC3	Reference
LLNA	Linalool oxidized (10 weeks)[3]	Ca. 10% hydroperoxide Ca. 80% linalool	0 % (AOO[1])		9.4	Sköld et al. 2004
			5 % (w/v)	1.4		
			10 % (w/v)	3.2		
			25 % (w/v)	12.7		
LLNA	Linalool oxidized (45 weeks)[3]	Ca. 15% hydroperoxide Ca. 30% linalool Ca 20% furan derivative	0 % (AOO[1])		4.8	Sköld et al. 2004
			2.5 % (w/v)	1.6		
			10 % (w/v)	6.4		
			25 % (w/v)	11.6		
FCAT (exp. 2)	Linalool oxidized (10 weeks)[3]	Ca. 80% linalool	Intradermal induction: 5.1% (w/w)		n.a.	Sköld et al. 2002
			Challenge: 1%. (w/w)	1/15		
			Challenge: 5.1% (w/w)	8/15		
			Re-challenge: 2.6% (w/w)	5/15		
			Re-challenge: 10.3% (w/w)	13/15		
			Re-challenge with non-purified linalool 5.1% (w/w)	3/15		

[1] acetone /olive oil (4/1)
[2] number of positive animals / number of animals tested
[3] linalool was air oxidized for the time given in brackets

Table 17 Synthesized oxidation products: results in LLNA and FCAT

Test type	Test substance	Chemical Structure	Concentration	Result (SI or number of positives[1])	EC3	Reference
LLNA	Linalool hydroperoxide mixture (5/3)		0 % (AOO[2]) 0.5 % (w/v) 2.5 % (w/v) 7.5 % (w/v)	 1.3 4.3 7.1	1.6	Sköld et al. 2004
LLNA	Linalool aldehyde		0 % (AOO[2]) 1 % (w/v) 5 % (w/v) 15 % (w/v)	 1.2 2.0 4.2	9.5	Sköld et al. 2004
LLNA	Linalool alcohol		0 % (AOO[2]) 1 % (w/v) 10 % (w/v) 30 % (w/v)	 1.0 1.3 1.3	n.a.	Sköld et al. 2004
LLNA	Linalool hydroperoxide (1)		0 % (DMF[3]) 1 % (w/v) 3 % (w/v) 9 % (w/v)	 2.2 13.8 16.9	n.i.	Bezard et al. 1997
LLNA	Linalool epoxide (2)		0 % (DMF[3]) 1 % (w/w) 3 % (w/w) 9 % (w/w)	 1.4 1.8 3.2	n.i.	Bezard et al. 1997
LLNA	Linalool epoxide (3)		0 % (DMF[3]) 1 % (w/w) 3 % (w/w) 9 % (w/w)	 0.9 1.4 1.3	n.a.	Bezard et al. 1997
LLNA	Linalool epoxide (4)		0 % (DMF[3]) 1 % (w/w) 3 % (w/w) 9 % (w/w)	 1.1 1.0 1.1	n.a.	Bezard et al. 1997

Test type	Test substance	Chemical Structure	Concentration	Result (SI or number of positives[1])	EC3	Reference
LLNA	Substance (5)		0 % (DMF[3]) 1 % (w/w) 3 % (w/w) 9 % (w/w)	 1.1 1.4 2.1	n.a.	Bezard et al. 1997
LLNA	Substance (6)		0 % (DMF[3]) 1 % (w/w) 3 % (w/w) 9 % (w/w)	 1.3 1.7 1.4	n.a.	Bezard et al. 1997
FCAT	Linalool hydroperoxide (1)		Induction 4% Challenge 3% 1% 0.3%	 10/10[4]* 7/10[4*] 1/10[4]	n.a.	Bezard et al. 1997
FCAT	Linalool epoxide (2)		Induction 4% Challenge 3% 1%	 9/9[4*] 9/9[4*]	n.a.	Bezard et al. 1997
FCAT	Linalool epoxide (3)		Induction 4% Challenge 3%	 2/10[4]	n.a.	Bezard et al. 1997
FCAT	Linalool epoxide (4)		Induction 4% Challenge 3%	 5/10[4]*	n.a.	Bezard et al. 1997

[1] number of positive animals / number of animals tested
[2] acetone /olive oil (4/1)
[3] DMF: dimethyl formamide
[4] reading at which the most animals on test reacted
n.i. not calculated in the publication
n.a. not applicable
* statistically significant

6.2. Human Data

In Table 18, Table 19 and Table 20, the data are summarized.

6.2.1. Skin sensitization studies in human volunteers

Harrison LB, Spey DR (2005) Final Report; Repeated Insult Patch Test (RIPT), Harrison Research Laboratories Inc, NJ 07083, USA, Report No. 49469, dated 3-October-2005

In a human RIPT, 135 healthy volunteers without any dermatological or other medical or interfering physical condition were placed on the study. Females were non-pregnant and non-nursing. Volunteers were induced with 0.3 mL of each of the 3 test materials (saline, 12.7% linalool in 1:3 EtOH:DEP[4], or 1:3 EtOH:DEP). The test materials were applied via occlusive patches at different sites on the left back. Patches were removed 24 h later followed by the next induction application 24 h or 48 h later. Patched sites were inspected and scored. In total, nine induction applications were done. After a rest period of 2 weeks following the last induction, subjects were challenged on the right back with the challenge patches for 24 h. Each subject was scored 24 h, 48 h, 72 h and 96 h post-patching

During the induction phase, the negative control saline produced low-level transient reactions in 4 subjects. During challenge phase, 3 subjects exhibited low-level transient reactions. The test material 12.7% linalool produced low-level transient reactions in two subjects during induction and in 1 subject during challenge phase. The vehicle 1:3 EtOH:DEP had 1 low-level transient reaction during induction and 2 low-level transient reactions after challenge.

It is concluded that none of the test materials induced dermal sensitization in human subjects.

Three human maximization tests are cited in the review of Belsito et al. 2008. In total 75 health volunteers were used; 50 of those were tested at 20% linalool, the remaining 25 volunteers were tested at 8%. None of the 75 volunteers showed skin reactions.

[4] Ethanol:Diethyl phthalate

Table 18 Linalool: studies in healthy human volunteers

Test item	Supplier	Con-centration	Vehicle	Subjects	Results	Pos. reactions	Reference
Linalool (HRIPT)	n.i.	12.7%	EtOH:DEP (1:3)	135 healthy volunteers	No sensitizing reactions	0%	Harrison & Spey 2005
Linalool (Max-Test)	n.i.	20%	Petrolatum	25 volunteers	No sensitizing reactions	0%	Belsito et al. 2008
Linalool (Max-Test)	n.i.	8%	n.i.	25 volunteers	No sensitizing reactions	0%	Belsito et al. 2008
Linalool (Max-Test)	n.i.	20%	n.i.	25 volunteers	No sensitizing reactions	0%	Belsito et al. 2008

HRIPT Human Repeated Insult Patch Test
Max-Test Maximization Test
n.i. not indicated

6.2.2. Diagnostic Patch Tests

Belsito et al. 2008 concluded that based on the available data, linalool does not have a sensitizing effect. Hostynek & Maibach 2008 also assessed the quality of the data in their review. Based on this, a clear cause-effect relationship has been established infrequently. It is emphasised that linalool is prone to oxidation and that the degree of oxidation occurring in the patch test material should be established (Hostynek & Maibach 2008). An overview of patch test data in patients can be found in Table 19 on page 40.

6.2.2.1. Patch tests with linalool

Newer patch test data with oxidized linalool and patch test data not yet considered by Belsito et al. 2008:

Matura et al. (2005), tested 20% linalool in two centres. 20% linalool produced no reactions in the 21 fragrance hypersensitivity patients and no positive reactions in the 66 hand eczema patients.

From April 2005 to June 2007 a total of 320 eczema patients suspected of being contact allergic to fragrances or cosmetics were interviewed and patch tested with linalool 10% in petrolatum (van Oosten et al. 2009). Two patients reacted (0.6%).

The German information network of departments of dermatology reports that from January 2003 to December 2004, stabilized linalool (10%) was tested in 2401 contact allergy patients. Of those only 7 (0.3%) showed positive skin reactions (Schnuch et al. 2007). The results from the same network obtained between 2005 and 2008 (Uter et al. 2010) revealed 2 positive reactions in 985 tested patients (i.e. 0.2%).

In a retrospective study based on data from the Copenhagen University Hospital Gentofte, only 1 clear positive reaction was found for non-oxidized linalool out of 1397 patients (Heisterberg et al. 2011).

Buckley 2011 tested 10% stabilized linalool in 88 selected patients suspected of having fragrance allergy. Of 88 patients tested with the 10% stabilised linalool, 4 (4.5%) had positive reactions.

108 consecutive patients were patch tested with linalool (30% w/w) in petrolatum. No irritant reactions were noted and only 1 patient (0.09%) showed an allergic reaction (Bruze et al. 2012).

Audrain et al. (2014) patch tested between August 2011 and December 2012 4731 consecutive patients with stabilised linalool (10%) in petrolatum. Only 12 patients (0.3%) reacted towards stabilized linalool.

6.2.2.2. Patch tests with oxidized linalool

In a multicenter study done in 6 different clinics (Matura et al. 2005), oxidized linalool (containing 30% linalool and 16% linalool hydroperoxide), and linalool hydroperoxide were used for patch testing in patients. The oxidation mixture used at 2% concentration produced 20 reactions in 1511 dermatitis patients and a concentration of 0.5% hydroperoxide produced 16 reactions in the 1511 dermatitis patients.

In a further patch test study (Christensson et al. 2010) examining the dose-response of oxidized linalool, the following concentrations were applied: 2, 4, 6, and 11%. For each

concentration more than 1000 patients were tested. The percentage of positive responses was 0.83%, 3.2%, 5.3%, and 7.2% at 2, 4, 6, and 11% oxidized linalool, respectively.

Buckley 2011 tested oxidized linalool at 3% in 483 consecutive patients and 10% stabilized linalool in 88 selected patients with known fragrance allergy. Of 483 patients tested 11 (2.3%) had positive patch test reactions to 3% oxidized linalool. Four of these patients were also tested with the extended fragrance battery and 3 had positive reactions to 10% stabilized linalool. Of 88 patients tested with the 10% stabilised linalool, 4 (4.5%) had positive reactions, including the 3 previously mentioned. There was 1 patient who reacted only to 10% stabilized linalool and not to 3% oxidized linalool.

Consecutive patients were screened with patch tests containing 6.0% oxidized linalool with a validated content of 0.8% of the major hydroperoxide 7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol (corresponding to a total content of 1% hydroperoxides). In total 2900 patients were tested from April 2010 to June 2011. 200 patients (6.9%) had positive patch test reactions and 37 showed irritant reactions (1.3%). The authors of the study stated that none of the patients was sensitized during the study because no late responses (usually indicating active sensitization) were seen (Christensson et al. 2012).

Friis et al. (2013) studied the occurrence of occupational allergic contact dermatitis (from January 2010 to August 2011) in the work environment. 228 consecutive patients diagnosed with occupational contact dermatitis; all patients underwent a clinical examination, the stepwise exposure assessment, and extensive patch and prick testing. 7 patients reacted towards oxidized linalool (3.1%).

Audrain et al. (2014) patch tested between August 2011 and December 2012 4731 consecutive patients with 1% hydroperoxides of linalool in petrolatum. 281 patients (5.9%) had positive patch tests to linalool hydroperoxides. 3 patients showed irritant reactions.

In a Repeated Open Application Test (ROAT) following an updated ROAT protocol, the elicitation threshold concentration was investigated in a limited number (6) of selected patients (Andersch Björkman et al. 2014). Oxidized linalool containing 18.8% linalool hydroperoxides (about 80% of the major hydroperoxide 7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol) was used to prepare creams (containing 0.56, 0.19, and 0.056% linalool hydroperoxides) and fine fragrance (0.19, 0.056, and 0.019% hydroperoxides in ethanol). Creams and fine fragrance were used for the ROAT i.e. the selected patients were treated twice daily for up to 3 weeks. The formulations each with 3 different concentrations and the negative controls were colour coded and tested in parallel in each individual which is a considerable deviation from the standard protocol. 0.1 mL of the cream formulations were applied to the right forearm, 0.1 mL of the fine fragrance on the left. The study was single-blinded, thus the participants were not informed on the colour codes. The skin was examined once weekly. All participants were patch tested two or three weeks after the ROAT. The patches contained 1.13, 0.38, 0.13, 0.038, and 0.013% hydroperoxide and were prepared from the same batch of oxidized linalool. During the ROAT applications, 5 out of the 6 participants reacted to the cream (0.56 % linalool hydroperoxides) and 4 reacted also to the fragrance (0.19% linalool hydroperoxides). 1 participant did not react at all both during the ROAT and during the final patch testing. No reaction was obtained at a concentration of 0.019% linalool hydroperoxides.

Table 19 Linalool: Summary of patch tests performed in patients

Test item	Supplier	Con-centration	Vehicle	Subjects	Results	Positive reactions	Reference
Linalool	n.i.	30%	Petrolatum	179 selected patients with suspected cosmetic allergy	No reactions	0%	DeGroot et al. 1985
Linalool	Chemotechnique, Sweden or Trolab-Hermal, Germany	n.i.	n.i.	75 selected patients	3 positive reactions	4%	De Groot 1987
Linalool	n.i.	10%	Petrolatum	119 selected patients with cosmetic allergy	1 positive reaction	0.8%	DeGroot et al. 1988
Linalool	n.i.	5%	n.i.	162 patients, 16 controls	No reactions	0%	Itoh et al. 1988
Linalool	n.i.	1% and 5%	Petrolatum	100 consecutive patients	No positive reaction	0%	Frosch et al. 1995
Linalool	n.i.	20%	Petrolatum	1825 unselected patients	3 positive reactions	0.2%	DeGroot et al. 2000
Linalool	Chemotechnique, Sweden or Trolab-Hermal, Germany	20%	n.i.	21 consecutive fragrance hypersensitivity patients	No positive reactions	0%	Matura et al. 2005
				66 consecutive hand eczema patients	No positive reactions	0%	
Linalool (stabilized)	Hermal /Trolab, Germany	10%	n.i.	2401 unselected patients	7 positive reactions	0.3%	Schnuch et al. 2007
Linalool	Unclear, the following supplier were used: Hermal/Trolab, IFF, Givaudan, Millendium, Bdeoukian, Rhodia, Symrise, Firmenich	10%	Petrolatum	320 patients with eczematous skin disease	2 positive reactions	0.6%	Van Oosten et al. 2009
Linalool (stabilized)	most likely Hermal /Trolab, Germany	10%	n.i.	985 consecutive patients	2 positive reactions	0.2%	Uter et al. 2010
Linalool (stabilized)	Trolab, Germany	10%	n.i.	88 selected patients suspected of having fragrance allergy	4 positive reactions, 3 of them reacted also towards oxidized linalool	4.5%	Buckley 2011

Test item	Supplier	Con-centration	Vehicle	Subjects	Results	Positive reactions	Reference
Non-oxidized linalool	Hermal, Germany	10	n.i.	1397 consecutive patients	1 positive reaction	0.1%	Heisterberg et al. 2011
Linalool (unknown whether oxidation products were formed)	Millenium speciality Chemicals	30% (w/w)	Petrolatum	108 consecutive patients with suspected allergic contact dermatitis	1 allergic reaction	0.09%	Bruze et al 2012
Linalool (stabilized)	Chemotechnique Diagnostics, Vellinge, Sweden	10%	Petrolatum	4731 consecutive patients	12 allergic reactions and 3 irritant reactions	0.3%	Audrain et al. 2014

n.i. not indicated

Table 20 Oxidized linalool: Summary of human patch tests in patients

Test item	Test item concentration	Vehicle	Subjects	Results	Positive reactions	Reference
Oxidized linalool[1]	2% (w/w)	petrolatum	1511 consecutive dermatitis patients	20 positive reactions	1.3%	Matura et al. 2005
Linalool hydroperoxide	0.5% (w/w)	petrolatum	1511 consecutive dermatitis patients	16 positive reactions	1.1%	Matura et al. 2005
Oxidized linalool[3]	2% (w/w)	petrolatum	1693 consecutive patients	14 positive reactions	0.83%	Christensson et al. 2010
Oxidized linalool[3]	4% (w/w)	petrolatum	2075 consecutive patients	67 positive reactions	3.2%	Christensson et al. 2010
Oxidized linalool[3]	6% (w/w)	petrolatum	1725 consecutive patients	91 positive reactions	5.3%	Christensson et al. 2010
Oxidized linalool[3]	11% (w/w)	petrolatum	1004 consecutive patients	72 positive reactions	7.2%	Christensson et al. 2010
Oxidized linalool[2] from Chemotechnique Diagnostics Sweden	3%	petrolatum	483 consecutive patients	11 positive reactions	2.3%	Buckley 2011
Oxidized linalool[4] from Chemotechnique Diagnostics	6% (1% hydroperoxides)	petrolatum	2900 consecutive patients	200 positive reactions 37 irritant reactions	6.9%	Christensson et al 2012
Oxidized linalool from Trolab or Chemotechique	Not indicated	Not indicated	228 subjects with occupational contact dermatitis	7	3.1%	Friis et al 2013
Linalool hydroperoxides from Chemotechnique Diagnostics, Vellinge, Sweden	1%	petrolatum	4731 consecutive patients	281 positive reactions (and 196 irritant reactions)	5.9%	Audrain et al. 2014

1 30% linalool and 16% linalool hydroperoxide
2 composition not specified
3 30 % linalool
4 contained 0.8% of the major hydroperoxide (7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol)

6.2.1. Case reports

Schubert 2006 reported that 4 out of 26 perfume factory workers were reacting towards 10% linalool in petrolatum when being patch tested. The author emphasis that the degree of automation in the factory and the degree of local exhaust ventilation was very low resulting in high exposure via air. Again exposure via air likely results in oxidation of linalool.

In another case report a male patient (Schaller & Korting 1995) is described who reacted positively to linalool (2%). This patient underwent extreme self-medication by aromatherapy (baths and aroma lamps). This extreme exposure via air and in baths also likely resulted in oxidation of linalool.

7. Discussion and Conclusion

7.1. Identity

This document and the substance identity presented in the DSM REACH Dossier (2010) refers to linalool with purity between $\geq$ 96.7 and $\leq$ 98.2 % (w/w) containing minor impurities structurally related to linalool. In addition, linalool is stabilized with an antioxidant (additive), alpha-tocopherol, in a concentration range of $\geq$ 0.02 to $\leq$ 0.03 % (w/w) which is part of the substance identity according to the definition of a substance in Regulation (EC) No 1907/2006 Article 3(1) and Regulation (EC) No 1272/2008 Article 2(7).

Data referring to non-relevant substances were used in the CLH report (2014). For example data on lavender oil containing linalool, artificially produced mixtures such as “oxidized” linalool, or data on linalool hydroperoxides were used to justify the proposed classification and labelling of linalool as skin sensitizer category 1a. All these substances/mixtures fail to meet the specifications of the substance identity as placed on the market. This is not in accordance with Regulation (EC) No. 1272/2008 Article 8(6) which states “ Tests that are carried out for the purposes of this Regulation shall be carried out on the substance or on the mixture in the form(s) or physical state(s) in which the substance or mixture is placed on the market and in which it can reasonably be expected to be used.” Thus, for the purpose of discussing the skin sensitization potential of linalool only such information should be used which specifically addresses the substance linalool as given in the substance identity.

7.2. Reactivity of linalool upon air exposure

It is known that substances with allylic structural elements which are also present in linalool can be autoxidized. Sköld et al. (2004) showed that pure non-stabilized linalool is degraded and that linalool hydroperoxides and degradation products thereof are formed: after 10 weeks 30% of the initial linalool was degraded.

To prevent oxidation in their products, the industry adds antioxidants to linalool as specified by the recommendation of IFRA (2009). The maximum peroxide content should be 20 mM; 0.1% antioxidant such as e.g. BHT or α-tocopherol has shown great efficiency. The content of the antioxidant is specified in the identity section of the REACH Dossier (2010) and repeated in section 2 of this document.

The conditions used by Sköld et al. (2002 and 2004) or Christensson et al. (2010 and 2012) are not realistic and do not reflect normal production and use conditions. Adequate protection is achieved by antioxidants or a combination of antioxidants and further stabilizers such as UV-filters and/or chelating agents prevent which prevent such oxidation both in the pure substance and in personal care products (DSM 2014, Kern et al. 2014).

In the CLH report (2014) it is argued (based on a paper by Karlberg et al. (1994)) that the use of antioxidants does not adequately protect against oxidation. However, the Karlberg paper studied limonene and its degradation but not linalool. We do not agree that such argumentation is adequate and refer to newer data which specifically addresses the question of potential degradation of linalool in the presence or absence of antioxidant both as pure substance or in personal care formulations (DSM 2014, Kern et al. 2014). These documents show that linalool is effectively protected from oxidation even under prolonged and accelerated storage conditions.

7.3. Dermal Penetration and metabolism

According to our interpretation of the available data, the key study on dermal absorption of linalool is the study of Green et al. (2007) in which ^{14}C-labelled linalool was used and which was conducted in compliance with existing OECD guidelines on the conduct of an in vitro dermal absorption study (OECD 428 (2004)). This study was available in DSM REACH Dossier (2010) but not taken into account in the CLH report (2014). In contrast, published papers (Cal 2006a or Cal & Sznitowska 2003) which do not comply with the OECD 428 guideline because not all required samples were investigated.

Once applied to the skin, linalool quickly evaporates (see section 4.1, Green et al. 2007) from skin with only 7% of applied dose remaining after 1h. The available data on dermal absorption of linalool into the viable skin (epidermis and dermis) show that only a minor amount of the applied substance is absorbed (about 4% of the applied substance under non-occluded conditions within 24h).

We are in addition surprised about the use of data on other substances in the CLH report (2014). For example Cal et al. (2001) addresses limonene, diterpene, terpinolene, and eucalyptol, Cal (2006b) studied lavender oil, and Kitahara et al. (1993) did investigations with several terpenes but not with linalool.

Up to date we found no information whether any form of oxidized linalool once applied dermally is systemically available and/or on dermal penetration. The only information is that forms of oxidized linalool can induce skin sensitization upon dermal application (e.g. Sköld et al. 2002, 2004, Bezard et al. 1997). In addition, we have no evidence that any form of oxidized linalool can be formed by metabolism in the skin. Any conclusion on this is highly speculative. We consider it therefore not justified to consider such opinion in the process of discussing linalool as a potential skin sensitizer.

7.4. Skin Sensitization

In the sections above the data related to skin sensitization were summarized. However, based on these data on linalool, classification with regard to skin sensitization has to be well considered. It is difficult to discriminate whether the positive responses seen in the reports are indeed the result of linalool itself or of oxidation products, which are strong skin sensitizers and/or whether responses are related to skin irritant properties.

7.5. Animal Data

It is emphasised that for most animal studies addressed in the present paper and in the REACH Dossier (2010), linalool was from other sources where it is neither known whether antioxidant was present nor information on the peroxide content is given. One tested batch of linalool contained oxidation products prior to the first application in the LLNA (Basketter et al. 2002 and Ryan et al. 2000, see Table 15 on page 32). This quality produced positive responses in the LLNA. Removal of these oxidative products by purification steps reduced significantly the extent of positive responses in the LLNA (Basketter et al. 2002). Consequently, purchased linalool was purified one day prior to the first application in the LLNA (Sköld et al. 2002 and 2004, Basketter et al. 2002). However, purification likely removed any antioxidant present initially, the tested linalool may have been oxidised again in the time between purification and application. Sköld et al. 2004 indicate that purification was done before the first application to the animals without providing further analysis data. In contrast in the study of Khan & Dearman (2010) pure linalool was used.

It should also be considered that concentrations of 50 and 100% linalool are known to be skin irritant (see Table 13 on page 29) and therefore, the observed responses in the LLNA may be related to proliferation of the lymph node cells as a response to skin irritation. This hypothesis was tested in more detail in a mechanistic study conducted by Khan & Dearman (2010). The linalool used in this study was used either in the presence or absence of antioxidant. In this mechanistic study, skin sensitisation as reaction to treatment can be excluded because no increase in the relative amount of B220+ cells – an indicator for sensitization – was obtained. The results of this mechanistic study indicate in addition that the positive responses seen only at concentration equal or greater than 50% in the LLNA by Sköld et al. (2004) and Basketter et al. (2002) using purified material may also be attributed to skin irritant properties. The impact of skin irritation on the result in the LLNA is even more pronounced in situations with oxidation products of linalool as oxidized linalool is the stronger skin irritant when compared to linalool in and human subjects (Christensson et al. 2009).

The available animal data show that linalool itself is not a skin sensitizer.

7.6. Human Data

Below we provide our view on the results with linalool. It is our understanding that oxidized linalool being it the hydroperoxides and/or degradation products thereof, do not comply with the substance identity (see section 2). Linalool is protected from oxidation by antioxidants. Further we showed that oxidised linalool and/or degradation products thereof are not formed during normal production and storage conditions and even after prolonged storage of linalool when it complies to the specifications.

Human Repeated Insult Patch Test (HRIPT) on 135 volunteers was negative (Harrison & Spey 2005). A similar outcome was obtained in earlier human Maximisation tests with in total 75 healthy volunteers (Belsito et al. 2008 and references therein).

General population studies are not available, however, it was noted in the CLH report (2014) that Dr. Christensson anticipated a 2% frequency of positive patch tests with linalool (p. 33 and p. 32 of the respective document) thereby referencing to the internet homepage medicalnewstoday[5] where an interview citation with Dr. Christensson is given. “I suspect that

[5] http://www.medicalnewstoday.com/releases/144041.php

about 2% of the complete population of Sweden are allergic to air oxidized linalool". The rational for this suspicion has not been given. Further, this would not have to be interpreted that 2% of the Swedish population are allergic to linalool (as stated on pp 32-33 of above mentioned document).

Reports from patch tests in clinics do not specify whether the linalool used for the patch tests was protected by antioxidant, however, some of the publications mention "stabilised" linalool. The results of these studies showed low frequency of positive responses as compared to positive reactions in patch tests using oxidized linalool (Table 20 on page 42): The frequency of positive reactions in consecutive patients to linalool (stabilized or non-stabilized) in the studies ranged from 0% to 0.6% (Table 19 on page 40) with an overall number of 28 positives out of 12132 patients tested (overall frequency of 0.23%). In selected patients 8 out of 461 reacted (1.7%) with a range from 0-4%. In the one publication on occupational contact dermatitis 4 out of 26 patients were positive to linalool (15.4%). However, it is not clear from the publication whether linalool was adequately protected from oxidation. Thus, overall the studies report a low frequency of reactions towards linalool (see Table 21) thereby considering ECHA guidance on the application of the CLP criteria (ECHA 2013).

7.7. Application of CLP criteria

According to Regulation (EC) No. 1272/2008, "Substances shall be classified as skin sensitizer (Category 1) in accordance with the following criteria:

(i) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons, or

(ii) if there are positive results from an appropriate animal test".

Table 21 summarizes the frequencies of positive reactions. Considering the widespread use of linalool the frequency is low.

The SCCS (2012) states in their document p. 46-47 that linalool has a frequency of reported reactions between 11 to 100 (++) whereas in the CLH report (2014) p. 34 the frequency greater than 100 is cited. This figure (+++ or 101-1000) was estimated for oxidised linalool by SCCS (2012) (see also Table 21).

Table 21 Overview on frequencies of occurrence of skin sensitization

Type of Human diagnostic patch test	CLH report (2014) evaluation on linalool	DSM evaluation on Linalool
General population study	2%[1]	No data
Dermatitis patients	0.2-0.3%	0.23%[4]
Selected dermatitis patients	0-4%[2]	1.7%[5]
Work place studies -All or randomly selected workers -Selected workers with known exposure or dermatitis	 No data 15%[6]	 No data 15%[6]
Number of published cases	101-1000[3]	11 – 100[3]

[1] interview with Dr. Christensson
[2] data from DeGroot 1987 being a review on published data
[3] estimated by SCCS (2012)
[4] see also Table 19, range 0-0.6%, overall number of 28 positives out of 12132 patients (0.23%)
[5] see also Table 19, range 0-4%, overall number of 8 positives out of 461 patients (1.7%)
[6] Schubert 2006, one factory; linalool source and specification unclear

The exposure to linalool is low based on the known use concentrations (see p. 34 of CLH report (2014)) but with a frequent repeated exposure and widespread use. Considering a low exposure together with a low frequency of occurrence of skin sensitisation (see Table 21) the harmonised classification and labelling as skin sensitizer is not justified.

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