

Can contact allergy to *p*-phenylenediamine explain the high rates of terpene hydroperoxide allergy? – An epidemiological study based on consecutive patch test results

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Summary

Background. Contact allergy to linalool hydroperoxides (Lin-OOHs) and limonene hydroperoxides (Lim-OOHs) is common. Similarly to what occurs with the terpene hydroperoxides, reactive intermediates formed from *p*-phenylenediamine (PPD) can cause oxidative modifications of tryptophan residues on proteins in mechanistic studies.

Objectives. To test the hypothesis that patients sensitized to PPD are at increased risk of concomitant reactivity to either of the terpene hydroperoxides, owing to a 'common pathway' of skin protein oxidation.

Methods. A database study of consecutively patch tested eczema patients ($n = 3843$) from 2012 to 2015, tested concomitantly with PPD, Lim-OOHs and Lin-OOHs, was performed. Associations were examined by level of concordance and odds ratios (ORs) adjusted for age, sex, and contact allergy to fragrance mix I and fragrance mix II.

Results. Concomitant reactions to PPD were seen in 2.2% of Lim-OOH-positive patients and in 4.9% of Lin-OOH-positive patients. Neither proportion was higher than expected by chance. No association existed between PPD and Lim-OOH patch test reactivity. In a multiple logistic regression analysis, PPD allergy was associated with an insignificantly increased risk (OR 2.11, 95%CI:0.92–4.80) of a positive patch test reaction to Lin-OOHs.

Conclusions. PPD sensitization cannot explain the high rates of sensitization to Lin-OOHs and/or Lim-OOHs. Contact allergy to oxidized linalool is more strongly associated with fragrance allergy than with PPD allergy.

Key words: concomitant reactivity; contact allergy; D-limonene; linalool; *p*-phenylenediamine; patch testing; terpene hydroperoxides.

The terpenes linalool and D-limonene are among the most commonly used fragrance ingredients in consumer products (1, 2). Upon air exposure, these fragrance ingredients

readily autoxidize, resulting in an increased allergenic potential. The main allergens present after oxidation of linalool and D-limonene are hydroperoxides (3, 4). Newer results from multicentre trials in Europe have shown contact allergy to limonene hydroperoxides (Lim-OOHs) and linalool hydroperoxides (Lin-OOHs) in 5.0–6.9% of consecutively tested patients (5–7). It has recently been hypothesized by the fragrance industry that positive patch test reactions to these terpene hydroperoxides may not always represent a hapten-specific sensitization state, but could also be associated with a state of reactivity to multiple degradation products formed under oxidative

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conditions. Thus, Natsch et al. proposed that oxidative modifications of tryptophan (Trp) residues on skin proteins, namely *N*-formyl kynurenine (NFK), caused by various sources, could lead to a sensitized state detected by patch testing with the terpene hydroperoxides (8).

It has previously been shown that oxidation derivatives of allergenic para-amino aromatic compounds, namely *p*-benzoquinone diimines, can react with nucleophilic residues on amino acids such as lysine (Lys) and Trp (9, 10). The degradation products of Trp formed during this process, which are identical to those formed with terpene hydroperoxides, could therefore, at least in theory, contribute to the high frequency of patch test reactions to terpene hydroperoxides, according to the hypothesis proposed above regarding the formation of non-specific antigens. *p*-Phenylenediamine (PPD), a colourless substance, is the typical example of a para-amino aromatic compound (11) acting as a primary intermediate in hair dyes. On the basis of the murine local lymph node assay, PPD is one of the most potent allergens, with an EC₃ value of 0.1%; among consecutively patch tested dermatitis patients in Europe, ~4% are sensitized to PPD (12).

The aim of the current study was to examine the presence of a possible observational association between concomitant positive patch test reactions to limonene/linalool hydroperoxides and PPD in consecutively tested dermatitis patients. We wanted to test the hypothesis that patients sensitized to PPD are at significantly increased risk of having concomitant positive patch test reactions to either of the terpene hydroperoxides.

Methods

The data for this retrospective study were obtained from the clinical database on contact allergy at the Department of Dermatology and Allergology, Copenhagen University Hospital Herlev-Gentofte, Denmark, as described elsewhere in detail (13). Consecutive patients who were suspected of suffering from allergic contact dermatitis, and who had been tested with our baseline patch test series between January 2012 and November 2015, were included. This time period was chosen because the oxidized terpenes became commercially available for patch testing in the beginning of 2012.

Baseline patch testing at our department is performed with the European baseline series (Almirall Hermal GmbH, Reinbek, Germany) including PPD 1% pet., and our fragrance series including Lim-OOHs 0.3% pet. and Lin-OOHs 1% pet. (Chemotechnique Diagnostics, Vellinge, Sweden). Patch testing was performed with Finn Chambers® (8 mm; Smartpractice, Phoenix, AZ, USA), applied on the upper back for 48 h with Scanpor®

tape (Norgesplaster, Vennesla, Norway). Readings were performed on day (D) 2, D3, and D7, and maximum reactions are presented here. Grading of positive reactions (+, ++, and +++) was performed according to international recommendations (14), and an irritant reaction, a doubtful reaction (?+) or a negative reading was interpreted as a negative (non-allergic) response. Because of prior testing, and known sensitivity, not all patients were tested with the complete baseline series according to the department's standard procedure. These patients are reported separately where relevant. All data were handled anonymously.

Data management and statistical analyses were performed with SAS® ENTERPRISE GUIDE® software, Version 7.1 (SAS Institute Inc., Cary, NC, USA). Confidence intervals (CIs) for proportions were calculated with the Wilson score test. The relationship between a positive patch test reaction to PPD and concomitant reactivity to Lim-OOHs or Lin-OOHs was assessed both by degree of association and concordance of positive patch test results according to statistical guidelines (15). The odds ratios (ORs) with 95% CIs were calculated for univariate risk estimates of association. Significant associations were adjusted for possible explanatory variables by use of a multiple logistic regression model. Sensitization to the screening markers of contact allergy to fragrances, that is, fragrance mix I (FMI) and fragrance mix II (FMII), was included in these analyses as a measure of fragrance allergy in a broader sense. Significant associations in univariate analysis were examined for trend stratified on level of exposure. Two-sided *p*-values of <0.05 were generally considered to be statistically significant.

For measure of concordance, proportions of concomitant sensitization to PPD, Lim-OOHs and Lin-OOHs were calculated with Cohen's kappa coefficients, including accompanying 95% CIs. Cohen's kappa is a widely used measure of chance-corrected agreement for categorical data. An interpretative scale has been proposed, with kappa values of zero or below suggesting no agreement or agreement less than chance, 0–0.2 slight agreement, 0.2–0.4 fair agreement, 0.4–0.6 moderate agreement, 0.6–0.8 substantial agreement and 0.8–1.0 (almost) perfect agreement between observations (16).

Results

A total of 3912 patients were patch tested with our baseline series from January 2012 to November 2015. Maximum reactions to PPD 1% pet., Lin-OOHs 1% pet., and Lim-OOHs 0.3% pet., along with patch test results for FMI and FMII, are shown in Table 1. Because of known sensitivity (0.1–0.6%) and for unknown reasons (0.1–0.6%), concomitant patch testing with the

Table 1. Patch test results for consecutively tested patients in the study period, N = 3912

Allergen	n (%)								% Positive (95%CI)*
	NT	Negative	?+	IR	NT-S	+	++	+++	
Lim-OOHs	2 (0.1)	3038 (77.7)	545 (13.9)	228 (5.8)	3 (0.1)	61 (1.6)	31 (0.8)	4 (0.1)	2.5 (2.0–3.0)
Lin-OOHs	24 (0.6)	2634 (67.3)	822 (21.0)	283 (7.2)	3 (0.1)	94 (2.4)	50 (1.3)	2 (0.1)	3.8 (3.2–4.4)
PPD	16 (0.4)	3691 (94.4)	85 (2.2)	19 (0.5)	24 (0.6)	28 (0.7)	44 (1.1)	5 (0.1)	2.0 (1.6–2.5)
FMI	3 (0.1)	2787 (71.2)	657 (16.8)	79 (2.0)	34 (0.9)	168 (4.3)	175 (4.5)	9 (0.2)	9.1 (8.2–10.0)
FMII	7 (0.2)	3345 (85.5)	344 (8.8)	45 (1.2)	18 (0.5)	58 (1.5)	93 (2.4)	2 (0.1)	3.9 (3.4–4.6)

FMI, fragrance mix I 8% pet.; FMII, fragrance mix II 1.4% pet.; IR, irritant reaction; Lim-OOHs, limonene hydroperoxides 0.3% pet.; Lin-OOHs, linalool hydroperoxides 1.0% pet.; NT, not tested; NT-S, not tested, known sensitized; PPD, *p*-phenylenediamine 1% pet.; ?+, doubtful reaction. *NT and NT-S were treated as missing.

Table 2. MOAHLFA index (percentage positive) for all patients tested concomitantly and patients reacting to *p*-phenylenediamine (PPD) 1% pet., linalool hydroperoxides (Lin-OOHs) 1% pet., and limonene hydroperoxides (Lim-OOHs) 0.3% pet.

	All patients (%) N = 3843	PPD-positive (%) N = 76	Lim-OOH-positive (%) N = 90	Lin-OOH-positive (%) N = 143
Male	31.8	28.9	23.3	33.6
Occupational	18.8	21.1	14.4	20.3
Atopic dermatitis	20.6	14.5	20.0	11.9
Hand dermatitis	37.6	46.1	31.1	41.3
Leg dermatitis	1.6	3.9	4.4	2.1
Face dermatitis	27.0	32.9	35.6	31.5
Age ≥40 years	65.8	71.1	58.9	74.8

terpene hydroperoxides and PPD was only performed in 3843 patients, who constituted the study population for further analyses. Demographic and clinical characteristics, defined by the MOAHLFA index, for the study population and patients reacting to PPD, Lim-OOHs and Lin-OOHs are shown in Table 2. Baseline characteristics were comparable between the subgroups of patients, with the exception of a higher proportion of patients aged ≥40 years who were Lin-OOH-positive than who were Lim-OOH-positive ($p = 0.01$).

Dichotomized patch test results (positive versus non-positive) for Lim-OOHs and Lin-OOHs, stratified on patch test results for PPD, are summarized in Table 3. No significant association could be found between positive patch test reactions to PPD and to Lim-OOHs in univariate analysis (OR 1.13, 95%CI: 0.27–4.68), whereas a significant association (OR 2.71, 95%CI: 1.22–6.00) was found between contact allergy to Lin-OOHs and contact allergy to PPD. The association remained significant after adjustment for sex and age (categorized in tertiles) in a multiple logistic regression analysis (OR 2.70, 95%CI: 1.22–6.00). Further adjustment for concomitant sensitization to FMI and FMII (Table 4) attenuated the association between a positive patch test reaction to Lin-OOHs and a positive patch test reaction to PPD to a borderline insignificant level (OR 2.11, 95%CI: 0.92–4.80).

Stratification on patch test results for PPD showed a stepwise increase in the frequency of contact allergy to Lin-OOHs. Among 3767 patients with a non-positive patch test result for PPD, 136 (3.6%) had contact allergy to Lin-OOHs, whereas 2 of 28 patients (7.1%) with a + PPD reaction and 5 of 48 patients (10.4%) with a ++/+++ PPD reaction had contact allergy to Lin-OOHs. The observed trend was statistically significant (Cochran–Armitage exact trend test, $p = 0.02$).

Concomitant reactivity to Lim-OOHs, Lin-OOHs and PPD is shown in Fig. 1, and corresponding percentages and Cohen's kappa coefficients of concordance are summarized in Table 5. Concomitant positive patch test reactions to PPD were found in 2.2% of Lim-OOH-positive patients and in 4.9% of Lin-OOH-positive patients. CIs for both corresponding kappa values included zero, indicating no agreement for concomitant sensitization better than expected by chance.

By chart review, the relevance of positive patch test reactions to Lin-OOHs and Lim-OOHs was reviewed for the 8 patients with concomitant positive reactions to one or both of the terpene hydroperoxides and PPD. Three patients had unknown relevance of their positive patch test reactions to Lin-OOHs, whereas the remaining 5 patients had current relevance in terms of exposure to cosmetic products containing linalool.

Table 3. Cross-tabulation of dichotomized test reactions to *p*-phenylenediamine (PPD) 1% pet. and limonene hydroperoxides (Lim-OOHs) 0.3% pet./linalool hydroperoxides (Lin-OOHs) 1% pet.

		Lim-OOHs 0.3%			Lin-OOHs 0.3%		
		Negative/?+/IR	Positive	Total	Negative/?+/IR	Positive	Total
PPD 1% pet.	Negative/?+/IR	3679	88	3767	3631	136	3767
	Positive	74	2	76	69	7	76
	Total	3753	90	3843	3700	143	3843

IR, irritant reaction; ?+, doubtful reaction.

Table 4. Odds ratios with 95% confidence intervals (CIs) for explanatory risk factors for a positive patch test reaction to linalool hydroperoxides 1% pet. in multiple logistic regression analysis

Factor	Odds ratio	95%CI
Age <40 years	1.00	Reference
Age 40–56 years	1.34	0.86–2.07
Age >56 years	1.40	0.90–2.16
Male	1.16	0.81–1.66
PPD-positive	2.11	0.92–4.80
Fragrance mix I-positive	2.27*	1.41–3.66
Fragrance mix II-positive	2.59*	1.43–4.69

PPD, *p*-phenylenediamine.

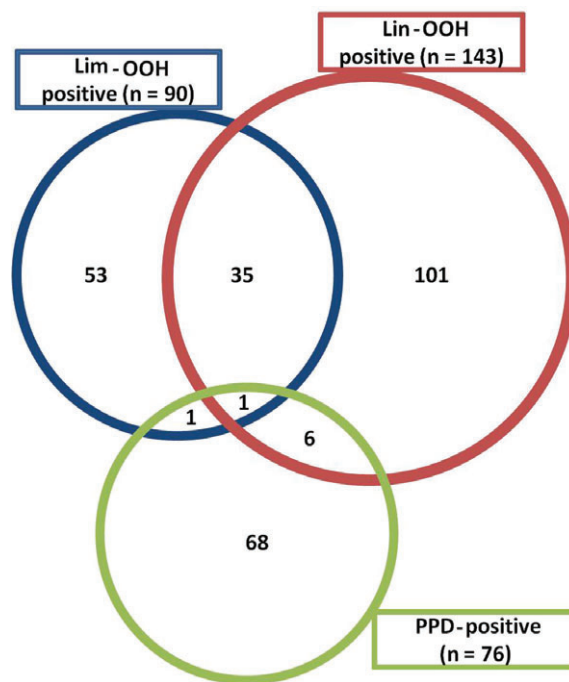
Odds ratios for males as compared with females, and PPD-positive, fragrance mix I-positive and fragrance mix II-positive patients as compared with non-positive patients.

* $p < 0.05$.

Discussion

The data from this observational study showed an unadjusted significant association (OR 2.71, 95%CI: 1.22–6.00) between positive patch test reactions to PPD 1% and to Lin-OOHs 1% in consecutive patch tested patients suspected of suffering from allergic contact dermatitis. However, when sensitization to PPD was treated as the exposure of interest, and sensitization to Lin-OOHs as the outcome, the association was attenuated to a borderline insignificant level (OR 2.11, 95%CI: 0.92–4.80), after adjustment for concomitant sensitization to FMI and FMII. Only 2.2% of Lim-OOH-positive patients and 4.9% of Lin-OOH-positive patients had concomitant positive patch test reactions to PPD. Kappa values for concomitant positive patch test reactions to either of the terpene hydroperoxides and PPD indicated no concordance better than expected by chance.

As summarized in Fig. 2, it has previously been shown that NFK [1] can be formed from Trp [2] when it is exposed to Lin-OOHs [3] and Lim-OOHs [4] (8, 17), *p*-benzoquinone diimines [5] (9) and ketoprofen under ultraviolet (UV) irradiation [6] (18). It is of note that the activation mechanism through UV irradiation is specific for photosensitizers such as ketoprofen, which

**Fig. 1.** Venn diagram of positive patch test reactions for patients tested concomitantly (N = 3843) with *p*-phenylenediamine (PPD) 1% pet., limonene hydroperoxides (Lim-OOHs) 0.3% pet., and linalool hydroperoxides (Lin-OOHs) 1% pet. See Table 5 for additional information.

absorbs UV light, leading to the formation of singlet oxygen that can react with amino acids such as Trp. In contrast, both hydroperoxides and PPD intermediates can directly degrade Trp residues without this initial activation through UV irradiation. It should be noted that NFK is also formed in the presence of radicals and other oxidative species, making this amino acid a good marker of oxidative stress (19). If NFK formed from very different sources led to the formation of antigenic structures, one would expect a significant level of concomitant positive patch test reactions to these sources. Therefore, patients sensitized to PPD should have a significantly higher frequency of positive patch test reactions

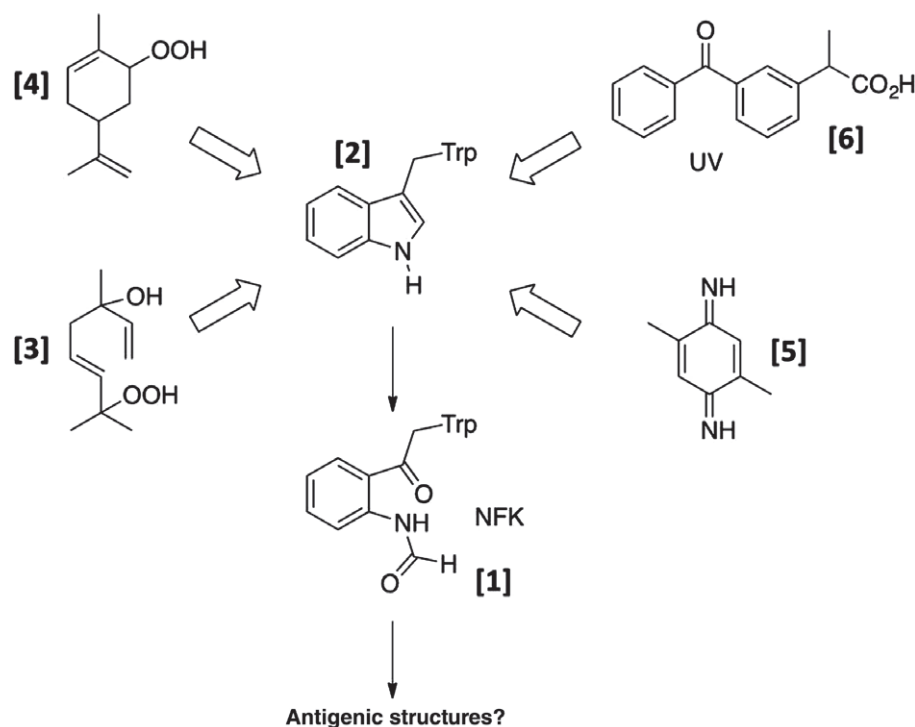
Table 5. Concomitant reactions between limonene hydroperoxides (Lim-OOHs) 0.3% pet., linalool hydroperoxides (Lin-OOHs) 1% pet., and *p*-phenylenediamine (PPD) 1% pet.

Concordance	Kappa (95%CI)*	Frequencies of concomitant reactions (%)†	
Lim-OOHs and PPD	0.003 (−0.03 to 0.04)	Lim-OOHs: 2.2	PPD: 2.6
Lin-OOHs and PPD	0.04 (−0.01 to 0.08)	Lin-OOHs: 4.9	PPD: 9.2
Lim-OOHs and Lin-OOHs	0.29 (0.21–0.37)	Lim-OOHs: 40.0	Lin-OOHs: 25.2

CI, confidence interval.

*Kappa interpretation (16): <0, less than chance agreement; 0.01–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement.

†Percentages of patients with concomitant reactions; that is, e.g., 2.2% of Lim-OOH-positive patients and 2.6% of PPD-positive patients reacted to both allergens.

**Fig. 2.** Scheme of various sources causing the formation of *N*-formyl kynurenine (NFK) [1] from tryptophan (Trp) [2], linalool hydroperoxide [3], limonene hydroperoxide [4], *p*-benzoquinone diimine [5], and ketoprofen [6].

to Lin-OOHs and Lim-OOHs than observed in the current study. The hypothesis regarding a hapten-unspecific reactive state resulting from oxidative modifications of skin proteins is based on experimental evidence, suggesting that photo-cross-reactivity to ketoprofen and structurally unrelated products could be explained by the oxidative formation of a specific immunogenic complex not containing the hapten itself (18). In a series of experimental studies, Karlsson et al. showed that the formation of a reaction product between the amino acids Trp and Lys was significantly enhanced in the presence of ketoprofen as compared with when the amino acids were UV-irradiated without ketoprofen being present (18). The authors hypothesized that the formation of a possible immunogenic molecule, enhanced by the presence of ketoprofen but not containing any ketoprofen derivatives,

could provide a possible explanation for the clinically observed cross-reactivity between ketoprofen and structurally unrelated molecules. These results have yet to be confirmed, and the application of the above hypothesis, as performed by Natsch et al. (8), to the case of frequent patch test reactivity to Lin-OOHs and Lim-OOHs has been criticized, for several reasons (20). Previously, both mechanistic studies (21) and animal studies have provided evidence against general cross-reactivity between terpene hydroperoxides (22, 23). The results from recently published clinical studies have also supported the specificity of allergenic reactivity to Lim-OOHs and Lin-OOHs (24, 25).

Patch test data from the German Information Network of Departments of Dermatology have previously shown that PPD is one of the top-ranking allergens

regarding the absolute number of significant associations with concomitantly patch tested allergens, with PPD sensitivity being significantly associated with positive patch test reactions to 19 of 23 other allergens from the baseline series (26). The unadjusted significant association between sensitization to Lin-OOHs and sensitization to PPD observed in the current study should be evaluated in this context. The very possible occurrence of co-sensitization to PPD and Lin-OOHs could be attributable to coupled exposure in consumer (hair) products (27). This is supported by the attenuation of the association after adjustment for fragrance sensitization in a broader sense (contact allergy to FMI and FMII); that is, contact allergy to Lin-OOHs is explained by fragrance allergy rather than PPD contact allergy. In view of this, we believe that contact allergies to Lin-OOHs and Lim-OOHs should be interpreted as specific fragrance contact allergies, in line with the existing evidence supporting the specificity of patch test reactions to these allergens.

Notably, we found that the proportion of patients with positive patch test reactions to Lin-OOHs showed a step-wise significant increase for patients with positive (+) and strong positive (++/+++) reactions to PPD as compared with PPD-non-positive patients. As the absolute number of Lin-OOH-positive patients was low in two of the three

groups, the observed trend should be evaluated in this context. However, as previously hypothesized, in patients sensitized to para-amino compounds, including PPD, this pattern of reactions could possibly identify a subset of patients who are genetically prone to multiple sensitizations (28, 29).

In summary, the data from this observational study do not support the hypothesis of unspecific patch test reactivity to the terpene hydroperoxides Lim-OOHs and Lin-OOHs. No concomitant sensitization to PPD and Lin-OOHs or Lim-OOHs beyond that expected by chance could be identified in the present study of >3800 consecutively patch tested dermatitis patients. On the basis of these results, it is most likely that the unadjusted increased risk of contact allergy to Lin-OOHs in PPD-positive patients is attributable to an increased risk of coupled exposure to fragrances in patients exposed to PPD in consumer products. This is supported by an attenuation of the association between PPD and Lin-OOH contact allergy after adjustment for fragrance allergy in a broader sense. On the basis of the current study, future measures aimed at reducing the prevalence of positive patch test reactions to the terpene hydroperoxides should be focused on reducing exposure to these common fragrance allergens.

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