Two decades of *p*-phenylenediamine and toluene-2,5-diamine patch testing – focus on co-sensitizations in the European baseline series and cross-reactions with chemically related substances

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Summary

Background. Cross-reactions and co-sensitizations are of great importance in understanding contact allergy and exposure sources.
Objectives. To investigate common cross-reactions and co-sensitizations in *p*-phenylenediamine (PPD)-sensitized and toluene-2,5-diamine (TDA)-sensitized individuals.
Methods. From our patch test population, 8036 patients patch tested with the European baseline series were extracted. Readings had to be performed at least on day 3 according to ICDRG guidelines.
Results. Two hundred and fifty-one patients were sensitized to PPD and/or TDA; 231 patients were sensitized to PPD, and 109 to TDA. Significant differences were observed regarding the strengths of patch test reactions to PPD and number of cross-reactions. For TDA, a difference was found between all reaction strengths, except between + and ++ strengths. PPD-sensitized individuals were more likely to be sensitized to carba mix, cobalt chloride, colophonium, *p-tert*-butyl phenolformaldehyde resin, paraben mix, and methylisothiazolinone. TDA-sensitized individuals were more often sensitized to carba mix.
Conclusions. Cross-reactivity was commonly found among individuals sensitized to PPD or TDA, and was strongly related to the strength of the patch test reaction. Regarding co-sensitizations, a frequently appearing or common exposure source could not be determined. However, modification of the allergen by, for example, the skin microbiota may have caused the formation of molecules that are, for the human immune system, indistinguishable from PPD.

Key words: toluene-2,5-diamine; allergic contact dermatitis; co-sensitization; cross-reaction; European baseline series; patch tests; *p*-phenylenediamine.

The aromatic amines *p*-phenylenediamine (PPD) (1,4-diaminobenzene, CAS no. 106-50-3) and toluene-2,5-diamine (TDA) (1,4-diamino-2-methylbenzene, CAS no. 95-70-5; synonym: *p*-toluenediamine) are both important components of permanent hair dye products. Their low molecular weight and strong protein binding capacity enable these haptens to penetrate deeply into the hair shaft, resulting in permanent staining of the hair. Moreover, these capacities make them extremely sensitizing components (1). For this reason, the PPD dose in permanent hair dyes may not exceed 2% in the final product [amending Annex III to Regulation (EC) No. 1223/2009 of the European Parliament and of the Council on Cosmetic Products]. The prevalences of

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contact allergy in both the general and the patch tested population remain high, at 1.3% and 3.3%, respectively, in the population of The Netherlands (2). Diepgen et al. reported a similar prevalence, namely 0.8%, in the general population of Europe (3).

Cross-reactions between PPD and TDA are often recognized, as described by Basketter and Goodwin in 1988 (4). Cross-reactivity can result from the almost identical molecular structures of the two chemicals. The immune system cannot differentiate between the chemical to which someone has been sensitized and molecularly similar chemicals. This phenomenon of cross-reactions of PPD with structurally related chemicals other than TDA is also well known, and was recently described by Thomas et al. (5). They investigated the relationship between the strength of the patch test reaction to PPD and cross-reactions with three structurally related substances, which were local anaesthetics: caine mix, an anti-ozonant in black rubber, that is, *N*-isopropyl-*N*′-phenyl-*p*-phenylenediamine (IPPD), and the textile dye Disperse Yellow 3. They found an increase in the likelihood of reactions to all three substances in relation to increasing strength of the PPD patch test reaction.

In the present study, we analysed the strengths of patch test reactions to both PPD and TDA in relation to the number of positive patch test reactions to cross-reacting substances in two decades.

Co-sensitization has been defined as simultaneously appearing sensitization to two or more molecularly unrelated substances (6). Co-sensitization is a less studied phenomenon regarding PPD and TDA contact allergy. Therefore, the secondary objective of this study was to identify concomitant sensitization in patients with PPD and TDA contact allergy.

Methods

Population

A total of 8036 consecutive patients were patch tested with an extended European baseline series between January 1994 and December 2013 at the dermatology department of the University Medical Centre Groningen. Data were retrieved from the local European Surveillance System of Contact Allergies (ESSCA) database regarding strength of patch test reactions, demographic information (sex and age), history of atopic dermatitis, and polysensitization.

Patch testing

Patch tests were performed with the European baseline series [TRUE test™ panels 1 and 2 (Mekos Laboratories, Hillerød, Denmark), supplemented with investigator-loaded allergens] and our extended baseline series, supplied by Chemotechnique, Vellinge, Sweden; tested in van der Bend chambers (van der Bend, Brielle, The Netherlands). PPD was tested with the TRUE test™ at a concentration of 0.090 mg/cm^2, and TDA was tested 1% pet. Tests were read, according to the criteria of the ICDRG, on day (D) 2 and D3 from January 1994 until November 2008. From November 2008 to December 2013, the readings were performed on D3 and D7, to identify late reactions. Weak (+), strong (++) and extreme (+++) positive patch test reactions were considered to be positive.

Data analyses and statistics

Data were analysed with IBM SPSS™ STATISTICS version 22 for Windows™. A p-value of <0.05 was considered to indicate a statistically significant difference. In cases of multiple testing, a Bonferroni correction was applied.

The prevalences of PPD and TDA contact allergies in the patch tested population were calculated. Demographic and clinical characteristics were compared between the PPD-sensitized and TDA-sensitized groups.

Cross-reactions with either PPD or TDA were determined within our extended European baseline series. The selected cross-reacting substances were caine mix (including the local anaesthetics benzocaine, cinchocaine hydrochloride, and tetracaine hydrochloride), black rubber mix (IPPD, *N*-cyclohexyl-*N*′-phenyl-*p*-phenylenediamine, and *N*-diphenyl-*p*-phenylenediamine), Disperse Orange 3, Disperse Yellow 3, 4-aminoazobenzene, and 4,4′-diaminediphenylmethane.

Caine mix and black rubber mix were both present in the European baseline series during almost the whole study period. All other cross-reacting chemicals were present in our extended European baseline series for shorter periods. To calculate differences in the strengths of patch test reactions to PPD or TDA regarding cross-reactivity of both PPD and TDA, the Kruskal–Wallis test was used.

Co-sensitizations were determined within the European baseline series [TRUE test (R) supplemented with investigator-loaded pet.- and aq.-based allergens (Table 3 and 4)], in the period January 1994 to December 2013. Individuals in whom <16 substances from the European baseline series were tested were excluded from analysis. Cases with excited skin reactions, the so-called 'angry back', were not included in our database. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated

Table 1. Demographic characteristics of 214 patients who were tested with both *p*-phenylenediamine (PPD) and toluene-2,5-diamine (TDA)

Characteristics	PPD-sensitized	TDA-sensitized	PPD-sensitized and TDA-sensitized
Male sex, n (%)	36 (33)	7 (47)	19 (21)
Atopic dermatitis, n (%)	31 (28)	5 (33)	22 (25)
Age >40 years, n (%)	51 (46)	4 (27)	42 (47)
Age (years): mean; median; minimum–maximum	39.5; 40.2; 12–69	36.2; 33.4; 20–77	39.0; 40.5; 14–70

One hundred and ten subjects were sensitized to PPD but not to TDA, 15 were sensitized to TDA but not to PPD, and 89 were sensitized to both PPD and TDA. Groups were comparable according to their demographic characteristics. No statistically significant differences were found, as calculated with Fisher's exact test (two-sided).

Table 2. Substances with well-known cross-reacting potential included in the extended European baseline series

Chemical	Test concentration (vehicle)	Overall prevalence (%) of contact allergy to the chemical (number tested)	Number reacting to PPD	Number (%) reacting to the chemical and PPD	Number reacting to TDA	Number (%) reacting to the chemical and TDA
Black rubber mix	75 μg/cm^2 [a]	0.9 (7780)	226	26 (11.5)	104	19 (18.3)
Caine mix	630 μg/cm^2 [a]	0.8 (7777)	225	18 (8.0)	105	9 (8.6)
Disperse Orange 3	1% (pet.)	2.1 (6267)	184	111 (60.3)	91	67 (73.6)
Disperse Yellow 3	1% (pet.)	0.6 (6260)	183	19 (10.4)	90	17 (18.9)
PPD	90 μg/cm^2 [a]	2.9 (8024)	NA	NA	105	90 (85.7)
TDA	1% (pet.)	1.4 (7124)	199	52 (43.5)	NA	NA
4-Aminoazobenzene	0.25% (pet.)	2.6 (6281)	182	141 (77.5)	90	77 (85.6)
4,4′-Diaminodiphenylmethane	0.5% (pet.)	1.2 (6344)	184	44 (23.9)	92	32 (34.8)

NA, not applicable.
Note that there is some overlap, as 89 subjects were sensitized to both PPD (*p*-phenylenediamine) and TDA (toluene-2,5-diamine).
[a]Substance tested as a part of the TRUE test™.

by the use of logistic regression analysis with adjustment for sex and age.

Results

Demographic characteristics

In the two investigated decades (1994–2014), 8036 subjects were patch tested. Of these, 251 had shown positive patch test reactions to either PPD or TDA, or to both PPD and TDA. Positive patch test reactions to PPD were seen in 231 subjects (prevalence: 2.9%), and positive patch test reactions to TDA in 109 (prevalence: 1.4%). Of 251 subjects, 214 were tested with both PPD and TDA. One hundred and ten subjects were sensitized only to PPD, 15 were sensitized only to TDA, and 89 were sensitized to both PPD and TDA.

Table 1 shows the characteristics of the groups that were tested with both PPD and TDA. On the basis of the demographic characteristics, namely sex, history of atopic dermatitis, and the hand, leg, face or arm as the primary location, no statistically significant differences were found between those sensitized to PPD only, to TDA only, or to both PPD and TDA.

Strength of the PPD and TDA reactions and the number of cross-reactions

The number of positive patch test reactions to cross-reacting substances is shown in Table 2, and is strongly related to the strength of the PPD patch test reaction (Fig. 1). A statistically significant difference was found between the different strengths of patch test reactions to PPD and the numbers of cross-reactions ($p < 0.001$ for all groups). The relationship between the number of cross-reactions and the strength of the patch test reaction to TDA was also obvious, although less so than with PPD (Fig. 2). Statistically significant differences were found between ++ and +++ reactors and between + and +++ reactors (respectively, $p = 0.049$ and $p = 0.003$). The difference between + and ++ reactors was not significant ($p = 0.081$).

The appearance of co-sensitizations with PPD and TDA in the European baseline series

Within the population of the 251 subjects with positive patch test reactions to either PPD or TDA, or to both PPD and TDA, all other sensitizations were analysed,

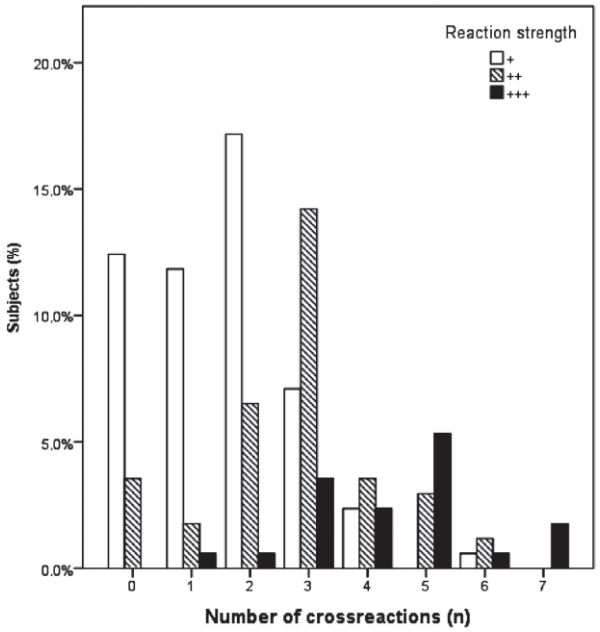

Fig. 1. Strength of patch test reactions to *p*-phenylenediamine in relation to the number of cross-reactions (black rubber mix, caine mix, 4-aminoazobenzene, Disperse Orange 3, Disperse Yellow 3, 4,4′-diaminodiphenylmethane, *p*-toluenediamine, or none). One hundred and forty-four subjects were included for analysis; 87 were weak reactors, 57 were strong reactors, and 25 were extremely strong reactors. A statistically significant difference was found in the number of cross-reacting substances between all reaction strengths ($p < 0.001$ for +/++, ++/+++, and +/+++).

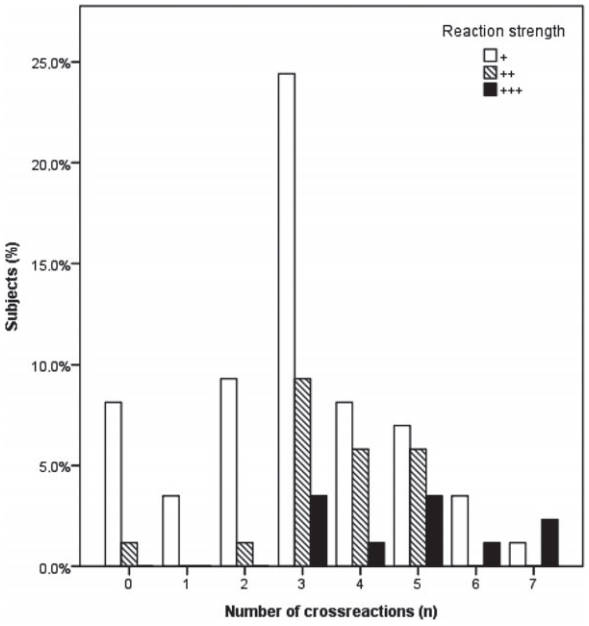

Fig. 2. Strength of patch test reactions to *p*-toluenediamine in relation to the number of cross-reactions (black rubber mix, caine mix, 4-aminoazobenzene, Disperse Orange 3, Disperse Yellow 3, 4,4′-diaminodiphenylmethane, *p*-phenylenediamine, or none). Eighty-six subjects were included for analysis; 56 were weak reactors, 20 were strong reactors, and 10 were extremely strong reactors. Differences were found in the number of cross-reacting substances between ++/+++ reactions and +/+++ reactions (respectively: $p = 0.049$ and $p = 0.003$). No significant difference was found between + and ++ reactions ($p = 0.081$).

and the frequencies of appearance of positive patch test reactions to chemically unrelated allergens in the European baseline series were compared with those in the entire patch test population. The results are shown in Tables 3 and 4.

Subjects who were at least sensitized to PPD had an approximately two-fold greater chance of being sensitized to carba mix (OR: 2.51, 95%CI: 1.41–4.50; $p = 0.002$), cobalt chloride (OR: 2.04, 95%CI: 1.31–3.19; $p = 0.002$), colophonium (OR: 2.36, 95%CI: 1.47–3.79; $p < 0.001$) and *p-tert*-butylphenol formaldehyde resin (PTBFR) (OR: 2.74, 95%CI: 1.56–4.81; $p < 0.001$) than the entire patch test population. Subjects with positive patch test reactions to PPD had a 10-fold greater chance of being sensitized to parabens mix as well (OR: 9.66, 95%CI: 3.51–26.6; $p < 0.001$). Since the recent introduction of MI into the European baseline series, frequent co-sensitization with PPD has occurred, and PPD-sensitized individuals have a 26-fold greater chance of being sensitized to MI as well (95%CI: 3.6–192; $p = 0.001$). However, MI has not been tested frequently as a part of the European baseline series (n = 245).

A higher chance of being sensitized to formaldehyde (OR: 2.74, 95%CI: 1.37–5.50; $p = 0.004$) and fragrance mix I (OR: 1.98, 95%CI: 1.26–3.12; $p = 0.003$) was observed in patients with PPD sensitization. These co-sensitizations appeared in, respectively, 3.9% and 9.5% of this group.

The 109 subjects who were at least sensitized to TDA had a 3.4-fold increased chance of being sensitized to carba mix as well (OR: 3.41, 95%CI: 1.63–7.16; $p = 0.001$). This co-sensitization was seen in 8 subjects (7.6%). In these 109 subjects, a 2.5-fold increased chance of colophonium sensitization (OR: 2.48, 95%CI: 1.28–4.83; $p = 0.007$) was found.

Discussion

In the present study, we aimed to investigate the relationship between the strength of the patch test reactions to PPD, to TDA, and to well-known cross-reacting chemicals. Furthermore, we investigated the presence of unexpected concomitant sensitizations to chemically unrelated substances.

Table 3. Co-sensitizations to *p*-phenylenediamine (PPD) and allergens in the European baseline series

Allergen from the departmental baseline series	Test concentration (vehicle)	Overall prevalence (%) of allergy to European baseline series allergens (number tested)	Number reacting to PPD	Number reacting to European baseline series allergens and PPD (%)	*p*-Value	OR (95%CI)
Myroxylon pereirae (balsam of Peru)	800 μg/cm^2	2.2 (8035)	231	8 (3.5)	0.25	1.5 (0.7–3.2)
Budesonide	0.1% (pet.)	0.8 (8036)	228	2 (0.9)	0.84	1.2 (0.3–4.8)
Carba mix	250 μg/cm^2	2.4 (7778)	225	13 (5.8)	0.002*	2.5 (1.4–4.5)
Methylchloroisothiazolinone/ methylisothiazolinone	4 μg/cm^2	4.3 (7777)	225	11 (4.9)	0.8081	1.1 (0.6–2.0)
Cobalt chloride	20 μg/cm^2	5.0 (8005)	229	23 (10)	0.002*	2.0 (1.3–3.2)
Colophonium (colophony)	850 μg/cm^2	3.8 (8017)	231	20 (8.7)	<0.001*	2.4 (1.5–3.8)
Epoxy resin	50 μg/cm^2	2.2 (8030)	231	9 (3.9)	0.046	2.0 (1.0–4.0)
Ethylenediamine dihydrochloride	50 μg/cm^2	0.7 (7770)	225	2 (0.9)	0.70	1.3 (0.3–5.5)
Formaldehyde	180 μg/cm^2	1.4 (7977)	231	9 (3.9)	0.004	2.7 (1.4–5.5)
Fragrance mix I	430 μg/cm^2	4.9 (8033)	231	22 (9.5)	0.003	2.0 (1.3–3.1)
Fragrance mix II	14% (pet.)	2.4 (8036)	92	11 (12.0)	0.065	1.8 (1.0–3.5)
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	5% (pet.)	1.2 (8036)	96	4 (4.2)	0.74	1.2 (0.4–3.3)
Lanolin alcohol	1000 μg/cm^2	1.0 (8033)	231	1 (0.4)	0.38	0.4 (0.1–3.0)
Mercapto mix	75 μg/cm^2	1.4 (8035)	231	5 (2.2)	0.38	1.5 (0.6–3.7)
Mercaptobenzothiazole	75 μg/cm^2	1.1 (8005)	229	5 (2.2)	0.44	1.5 (0.5–4.1)
Methyldibromo glutaronitrile	0.5% (pet.)	3.1 (8036)	202	12 (5.9)	0.056	1.8 (1.0–3.3)
Methylisothiazolinone	0.01% (aq.)	7.8 (245)	5	3 (60.0)	0.001*	26.3 (3.6–192.4)
Neomycin sulfate	230 μg/cm^2	0.7 (8036)	231	4 (1.7)	0.11	2.3 (0.8–6.5)
Nickel sulfate	200 μg/cm^2	18.3 (7918)	226	61 (27)	0.022	1.4 (1.1–2.0)
Paraben mix	1000 μg/cm^2	0.3 (8029)	230	5 (2.2)	<0.001*	10.0 (3.5–26.6)
Potassium dichromate	23 μg/cm^2	3.7 (7999)	231	16 (6.9)	0.017	1.9 (1.1–3.2)
p-tert-Butylphenol formaldehyde resin	40 μg/cm^2	2.2 (7982)	231	14 (6.1)	<0.001*	2.7 (1.69–4.8)
Quaternium-15	100 μg/cm^2	1.6 (8035)	231	9 (3.9)	0.014	2.4 (1.2–4.8)
Quinoline mix	190 μg/cm^2	0.3 (8035)	231	2 (0.9)	0.10	3.4 (0.8–14.8)
Sesquiterpene lactone mix	0.1% (pet.)	1.1 (8036)	229	7 (30.6)	0.013	2.7 (1.2–6.0)
Thiomersal	8 μg/cm^2	1.2 (8022)	231	3 (1.3)	0.90	1.1 (0.3–3.4)
Thiuram mix	25 μg/cm^2	2.2 (8034)	231	7 (3.0)	0.21	1.6 (0.8–3.3)
Tixocortol-21-pivalate	0.1% (pet.)	0.7 (8036)	229	2 (8.7)	0.78	1.2 (0.3–5.0)

CI, confidence interval; OR, odds ratio.
ORs were calculated by the use of logistic regression analysis with 95%CIs, age- and sex-adjusted.
*Statistical significant differences are based on a corrected *p*-value threshold of 0.002, owing to multiple testing.

Of all PPD-sensitized subjects, 45% were also sensitized to TDA; of all TDA-sensitized subjects, 86% were also sensitized to PPD. On the basis of the chemical structures of PPD and TDA, the percentage of cross-reactions with PPD in TDA-sensitized individuals should be high, as in our study. It is a possibility that the 15% of subjects with positive patch test reactions to TDA, but not to PPD, are sensitized to this specific part of the molecule, so that the immune system does not recognize PPD. A comparable percentage was also found by Basketter and English in 2009 (7). Although this could explain the difference, the question remains of whether the number of false-negative test results obtained with the TRUE test™ or the number of false-positive test results obtained with chamber-loaded tests biases the findings (8).

The strength of the patch test reaction to PPD or TDA is related to the number of cross-reacting substances

A stronger elicitation reaction to PPD is associated with significantly more positive reactions to cross-reacting, *para*-amino compounds, as shown in Fig. 1. This phenomenon is also seen in TDA-sensitized individuals, in whom significantly more cross-reactions were found among the extremely strong reactions.

The relationship between the strength of the patch test reaction and cross-reactivity to related molecules, as we

Table 4. Co-sensitizations to toluene-2,5-diamine (TDA) and allergens in the European baseline series

Allergen from the departmental baseline series	Test concentration (vehicle)	Overall prevalence (%) of allergy to European baseline series allergens (number tested)	Number reacting to TDA	Number reacting to European baseline series allergens and TDA (%)	*p*-Value	OR (95%CI)
Myroxylon pereirae (balsam of Peru)	800 μg/cm^2	2.2 (8035)	108	3 (2.8)	0.65	1.3 (0.4–4.2)
Budesonide	0.1% (pet.)	0.8 (8036)	108	2 (1.9)	0.18	2.7 (0.6–11.2)
Carba mix	250 μg/cm^2	2.4 (7778)	105	8 (7.6)	0.001*	3.4 (1.6–7.2)
Methylchloroisothiazolinone/ methylisothiazolinone	4 μg/cm^2	4.3 (7777)	105	3 (2.9)	0.48	0.7 (0.2–2.1)
Cobalt chloride	20 μg/cm^2	5.0 (8005)	108	10 (9.3)	0.081	1.8 (0.9–3.5)
Colophonium (colophony)	850 μg/cm^2	3.8 (8017)	108	10 (9.3)	0.007	2.5 (1.3–4.8)
Epoxy resin	50 μg/cm^2	2.2 (8030)	108	1 (0.9)	0.44	0.5 (0.1–3.3)
Ethylenediamine dihydrochloride	50 μg/cm^2	0.7 (7770)	106	1 (1.0)	0.69	1.5 (0.2–11.1)
Formaldehyde	180 μg/cm^2	1.4 (7977)	108	3 (2.8)	0.31	1.8 (0.6–5.9)
Fragrance mix I	430 μg/cm^2	4.9 (8033)	108	7 (6.5)	0.59	1.2 (0.6–2.7)
Fragrance mix II	14% (pet.)	2.4 (8036)	35	4 (11.4)	0.32	1.7 (0.6–5.0)
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	5% (pet.)	1.2 (8036)	34	0 (0)	>0.99	NA
Lanolin alcohols	1000 μg/cm^2	1.0 (8033)	108	1 (0.9)	0.97	1.0 (0.1–7.0)
Mercapto mix	75 μg/cm^2	1.4 (8035)	108	4 (3.7)	0.079	2.5 (0.9–6.9)
Mercaptobenzothiazole	75 μg/cm^2	1.1 (8005)	108	3 (2.8)	0.55	1.5 (0.4–6.3)
Methyldibromo glutaronitrile	0.5% (pet.)	3.1 (8036)	89	4 (4.5)	0.61	1.3 (0.5–3.6)
Methylisothiazolinone	0.01% (aq.)	7.8 (245)	6	2 (33.3)	0.032	7.8 (1.2–51.1)
Neomycin sulfate	230 μg/cm^2	0.7 (8036)	108	1 (0.9)	0.81	1.3 (0.2–9.4)
Nickel sulfate	200 μg/cm^2	18.3 (7918)	107	28 (26.2)	0.27	1.3 (0.8–2.0)
Paraben mix	1000 μg/cm^2	0.3 (8029)	107	2 (1.9)	0.011	6.9 (1.6–30.3)
Potassium dichromate	23 μg/cm^2	3.7 (7999)	108	7 (6.5)	0.16	1.8 (0.8–3.8)
p-tert-Butylphenol formaldehyde resin	40 μg/cm^2	2.2 (7982)	107	4 (3.7)	0.33	1.7 (0.6–4.6)
Quaternium-15	100 μg/cm^2	1.6 (8035)	108	2 (1.9)	0.92	1.1 (0.3–4.4)
Quinoline mix	190 μg/cm^2	0.3 (8035)	108	1 (0.9)	0.21	3.6 (0.5–27.5)
Sesquiterpene lactone mix	0.1% (pet.)	1.1 (8036)	109	3 (2.8)	0.18	2.3 (0.7–7.4)
Thimerosal (thiomersal)	8 μg/cm^2	1.2 (8022)	109	0	NA	NA
Thiuram mix	25 μg/cm^2	2.2 (8034)	108	2 (1.9)	0.73	1.2 (0.4–3.9)
Tixocortol-21-pivalate	0.1% (pet.)	0.7 (8036)	109	1 (0.9)	0.85	1.2 (0.2–8.8)

CI, confidence interval; OR, odds ratio; NA, not applicable.
ORs were calculated by the use of logistic regression analysis with 95%CIs, age- and sex-adjusted.
*Statistically significant differences are based on a corrected *p*-value threshold of 0.002, owing to multiple testing.

found in the present study, has already been described by Thomas et al. (5).

Uter et al. described how it is often difficult to discriminate between cross-reactivity and co-sensitization, as not all individuals with PPD sensitization react to all *para*-amino compounds. As these compounds often appear together, such as PPD, TDA, *m*-aminophenol, and *p*-aminophenol, it is difficult to determine whether the immune system recognizes a similar molecular structure. Alternatively, previous exposure with independent sensitization can occur. This seems more plausible, as not all individuals are sensitized to all cross-reacting molecules (9). As mentioned before, the individual can be sensitized to another part of the molecule. Furthermore, haptens must have different spacial geometries and sizes, and allergens are not recognized by the same receptor (10). Accurate study of cross-reactivity is only possible when the exposure to suspected haptens is controlled.

A probable explanation for the less marked relationship between the the strength of the TDA patch test reaction and the number of cross-reactions, especially in weakly sensitized individuals, is the difference in molecular structure between TDA and PPD, namely the methyl group being next to one of the two amino groups, which makes the molecule a slightly less potent sensitizer (7). However, owing to the low number of

investigated individuals in our study, it is difficult to draw firm conclusions.

Co-sensitizations to PPD and TDA with baseline series allergens

When co-sensitization is studied, the relationship between positive patch test reactions can be unclear, as common exposure sources are often difficult to determine.

PPD-sensitized subjects had a statistically significant higher chance of being sensitized to both carba mix, cobalt chloride, colophonium, paraben mix and PTBFR than the entire patch test population. Furthermore, sensitization to fragrance mix I and formaldehyde showed an almost statistically significant higher chance of appearing more often in the PPD-sensitized population.

It is possible that subjects who dyed their hair are more exposed to hair styling, makeup or skin care products. This could explain why patients with PPD sensitization are more likely to be sensitized to fragrance mix I.

The higher chance of PPD-sensitized individuals being sensitized to parabens mix has already been described by Turchin *et al.* (11). Although the odds ratio they found was lower than in our population (OR: 10.0, 95%CI: 3.5–26.6), they found a similar prevalence of PPD/parabens mix sensitization, namely 2.4%. A possible explanation for the appearance of co-sensitization with PPD but not with TDA could be the similar molecular structures, whereby parabens have a hydroxyl group instead of an amino group in the *para* position. However, this is debatable, as research on this topic has shown some lack of consistency so far.

Recently, an epidemic of MI sensitization has been observed. In the present study, we found that subjects were 26 times more likely to be sensitized to both PPD and MI than to MI alone. However, the numbers are very small, as only 5 subjects reacted to PPD and were tested with MI, of whom 3 reacted to MI. This relationship was not observed when testing was performed with methylchloroisothiazolinone/MI. MI can be present in hair dye products, and these hair dye products are often used in combination with other hair cosmetics that may also contain MI, in both an occupational setting (hairdressers) and a non-occupational setting (consumers) (12).

It is unclear whether there is a relation between sensitization to colophonium, PTBFR, formaldehyde, and cobalt chloride. Schnuch et al. also found co-sensitization between PPD and colophonium and PTBFR. They attributed this concomitant sensitization to occupational exposure in the leather/textile industry (13). In our population this is unlikely, as there is no leather/textile industry in our region.

Both individuals with PPD sensitization and those with TDA sensitization are more likely to be sensitized to carba mix. This co-sensitization can occur in an occupational setting, owing to exposure to rubber chemicals, or in a non-occupational setting (14). Concomitant exposure to the accelators present in the carba mix and to rubber additives, which are present in the black rubber mix, could also have occurred.

When concomitant exposure to substances is not plausible, other mechanisms might underlie these co-sensitizations. One of these mechanisms could be the conversion of two very different molecules into two similar metabolites (10, 15). A well-known example of this conversion is that of the synthetic azo dyes being converted into aromatic amines. Around the late 1990s and early 2000s, it was reported that skin bacteria can cleave these azo dyes into, sometimes very toxic, aromatic amines, among which are PPD (16, 17).

Some of the chemicals that appeared to co-sensitize with PPD have an aromatic group. The role of the microbiota of the skin in the development and the prevention of diseases is increasingly recognised. One can presume that this microbiota can also convert or reduce the co-sensitizing molecules into molecules that are, for the human immune system, indistinguishable from PPD. Thus, the microbiota can also play a role in the development of contact allergy.

Conclusion

The well-known phenomenon of cross-reactivity between chemically related molecules and PPD and TDA was confirmed in the present study. Cross-reactivity was strongly related to the strength of the patch test reaction. Co-sensitization to cobalt chloride, colophonium, paraben mix and PTBFR is found more often in PPD-sensitized patients, and both PPD-sensitized and TDA-sensitized individuals have a significantly higher chance of being sensitized to carba mix as well. These co-sensitizations cannot be attributed to a frequently appearing or well-known exposure source. However, there is a possibility that different molecules are converted into chemically similar molecules, for example by the human skin microbiota, and this requires investigation.

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