

Contact allergy to the monomers in *p*-*tert*-butylphenol-formaldehyde resin

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In many adhesive formulations *p*-*tert*-butylphenol-formaldehyde resin (PTBP-F-R) is used as a binder. Contact allergy to this resin is not rare. In patients hypersensitive to PTBP-F-R, and butylphenol derivatives therein, it is for diagnostic and preventive reasons necessary to know the nature of the primary sensitizing substances, as well as the cross-reaction patterns for these. The aim of this study was to investigate contact allergy to monomers in PTBP-F-R and potential cross-reacting substances. 12 patients hypersensitive to PTBP-F-R were patch tested with 2 monomers, the raw materials formaldehyde and *p*-*tert*-butylphenol, and 3 closely related substances. High pressure liquid chromatography (HPLC) was used to investigate the purity of the test substances. It was shown that the monomers 2-methylol *p*-*tert*-butylphenol and 2,6-dimethylol *p*-*tert*-butylphenol could elicit allergic reactions in humans hypersensitive to PTBP-F-R. No simultaneous reactions or cross-reactions were shown to formaldehyde, *p*-*tert*-butylphenol, *p*-*tert*-butylcatechol, 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) or 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT). It was also shown that low amounts of contaminants in the test substances, if not taken into account, could influence the conclusions drawn from the test results obtained.

Key words: 2(3)-*tert*-butyl-4-hydroxyanisole; 2, 6-dimethylol *p*-*tert*-butylphenol; 2-methylol *p*-*tert*-butylphenol; 3, 5-di-*tert*-butyl-4-hydroxytoluene; BHA; BHT; contact allergy; contamination; formaldehyde; *p*-*tert*-butylcatechol; *p*-*tert*-butylphenol. © Blackwell Munksgaard, 2002.

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In many adhesive formulations *p*-*tert*-butylphenol-formaldehyde resin (PTBP-F-R) is used as a binder. Contact allergy to this resin is not uncommon(1–3). Allergy has been reported from contact with, among other things, glues, shoes, watch straps, athletic tape, a hearing aid and ink of a marking pen and the following substances have been reported as allergens in the resin: 2-methylol *p*-*tert*-butylphenol (1);

- 2,6-dimethylol *p*-*tert*-butylphenol (2);
- *p*-*tert*-butylphenol (3);
- some degradation products and one larger phenolic compound(4–9).

Chemical structures of the compounds are shown in Fig. 1. Simultaneous patch test reactions to Myroxylon Pereirae resin, hydroabietyl alcohol, phenol-formaldehyde resin (P-F-R) and PTBP-F-R have been reported (10).

In many cases of hypersensitivity to PTBP-F-R it is difficult to find a clinically relevant exposure to the resin and its sensitizers, which is

partly due to insufficient knowledge of the chemical identity of sensitizers in PTBP-F-R. Improved knowledge of the identity of the allergens, their sensitizing potential, cross-reaction patterns and analytical methods for the detection of the sensitizers in the environment can contribute to improved diagnosis and prevention of allergic contact dermatitis from PTBP-F-R. This study is part of a larger investigation aiming at isolation and identification of PTBP-F-R allergens. The purpose of this study was to investigate the presence of contact allergy to the monomers in PTBP-F-R and cross-reaction patterns to these in patients allergic to PTBP-F-R.

Materials and Methods

Substances

The following substances were used: methanol, diethyl ether, tetrahydrofuran (THF) and acetone *in pro analysi* quality manufactured by Laboratory-Scan (Ireland); formaldehyde 37%, sulphuric acid conc., *p*-*tert*-butylcatechol (4) 99% and 3 were

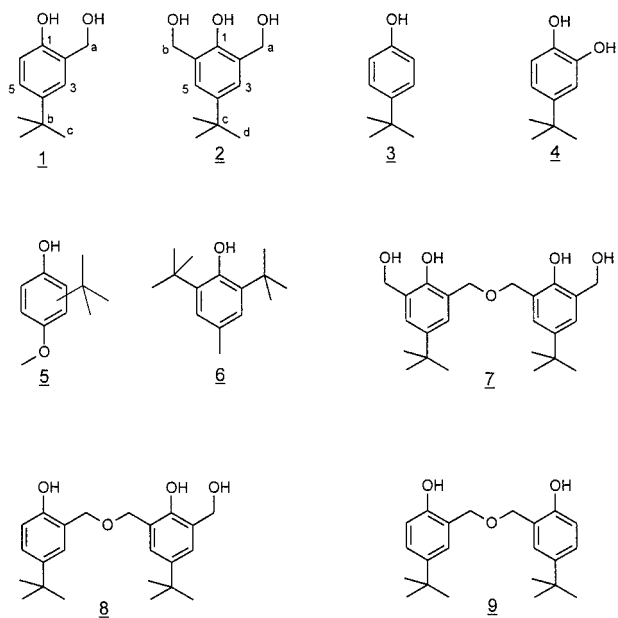


Fig. 1. Chemical structures of 2-methylol p-tert-butylphenol (1), 2,6-dimethylol p-tert-butylphenol (2), p-tert-butylphenol (3), p-tert-butylcatechol (4), 2(3)-tert-butyl-4-hydroxyanisole (5), 3,5-di-tert-butyl-4-hydroxytoluene (6), 4-tert-butyl-2-(5-tert-butyl-2-hydroxy-3-hydroxymethyl-benzyloxymethyl)-6-hydroxymethyl-phenol (7), 4-tert-butyl-2-(5-tert-butyl-2-hydroxy-benzyloxymethyl)-6-hydroxymethyl-phenol (8) and 4-tert-butyl-2-(5-tert-butyl-2-hydroxy-benzyloxymethyl)-phenol (9).

manufactured by Acros Organics (Gell, Belgium); 2(3)-tert-butyl-hydroxyanisole (BHA) (5) and di-tert-butyl-hydroxytoluene (BHT) (6) were manufactured by ICN Biomedicals Inc. (Aurora, OH, USA); PTBP-F-R 1%w/w in petrolatum (pet.) from Chemotechnique (Malmö, Sweden); sodium hydroxide from Fluka Chemie AG (Buchs, SG, Switzerland). 4-tert-Butyl-2-(5-tert-butyl-2-hydroxy-3-hydroxymethyl-benzyloxymethyl)-6-hydroxymethyl-phenol (7), 4-tert-butyl-2-(5-tert-butyl-2-hydroxy-benzyloxymethyl)-6-hydroxymethyl-phenol (8) and 4-tert-butyl-2-(5-tert-butyl-2-hydroxy-benzyloxymethyl)-phenol (9) were synthesized at our department as reported elsewhere (11). Compounds 1 and 2 were synthesized at the department as described below.

High pressure liquid chromatography (HPLC)

Analytical HPLC was performed using a solvent delivery system consisting of an LDC Constametric I pump (Laboratory Data Control, Riviera Beach, FL, USA), a Rheodyne 7010 injector (Rheodyne, Cotati, CA, USA) equipped with a 20- μ L loop, an LDC SpectroMonitor III UV detector (Laboratory Data Control) operating at a wavelength of 280 nm and a column (4 mm i.d. $\times$ 200 mm) packed with 5 μ m Nucleosil C₁₈ (Macherey-

Nagel & Co., Dorën, Germany). A mixture of methanol and water was used as mobile phase and the flow rate was 1 mL/min.

Semipreparative HPLC (I) was performed using a solvent delivery system consisting of an LDC Gradient Master and 2 LDC Constametric III pumps (Laboratory Data Control). The automatic process was controlled by an F-AV electronic process control unit (Film och AV, Malmö, Sweden). The injector, allowing an injection volume of 10 mL, was a Rheodyne 7010 (Rheodyne) equipped with an electric actuator (Film och AV). Samples were collected by a pneumatic 6-position valve, Rheodyne model 5011 A (Rheodyne). The process was monitored using an LKB Uvicord S UV detector (LKB, Uppsala, Sweden) operating at a wavelength of 280 nm and an F-AV computer-system (Film och AV). The column (22 mm i.d. $\times$ 300 mm) was packed with 5 μ m Nucleosil C₁₈ (Macherey-Nagel & Co., Dorën, Germany).

Semipreparative HPLC (II) was performed using a solvent delivery system consisting of an LDC Gradient Master and 1 LDC Constametric III pump and 1 LDC Constametric I pump (Laboratory Data Control). The automatic process was controlled by an F-AV electronic process control unit (Film och AV). The injector, allowing an injection volume of 1 mL, was a Rheodyne 7010 equipped with a pneumatic actuator (Rheodyne). Samples were collected by a pneumatic 6-position valve, Rheodyne model 5011 A (Rheodyne). The process was monitored using an LDC Spectro-Monitor III UV detector (Laboratory Data Control) operating at a wavelength of 280 nm. The column (11 mm i.d. $\times$ 100 mm) was packed with 5 μ m Nucleosil C₁₈.

Nuclear magnetic resonance spectrometry (NMR)

¹H and ¹³C NMR spectra were recorded from samples of compounds 1 and 2 dissolved in CDCl₃ using a Bruker ARX, 500 MHz spectrometer. Chloroform was used as an internal reference (δ = 7.27 p.p.m. and 77.0 p.p.m.). Gradient selected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple-bond correlation (HMBC) was used.

Mass spectrometry (MS)

A Jeol SX 102 mass spectrometer was used with direct inlet to obtain mass spectra. Fast atomic bombardment with xenon (FAB+, 6 kV) in the presence of 3-nitrobenzyl alcohol and sodium iodide was used.

Synthesis of compounds 1 and 2

Synthesis was done according to a method used by Agatha & Schubert (12), though with some modification. Compound 3 (50.0 g, 330 mmol) was dissolved in a mixture of THF (300 mL), water (100 mL) and aq. sodium hydroxide (100 mL, 1.0 M). Formaldehyde (100 mL, 37%) was added to the solution. HPLC analysis of the reaction mixture was repeated several times. The reaction was allowed to continue until near maximal production of the 2 major reaction products was reached (normally around 48 h). The reaction was stopped by neutralization with sulphuric acid (2 M). The reaction mixture separated spontaneously into 2 phases. The upper yellow phase was evaporated under vacuum (30°C) and dissolved in methanol/water (50/50 v/v). The reaction products in this solution were separated by semipreparative HPLC (I) into fractions. The eluant was a mixture of methanol and water. An exponential gradient (exp. 5) was used starting at 55% v/v methanol, reaching 100% methanol after 15 min, remaining at 100% methanol for 15 min and returning to 55% v/v methanol after a total time of 31.5 min. The injected volume was 10 mL and the flow rate was 20 mL/min. 3 fractions were evaporated under vacuum (30°C). Fractions 1 and 2 were evaporated until most of the methanol had disappeared and crystals began to form. The samples were cooled, filtered and white crystalline residues were obtained. Fraction 3 was evaporated to dryness and formed an oily residue. Compounds 1 and 2 were further purified by using the semipreparative HPLC system I described above. The substances were analysed by MS and NMR.

1: ^1H NMR (CDCl_3): δ 1.29 (s, 9 H, H-c), 2.23 (t, 1 H, J 5.7 Hz, H-CH₂OH), 4.87 (d, 2 H, J 5.4 Hz, H-a), 6.84 (d, 1 H, J 8.5 Hz, H-6), 7.04 (s, 1 H, H-ArOH), 7.06 (d, 1 H, J 2.5 Hz, H-3), 7.24 (dd, 1 H, J 2.4 8.5 Hz, H-5); ^{13}C NMR (CDCl_3): δ 31.51 (C-c), 34.03 (C-b), 65.05 (C-a), 116.06 (C-6), 123.96 (C-2), 124.74 (C-3), 126.31 (C-5), 142.92 (C-4), 153.66 (C-1); HRMS (FAB+) calcd for $\text{C}_{11}\text{H}_{16}\text{O}_2\text{Na}$ $[\text{M} + \text{Na}]^+$: 203.1048, found: 203.1048.

2: ^1H NMR (CDCl_3): δ 1.28 (s, 9 H, H-d), 2.68 (s, 2 H, H-CH₂OH), 4.80 (s, 4 H, H-a and H-b), 7.08 (s, 2 H, H-3 and H-5), 7.94 (s, 1 H, H-ArOH); ^{13}C NMR (CDCl_3): δ 31.49 (C-d), 34.04 (C-c), 63.93 (C-a and C-b), 124.87 (C-3 and C-5), 125.50 (C-2 and C-6), 142.68 (C-4), 152.37 (C-1); HRMS (FAB+) calcd for $\text{C}_{12}\text{H}_{18}\text{O}_3\text{Na}$ $[\text{M} + \text{Na}]^+$: 233.1154, found: 233.1169.

The ^1H NMR spectrum corresponded to published data (13, 14).

Patch test 1

In all, 12 patients with contact allergy to PTBP-F-R detected by routine patch testing at our department participated in this study, which was approved by the Ethics Committee of the Lund University Medical Faculty.

Patch testing was performed with the Finn Chamber technique using Scanpor as adhesive and with 15 μL of the test solution on each patch unit. The patches remained on the back for 2 days and the reading was performed after a further 1 day according to ICDRG criteria.

Patch test 1 included testing with 1.0% w/v PTBP-F-R in pet. and 1.0% w/v formaldehyde in water (aq.). The monomers 1 (batch 1) and 2 (batch 1) were patch tested in 1.5 and 1.7% w/v, respectively, with 4 10-fold serial dilutions. The concentrations of the monomers were equimolar to the concentrations used when the monomers in P-F-R were patch tested, 81 mmol/L (15). Compounds 3, 5 and 6 were patch tested in 1.2, 1.5 and 1.8% w/v, corresponding to 81 mmol/L. Compounds 5 and 6 were also patch tested in 1 10-fold dilution. Compound 4 was tested in a concentration of 0.10% w/v (6.0 mmol/L) to avoid active sensitization (16). The sample BP was patch tested in 2 concentrations, 1.0% and 0.10% w/v. All test solutions were prepared in acetone (acet.). The chemical structures of the substances are shown in Fig. 1.

Purity of compound 1 (batch 1) and compound 2 (batch 1)

An estimation of the presence of the substances included in BP was made using analytical HPLC. 1 (batch 1) and 2 (batch 1) were analysed and BP used as reference.

Purification of the test substances

Semipreparative HPLC (I), with the same chromatographic conditions as described above, was used to purify 1 and 2 further, giving 1 (batch 2) and 2 (batch 2).

Investigations of the substances in BP

BP was investigated by analytical HPLC. Reference samples of 7, 8 and 9 (11) were used to determine if these were present in BP. The analysis indicated the presence of all of these substances in BP, as well as several others, which were seen as multiple peaks around 9 with only little separation. HPLC system (II) was used to divide BP into 3 fractions corresponding to 7, 8 and 9. Separation was achieved by isocratic elution with 87% v/v

Table 1. Patch test 1. Results from patch testing with p-tert-butylphenol-formaldehyde resin (PTBP-F-R), 2-methylol p-tert-butylphenol (**1**) (batch 1), 2,6-dimethylol p-tert-butylphenol (**2**) (batch 1), formaldehyde, p-tert-butylphenol (**3**), p-tert-butylcatechol (**4**), 2(3)-tert-butyl-hydroxyanisole (**5**), di-tert-butyl-hydroxytoluene (**6**) and by-products (BP) in 12 patients hypersensitive to PTBP-F-R

Compound	%* w/v and (mmol/L)	1	Patient no.										
			2	3	4	5	6	7	8	9	10	11	12
PTBP-F-R	1.0 w/w pet.	+	++	++	+++	++	++	+	+++	+++	++	++	++
Formaldehyde	1.0 aq. (333)	-	-	-	-	-	-	-	-	-	-	-	-
1	1.45 (81)	++	+	-	+++	+	+++	++	+++	+++	++	+++	++
(batch 1)	0.145	(+)	-		+++	++	++	+	+++	+++	+	+++	++
	0.0145	-			+	-	++	-	+++	+	-	++	-
	0.00145				(+)		(+)		++	-		++	
	0.000145				-		-		++			+	
2	1.69 (81)	++	++	++	(+)	++	+++	(+)	+++	+++	++	+	++
(batch 1)	0.169	(+)	(+)	(+)	+	+	++	-	++	+++	-	-	+
	0.0169	-	-	-	-	-	-		-	+			-
	0.00169									-			
3	1.21 (81)	-	-	-	-	-	-	-	-	-	-	-	-
4	0.10 (6.0)	-	-	-	-	-	-	-	-	-	-	-	-
5	1.45 (81)	-	-	-	(+)	-	-	-	-	-	-	-	(+)
	0.145				-								-
6	1.77 (81)	-	-	-	-	-	-	-	-	-	-	-	-
BP	1.0	++	++	+++	+++	++	+++	++	+++	+++	++	NT	+++
	0.10	++	+	++	+	++	+++	++	+++	+++	++	NT	+++

*Acetone was used as vehicle, if not otherwise indicated; +++, ++, + = patch test reaction of an allergic nature; (+) = only erythema or a few papules; (-) = negative test reaction; NT = not tested.

methanol for 8 min. The system was washed with 100% methanol for 2 min between each injection. The injected volume was 1 mL and the flow rate was 7 mL/min. ¹H NMR showed that the first 2 fractions were **7** and **8**, respectively. Fraction 3 (BP3) was collected and evaporated. **7**, **8** and BP3 were used for patch testing, as described below.

Patch test 2

In patch test 2, 6 of the patients tested in patch test 1 participated. Patch testing with **1** (batch 2) and **2** (batch 2) was performed in the same way as described above for patch test 1. Additional patch testing was performed with samples of **7**, **8** and BP3 in a concentration of 1% w/v in acet. and with 5 10-fold serial dilutions of each of the substances.

Purity of compound **1** (batch 2) and compound **2** (batch 2)

Analytical HPLC was used to determine the purity of **1** (batch 2) and **2** (batch 2). The contamination of one monomer in the other, as well as the contents of **7**, **8** and BP3 in the purified monomers, were determined.

Control tests

Test solutions of **1** (batch 2) 0.15% and **2** (batch 2) 0.17% were patch tested in 12 controls.

Statistical calculations

Fisher's exact test was used.

Results

The results of patch test 1 are given in Table 1. All patients hypersensitive to PTBP-F-R except 2 (patient no. 3 and no. 7) reacted positively to both **1** (batch 1) and **2** (batch 1). Patient no. 3 reacted positively to **2** but not to **1** and patient no. 7 reacted positively to **1** but not to **2**. There were no positive patch test reactions to formaldehyde, **3**, **4**, **5** or **6**. 2 patients showed only erythematous reactions to **5**. All patients tested with BP reacted positively to this sample and most of them showed strong reactions to both 1.0 and 0.10% w/v solutions.

HPLC analysis of **1** (batch 1) and **2** (batch 1) revealed the presence of substances from BP in both. BP substances constituted 0.04 and 1.3% w/w of the samples of **2** (batch 1) and **1** (batch 1), respectively. **2** (batch 1) contained 0.50% **1** and **1** (batch 1) contained 1.0% w/w **2**.

Results from patch test 2 are given in Table 2. All 6 patients reacted positively to all test substances with varying reactivity. Reactions to **1** (batch 2) were in most patients of the same strength, or weaker, as in patch test 1. Test reactions to **2** (batch 2) were in most cases somewhat stronger than in patch test 1. All the patients reacted to more di-

Table 2. Patch test 2. Results from patch testing with 2-methylol p-tert-butylphenol (**1**) (batch 2), 2,6-dimethylol p-tert-butylphenol (**2**) (batch 2) and the contaminants 4-tert-butyl-2-(5-tert-butyl-2-hydroxy-3-hydroxymethyl-benzyloxymethyl)-6-hydroxymethyl-phenol (**7**), 4-tert-butyl-2-(5-tert-butyl-2-hydroxy-benzyloxymethyl)-6-hydroxymethyl-phenol (**8**) and BP3 in 6 patients hypersensitive to p-tert-butylphenol-formaldehyde resin (PTBP-F-R)

Compound	%# w/v and (mmol/L)	Patient no.					
		4	5	7	8	11	12
1 (batch 2)	1.45 (81)	+++	+++	+++	NT	NT	NT
	0.145	+++	+++*	++	NT	+++	++
	0.0145	++**	—	—	++*	++	(+)
	0.00145	—	—	—	—	+	—
	0.000145	—	—	—	—	—	—
2 (batch 2)	1.69 (81)	+++	+++	+++	NT	NT	NT
	0.169	++**	+++**	++*	NT	+++	+
	0.0169	—	—	—	++**	+	—
	0.00169	—	—	—	—	—	—
7	1.0 (25)	+++	+++	+++	NT	NT	NT
	0.10	+++	+++	+++	NT	+++	+++
	0.010	+	++	+++	+++	+++	+++
	0.0010	(+)	—	++	++	+++	+++
	0.00010	—	—	+	—	++	++
	0.000010	—	—	—	—	(+)	++
8	0.9 (25)	+++	+++	+++	NT	NT	NT
	0.09	+++	+++	+++	NT	+++	+++
	0.009	++	++	+++	+++	+++	+++
	0.0009	(+)	+	++	+++	++	+++
	0.00009	—	—	+	++	—	+
	0.000009	—	—	—	—	—	(+)
BP3	1.0 (na)	+++	++	+++	NT	NT	NT
	0.10	++	++	+++	NT	+++	+++
	0.010	+	+	+++	+	+++	+++
	0.0010	—	+	(+)	++	+	++
	0.00010	—	—	—	(+)	—	—
	0.000010	—	—	—	—	—	—

#Acetone was used as vehicle; + + +, ++, + = patch test reaction of an allergic nature; (+) = only erythema or a few papules; (—) = negative test reaction; NT = not tested; na = not applicable; ** = a patch test shown to be due to the test substance and not a contaminant; * = a patch test highly likely to be due to the test substance and not a contaminant.

luted solutions of **7**, **8** and BP3 as compared to **2** (batch 2) and **1** (batch 2), except patient 4, who reacted positively to almost equally diluted solutions of **1** (batch 2) and BP3.

HPLC analysis was performed to investigate the purity of **2** (batch 2) and **1** (batch 2). Compound **2** (batch 2) contained <0.01% w/w of each of **7**, **8** and BP3 and 0.06% w/w of **1**. Compound **1** (batch 2) contained 0.05, 0.04 and 0.14% w/w of **7**, **8** and BP3, respectively, and 0.30% w/w of **2**.

The compounds **1** (batch 2) and **2** (batch 2) were patch tested in 12 controls, none of whom showed positive patch test reactions. The difference between test and control groups was statistically significant for **1** ($p = 0.049$) as well as for **2** ($p = 0.0098$).

Discussion

The first allergen reported in PTBP-F-R was **3** by Malten in 1958 (17). Agatha & Schubert mentioned **1** and **2** in a paper in 1979. **1** was considered to be a new main allergen in the resin (9, 12). They

found only weak or doubtful reactions to **2**. In 1985, Malten & Seutter used these compounds for an investigation in which 8 of 8 patients reacted positively to both substances (5). Later, in 1994, Hagdrup et al. investigated PTBP-F-R allergy in 1 patient and used the 2 monomers for patch testing (7). They found that both substances were allergens. However, in all the investigations mentioned above sparse data are given on the identity and the purity of the test substances. Agatha & Shubert gave some indication of the purity of one of test substances, **1**, and showed a melting point of the substance of 92°C, which corresponds well to other published data. Hagdrup et al. showed a purity of >99% for **1** and **2**.

In our efforts to synthesize **1** and **2** we noticed how difficult it was to obtain these substances at a high purity. They are reactive monomers and prone to react with each other just as they do when PTBP-F-R is manufactured.

In patch test 1, all patients but 2 tested positively to **1** (batch 1) and **2** (batch 1), which indicated that these monomers are allergens. 2 of the patients re-

acted only to one of the monomers. None of the 12 patients reacted positively to formaldehyde, **3**, **4**, **5** or **6**. Formaldehyde, **3**, **4**, **5** and **6** are all known as sensitizers but our results show that these substances are not frequent allergens among our PTBP-F-R-allergic patients. Regarding formaldehyde and **3**, these findings are in agreement with results from other studies (18, 19).

Before ascribing allergic reactions to chemically defined compounds, purity investigations should be performed to exclude contaminants as the cause of the observed reactions. Purity investigations of **1** (batch 1) and **2** (batch 1) revealed the presence of BP substances in both, though most in **1** (batch 1). The strong patch test reactions to BP and the detection of BP in both **1** (batch 1) and **2** (batch 1) indicated that the contaminants could be the cause of some or maybe all of the observed positive reactions.

As we had not unambiguously shown whether or not the monomers **1** or **2** were allergens in PTBP-F-R, it was considered necessary to retest some of the patients with further purified test substances. The sparse data on purity in other papers describing **1** or **2** as allergens (5, 7, 9, 12) further encouraged this effort. Simultaneous patch testing with serial dilutions of the putative sensitizers and the contaminants was used as a method to quantify the degree of contamination that could explain a patch test reaction. For this purpose, purified samples of **1** and **2** were tested in patch test 2 as well as the main impurities **7**, **8** and BP3.

In all, 6 patients were patch tested with **1** (batch 2), **2** (batch 2) and the impurities in patch test 2, and all reacted positively to both the monomers as well as to **7**, **8** and BP3 (Table 2). By HPLC analysis of **1** (batch 2) and **2** (batch 2) their contamination by **7**, **8** and BP3 was determined. With knowledge of the individual reactivities to the contaminants and the concentrations of these in the test substances, the patch test reactions due to the contaminants can be estimated. The number of positive reactions in the dilution series was used as a measure of reactivity regardless of the strength of the positive reaction. If the difference between the observed and the estimated reactivity was at least 2 steps in favour of the test substance, the degree of contamination was considered to be significantly lower than the level that could explain the test reaction. It was considered highly probable that the reaction was due to the test substance if the difference in reactivity was 1 dilution step in favour of the test substance, and due to the contaminant when there was no difference or a difference in favour of the contaminant.

Contamination with **2** can in no case explain the test reactions to **1** (batch 2) in patch test 2. Con-

tamination of **1** (batch 2) with **7**, **8** and BP3 was significantly lower than the degree of contamination required to explain the test response for 1 patient (no. 4) and low enough to make it highly probable that reactions in 2 more patients (no. 5 and no. 8) were not caused by contaminants. Still, in 3 patients (no. 7, no. 11 and no. 12) contaminants could explain the patch test reactions to **1** (batch 2).

The contamination of **2** (batch 2) with **1** was for all patients significantly lower than the level of contamination that could explain the test responses. The contamination of **2** (batch 2) with **7**, **8** and BP3 was significantly lower than the degree of contamination required to explain the test response for 3 patients (no. 4, no. 5 and no. 8) and low enough to make it highly probable that reaction in 1 more patient (no. 7) was caused by **2** and not the contaminants. Still, in 2 patients (no. 11 and no. 12) the contaminants could explain the patch test reactions to **2** (batch 2). Patch test 2 also shows that **7** and **8** are allergens, and our group has published a separate paper on this subject (20). Patch testing of **1** and **2** in controls strongly supported the conclusion that the observed positive reactions were allergic. Compounds **7** and **8** have previously been tested in controls without causing positive reactions (20).

This study shows that **1** and **2** are allergens. In broad outline this is in agreement with the previously reported reactions to **1** and **2** (5, 7, 9, 12). However, it seems very likely that some of the previous positive reactions were also caused by contaminants. Our results show that even a contamination level of 0.01% of **7** in **2** could explain some test results.

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