

Sensitizing capacity of 5,5'-di-tert-butyl-2,2'-dihydroxy-(hydroxymethyl)-dibenzyl ethers in the guinea pig

ERIK ZIMERSON AND MAGNUS BRUZE

Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, Sweden

In patients hypersensitive to p-tert-butylphenol-formaldehyde resin (PTBP-F-R), it is for diagnostic, therapeutic and preventive reasons necessary to know the identity of the primary sensitizing substances, their sensitizing capacities as well as their cross-reaction patterns. We have recently shown that the 2 dimers in PTBP-F-R, 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether (X) and 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether (IX) are contact sensitizers in man. The aim of this study was to investigate the sensitizing capacities of these dimers in PTBP-F-R and potential cross-reacting substances in the guinea pig with the guinea pig maximization test. IX, X, 2,6-dimethylol-p-tert-butylphenol (2,6-MPTBP), 2-methylol-p-tert-butylphenol (2-MPTBP), p-tert-butylcatechol (PTBC), 5,5'-di-tert-butyl-2,2'-dihydroxy-dibenzyl ether (XI) were used as possible cross reacting substances. IX and X were shown to be sensitizers. When compared to the sensitizers in phenol-formaldehyde resin, IX is a strong sensitizer ($p=0.00052$) and X a moderate sensitizer ($p=0.0053$). Animals sensitized to IX showed cross-reactions to X ($p=0.010$), 2,6-MPTBP ($p=0.0011$) and PTBC ($p=0.0498$). Animals sensitized to X showed no cross-reactions to the substances that were tested. The results indicate that IX is a main allergen in PTBP-F-R, with possibly also X.

Key words: cross-sensitization; PTBP-F-R; GPMT; 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether, 35851-08-2; 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether; 5,5'-di-tert-butyl-2,2'-dihydroxy-dibenzyl ether. © Munksgaard, 2000.

Accepted for publication 18 January 2000

Contact allergy to p-tert-butylphenol formaldehyde resin (PTBP-F-R), a resin used as a binder in many adhesive formulations, is not rare (1–4). The resin consists of hundreds of thousands of substances, of which the vast majority are not chemically defined. PTBP-F-R has been used since around 1950 but only a few allergens have been identified. For diagnostic and preventive reasons, the chemical identity of the sensitizers should be known as well as their sensitizing capacities, cross-reaction patterns and presence in the environment.

In recent years, we have identified several new allergens in this resin and the 5,5'-di-tert-butyl-2,2'-dihydroxy-(hydroxymethyl)-dibenzyl ethers seem to be potent allergens in PTBP-F-R, according to results from patch testing in patients hypersensitive to PTBP-F-R. Positive reactions to a patch test concentration as low as 0.01 ppm have

been recorded (5). For these substances, it is important to know their sensitizing capacities and this can best be investigated in animals. It is also possible to investigate the cross-reaction pattern for each sensitizer in animals, as these are sensitized to each individual substance.

This study is part of a larger project, the aim of which is to identify the main sensitizers in PTBP-F-R. As a part of this project we have isolated 2 dimers from PTBP-F-R: 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether (IX), 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether (X). Patch testing in patients hypersensitive to PTBP-F-R has shown that both of them are allergens (5). The purpose of this study is to determine the sensitizing capacities of IX and X and to investigate the cross-reaction patterns using the guinea pig for sensitization.

Materials and Methods

High-pressure liquid chromatography (HPLC)

Semipreparative HPLC was performed using a solvent delivery system consisting of a LDC Gradient Master and 2 LDC Constametric III pumps (Laboratory Data Control, USA). An F-AV electronic process control unit (Film och AV, Sweden) controlled the automatic process. The injector, allowing an injection volume of 10 ml, was a Rheodyne 7010 (Rheodyne, USA) 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, 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., Germany). Solvent A consisting of methanol/water (50/50% v/v) and solvent B consisting of methanol (100%) were used as mobile phases and the flow rate was 8.0 ml $\times$ min⁻¹. Elution (gradient, linear) started at 70% v/v B, reached 100% v/v B after 12 min, remained at 100% v/v B for 15 min and ended at 70% v/v B after a total time of 28 min.

Analytical HPLC was performed using a Varian 5000 (Varian, USA) pump system equipped with a Rheodyne 7010 injector (Rheodyne) with a 20 μ l loop and a column (4.6 mm i. d. $\times$ 250 mm) packed with 5 μ m Nucleosil C₁₈ (Macherey-Nagel & Co.). The built in UV-100 detector (Varian) was operated at a wavelength of 280 nm. Different mixtures of methanol and water were used as mobile phases and the flow rate was 1 ml/min.

Nuclear magnetic resonance spectrometry (NMR)

¹H and ¹³C NMR-spectra were recorded from samples dissolved in CDCl₃, using a Bruker ARX, 500 MHz NMR spectrometer. Tetramethylsilane was used as an internal reference (δ =0.00 ppm).

Mass spectrometry (MS)

A Jeol JMS-SX 102 mass spectrometer was used with direct inlet to obtain mass spectra of the synthesized substances described below. EI (70 eV), CI-CH₄ and FAB+Na was used.

Substances

The following substances were used: methanol, ethylacetate, tetrahydrofuran (THF) and acetone in pro analysi quality manufactured by Lab-Scan (Ireland); p-tert-butylcatechol (PTBC) 99%, 2-methylol phenol (2-MP), sodium hydroxide and

sulfuric acid, conc., were manufactured by Acros Organics (Belgium); 2-methylol-p-tert-butylphenol (2-MPTBP) and 2,6-dimethylol-p-tert-butylphenol (2,6-MPTBP) were synthesized at our department (to be published). IX, X and 5,5'-di-tert-butyl-2,2'-dihydroxy-dibenzyl ether (XI) were synthesized at our department as described below. Reference samples of IX and X had earlier been isolated from PTBP-F-R and identified at our laboratory (5). A reference sample of XI had been obtained in the same way (to be published).

Synthesis of 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether (IX)

2.0 g (9.5 mmol) 2,6-MPTBP was dissolved in a mixture of 3 ml THF and 0.012 ml sulfuric acid, conc. The reaction was carried out at room temperature. The reaction mixture was analyzed by analytical HPLC several times each day. A sample of IX earlier isolated from PTBP-F-R was used as a reference to establish the position of the IX in the HPLC chromatogram. The reaction was allowed to continue until maximal production of IX was reached (normally around 96 h). The reaction mixture was then neutralized by the addition of sodium hydroxide solution (1 M and 0.1 M). This solution was diluted with a mixture of methanol and water 70/30 v/v to a final concentration of 2%, dry weight. The reaction product IX in this solution was separated by semipreparative HPLC, the collected sample was evaporated under vacuum (30°C) and IX was obtained as a pale-yellow oily liquid. The product was analyzed by MS and NMR. MS and NMR data were in agreement with the results obtained from a reference sample of IX.

Synthesis of 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether (X)

1.0 g (5.6 mmol) 2-MPTBP and 1 g (4.8 mmol) 2,6-MPTBP were dissolved in a mixture of 3 ml THF and 0.012 ml sulphuric acid. The reaction was carried out at room temperature. The reaction mixture was analyzed by analytical HPLC several times each day. A sample of X earlier isolated from PTBP-F-R was used as a reference to establish the position of the X in the HPLC chromatogram. The reaction was allowed to continue until maximal production of X was reached (normally around 72 h). The reaction mixture was neutralized by the addition of sodium hydroxide solution (1 M and 0.1 M). This solution was diluted with a mixture of methanol and water 75/25 v/v to a final concentration of 4%, dry weight. The reaction product X in this solution was separated by semipreparative HPLC, the collected sample was evaporated under

vacuum (30°C) until most of the methanol had disappeared and crystals began to form. The sample was cooled, filtered and X was obtained as white crystals. The product was analyzed by MS and NMR. MS and NMR data were in agreement with the results obtained from a reference sample of X.

Synthesis of 5,5'-di-tert-butyl-2,2'-dihydroxy-dibenzyl ether (XI)

2 g (11 mmol) 2-MPTBP was dissolved in a mixture of 3 ml THF and 0.012 ml sulfuric acid, conc. The reaction was carried out at room temperature. The reaction mixture was analyzed by analytical HPLC several times a day. A sample of XI earlier isolated from PTBP-F-R was used as a reference to establish the position of the XI in the HPLC chromatogram. The reaction was allowed to continue until maximal production of XI was reached (normally around 72 h). The reaction mixture was neutralized by the addition of sodium hydroxide solution (1 M and 0.1 M). This solution was diluted with a mixture of methanol and water 75/25 v/v to a final concentration of 2%, dry weight. The reaction product XI in this solution was separated by semipreparative HPLC, the collected sample was evaporated under vacuum (30°C) until most of the methanol had disappeared and crystals began to form. The sample was cooled, filtered and XI was obtained as white crystals. The product was analyzed by MS. MS data were in agreement with the results obtained from a reference sample of XI.

Purity of test substances

Analytical HPLC was used to investigate the purity of the test substances, IX, X, 2,6-MPTBP, 2-MPTBP and PTBC.

Guinea pig maximization test (GPMT)

The GPMT was performed in accordance with the original description (6), but with some modifications in order to increase the standardization of the test and also to create conditions for objective evaluation, including statistical calculations of the patch test reactions and the inclusion of a positive control group (7, 8). The test and control animals, also the animals in the positive control group, were randomly distributed to the cages.

Animals

Albino female guinea pigs of the Dunkin-Hartley strain (J.A. Sahlin, Sweden) weighing 300–400 g were used. For each one of the sensitization series

42 animals were used, 36 animals participated in the actual sensitization study, 12 in the control group and 24 in the test group, while the remaining 6 animals comprised an additional control group. These 6 guinea pigs were sensitized to and challenged with the known sensitizer 2-MP and used as a positive control group, to eliminate the possible influence of expectations on the evaluation of test reactions that would result in underestimations, and to serve as an indicator of the accuracy of the induction procedure (8). The animals included in these procedures were not engaged in tests for topical irritancy.

Topical irritancy

The topical irritancy of the substances used for inductions and challenges was studied by means of a 48-h closed patch test in 4–8 animals for each compound (8). The compound was applied to each animal on 4 patches on the flank; 2 near the back, 2 near the abdomen. The induction substances were also applied on the neck. Testing was performed 1 week after pre-treatment with Freund's complete adjuvant (FCA) (Difco Lab., USA).

Induction procedure

For intradermal sensitization 3 injections were given in a row, on each side of the shoulder. (I) 0.1 ml of FCA in water 40% w/v (corresponds to FCA/water 50/50 v/v). (II) 0.1 ml of the test substance in propylene glycol. (III) 0.1 ml of the preparation consisting of a mixture of the test substance and FCA in acetone/propylene glycol, where the concentration for FCA was the same as in (I) and the concentration for the test substance was the same as in (II).

24 h before the topical sensitization, all the animals were treated with 200 μ l of a preparation consisting of sodium lauryl sulfate (SLS) 10.0% w/v in dimethyl acetamide/acetone/ethanol 99.5% 4/3/3 v/v. Topical sensitization was then performed by transferring 200 μ l of a solution of the suspected sensitizer to a 2×4 cm patch of Munktell 1002 filter paper (Munktell Filter AB, Sweden). The patch was covered with overlapping, impermeable plastic adhesive tape (Acrylastic, Beiersdorf AG, Germany). This in turn was firmly secured by an adhesive bandage (Durapore, 3M Health Care, USA). The dressing was left in place for 48 h. To enable comparisons of the sensitizing capacities among the test compounds themselves and with those of the earlier investigated dimeric methylol phenols of resins based on phenol and formaldehyde (P-F-R), concentrations equimolar to the ones used for the dimers of P-F-R were chosen (9).

Table 1. Concentrations (% w/v/mole $\times 1^{-1}$) and vehicles used for induction of guinea pigs with 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether (IX) and 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether (X) and challenge with IX, X, 5,5'-di-tert-butyl-2,2'-dihydroxy-dibenzyl ether (XI), 2-methylol-p-tert-butylphenol (2-MPTBP), 2,6-dimethylol-p-tert-butylphenol (2,6-MPTBP) and p-tert-butylcatechol (PTBC)

Procedure (vehicle)	Substance					
	IX	X	XI	2-MPTBP	2,6-MPTBP	PTBC
Induction						
ID (ac/FCA/pg)	1.6/0.039	1.5/0.039				
topical (ac)	4.0/0.099	3.7/0.099				
challenge I (ac)	3.2/0.079	2.9/0.079				
challenge II (ac)	3.2/0.079	2.9/0.079	2.7/0.079	22/1.2	25/1.2	7.5/0.45
(ac)	0.10/0.0025	0.10/0.0027				

ID=intradermal; ac=acetone; pg=propylene glycol; FCA=Freund's complete adjuvant.

Concentrations of substances used for inductions are given in Table 1.

Challenge procedure

Challenge I was performed 2 weeks after the 2nd stage of sensitization and a 24-h occluded patch test was performed on the right flank with 25 μ l of the test solutions on each of 2 patches near the back. For patch testing Al-test (Astra Agency, Sweden) on Durapore was used, which was firmly secured by Acrylastic and an outer layer of Durapore. In each sensitization study, 12 test animals received the suspected sensitizer on both patches, 6 animals received the suspected sensitizer only on the cranially located patch, while the vehicle alone was applied to the other patch. The same number of test animals received the suspected sensitizer and the vehicle in the reverse way.

Challenge II was performed at the same time as challenge I but on the other, left, side of the flank. 6 patches were tested on 24 test and 12 control animals, 2 patches being near the back, 2 near the abdomen and 2 between these. The animals were challenged with the sensitizer and possible cross-reacting substances. A distribution pattern based on a Latin square table was used.

Challenge was performed with non-irritant solutions for all substances. To enable comparisons of the sensitizing capacities among the test compounds themselves and with those of the previously investigated methylol phenols of P-F-R (MPs), concentrations equimolar to the ones used for the monomers and dimers of P-F-R, respectively, were chosen, if possible (9, 10). The solutions were equimolar except for PTBC, which was tested in a more diluted solution due to high topical irritancy. The concentrations of the test substances are given in Table 1. The structures of the test substances are shown in Fig. 1.

Controls

The animals in each control group were treated in the same way, concerning the induction and challenge procedures, as the corresponding animals in the test group, except that the suspected sensitizer was not administered during the induction.

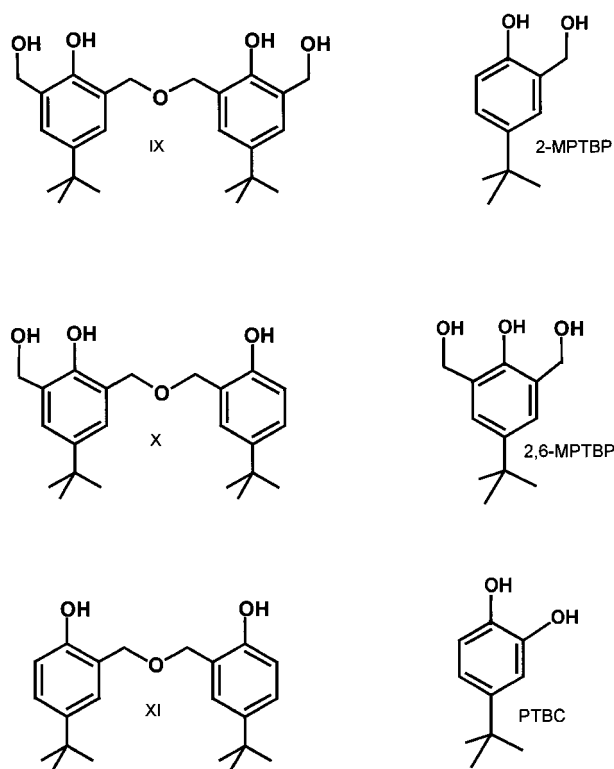


Fig. 1. Chemical structures of substances used for induction and challenge: 2-methylol p-tert-butylphenol (2-MPTBP), 2,6-dimethylol p-tert-butylphenol (2,6-MPTBP), p-tert-butylcatechol (PTBC), 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether (IX), 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether (X), 5,5'-di-tert-butyl-2,2'-dihydroxy-dibenzyl ether (XI).

Table 2. Challenge I; test reactions after induction and challenge with 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether (IX) and 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether (X)

Induction substance	No. positive animals			
	C/n	T/n	V/n	P/n
IX	0/12	14/24 ^a	0/12	4/6
X	2/12	16/24 ^b	1/12	1/6

C: test reactions to the suspected sensitizer in control animals; T: test reactions to the suspected sensitizer in test animals; V: test reactions to the vehicle in test animals; P: test reactions in positive control animals after induction and challenged with 2-methylol phenol; n: number of tested animals in the 4 groups C, T, V and P; ^a $p=0.00052$; ^b $p=0.0053$.

For challenge I, 6 control animals received the suspected sensitizer on both patches. 3 others received the suspected sensitizer and the vehicle alone on the patches. The same number of animals received the suspected sensitizer and the vehicle in the reverse way.

For challenge II, the 12 animals in the control group received the suspected cross-reacting substances in the same way and in the same concentrations as the animals in the test group.

The 6 animals in the particular "positive" control group were sensitized to and challenged with 2-MP, according to procedures described in detail elsewhere (8).

Evaluation

The reactions were evaluated blind 24 h after the removal of the patches. First the left flanks (challenge I), and with the right flanks shielded, of all animals were read and thereafter, still blind and without knowledge of the readings of the left flanks, the right flanks (challenge II) were read. The minimum criterion of an allergic (positive) reaction was a confluent erythema. The number of positive animals in each test group was statistically compared to the number of positive animals in the

corresponding control group, and also to the number of positive test animals tested with the vehicle alone. The assessment of whether an animal was positive or not was based on the result for only one patch chosen in advance for those animals which had received the test solution on both patches (12 test animals and 6 control animals). When both comparisons yielded significant values the compound was considered to be a contact sensitizer.

For challenge II, a comparison was made only between the number of positive animals in the test and control groups for each substance.

Statistical calculation

The Fisher exact test was used.

Results

Investigation of the purity of the test substances showed that the highest possible concentration of IX in X and of X in IX was less than 0.10% w/w. The concentration of IX in 2,6-MPTBP was 0.10% w/w and in PTBC less than 0.01% w/w (detection limit). The amount of IX and X in 2-MPTBP was less than 0.05% w/w.

Results of challenge I after induction with IX and X, respectively, are given in Table 2. In the group of animals subjected to induction with IX, 14 test animals showed positive test reactions. The difference in the number of positive animals for IX in the test and control groups was significant ($p=0.00052$). In the group of animals subjected to induction with X, 16 test and 2 control animals showed positive test reactions. The difference in the number of positive animals for X in the test and control groups was also significant, but with a lower significance level ($p=0.0053$).

Results from challenge II after induction with IX and X, respectively, are given in Table 3. In animals subjected to induction with IX, 15 test animals and

Table 3. Challenge II; test reactions in animals after induction with 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether (IX) and 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether (X) and challenge with IX, X, 5,5'-di-tert-butyl-2,2'-dihydroxy-dibenzyl ether (XI), 2-methylol-p-tert-butylphenol (2-MPTBP), 2,6-dimethylol-p-tert-butylphenol (2,6-MPTBP) and p-tert-butylcatechol (PTBC)

Induction substance	Group	n	No. of positive animals after challenge with							
			IX		X		XI	2-MPTBP	2,6-MPTBP	PTBC
			3.2%	0.10%	2.9%	0.10%	2.7%	22%	25%	7.5%
IX	test	24	NT	8	15 ^{a)}	NT	4	12	13 ^{b)}	16 ^{c)}
	control	12	NT	2	2	NT	2	3	0	4
X	test	24	5	NT	NT	2	4	5	1	6
	control	12	2	NT	NT	4	3	0	1	2

n=number of animals; NT=not tested; ^{a)} $p=0.010$; ^{b)} $p=0.0011$; ^{c)} $p=0.0498$.

2 control animals reacted to X ($p=0.010$), 13 test animals and 0 control animal reacted to 2,6-MPTBP ($p=0.0011$), and 16 test animals and 4 control animal reacted to PTBC ($p=0.0498$). Induction with IX also gave an increase in positive reactions to 2-MPTBP in the test group, but the difference between the test and control groups was statistically non-significant ($p=0.11$). This, however, indicates a possible cross-reactivity to 2-MPTBP. There was also an increase in the number of animals reacting positively to the low concentration of IX (0.10% w/w) compared to the control group, but the difference was statistically non-significant ($p=0.19$). In the group of animals subjected to induction with X no reactions to the putative cross-reacting substances were statistically significant. Possible cross-reactivity was, however, indicated to 2-MPTBP ($p=0.11$). No increase in the number of positive animals was noted in animals tested with the low concentration of X (0.10% w/w).

Discussion

We have recently demonstrated that IX and X can elicit allergic contact reactions in humans hypersensitive to PTBP-F-R (5). Patients hypersensitive to PTBP-F-R have shown an extremely high sensitivity to IX and X, with positive reactions to patch test solutions with a concentration of 0.01 ppm. In our research on allergens in PTBP-F-R we have isolated several allergens in recent years and there are indications of many more to come. Among so many allergens, it becomes important to distinguish between main, moderate and weak sensitizers, and substances that elicit allergic reactions mainly by cross-reaction. The sensitizing capacities and cross-reaction patterns of these allergens can virtually only be investigated in animals.

In this study, it is demonstrated that IX and X are sensitizers in the guinea pig. With IX as the sensitizer, cross-reactions to X, 2,6-MPTBP and PTBC could be demonstrated, while cross-reactions to 2-MPTBP were indicated ($p=0.11$). With X as the sensitizer, no cross-reactions could be demonstrated.

To investigate the influence of the detected low degree of contamination of IX and X in each other, challenge II also included preparations with these substances in concentrations equal to their highest possible level of contamination. Testing with the low concentrations of IX and X demonstrated that the impurities of these substances in each other, or in any of the other test substances, could not explain the observed positive challenge reactions.

The monomers and dimers of PTBP-F-R are chemically related to the monomers and dimers of P-F-R as all of them are methylol phenols (hydro-

xymethyl phenols). To enable comparisons of the sensitizing capacities of the dimers from the 2 resins, the concentrations of IX and X used for induction and challenge were equimolar to those used when the dimers of P-F-R were tested with the GPMT (9). Solutions of monomers and dimers for challenge were equimolar to solutions previously used for challenge with monomers and dimers in P-F-R, respectively (9, 10). In P-F-Rs there are several dimers which can all elicit allergic reactions in humans hypersensitive to this type of resin (11). 3 of the dimers in P-F-R were tested with the GPMT and all were demonstrated to be sensitizers (9). The significance levels $p<0.05$, $p<0.01$ and $p<0.001$ were used to designate the sensitizers as weak, moderate or strong, respectively (9). When compared to the dimers of P-F-R, IX can be classified as a strong sensitizer and X as a moderate sensitizer (8, 9).

When the dimers in P-F-R were assigned levels of sensitizing capacity according to the p -values, the dimer with 2 reactive groups (1 hydroxymethyl group on each aromatic rings) was a strong sensitizer and the P-F-R dimer with 1 reactive group was a moderate sensitizer. When the corresponding dimers in PTBP-F-R are tested and classified in the same way we get similar results. IX with 2 reactive groups is classified as a strong sensitizer while X with 1 reactive group is classified as a moderate sensitizer. This is an indication of a relationship between number of reactive groups and degree of sensitizing capacity. A theoretical background for this could be that molecules with 2 reactive centres can cause more structural changes in macromolecular endogenous substances such as proteins, RNA or DNA as they have the capability to create cross-bindings. It has previously been recognized that epoxy substances with 2 epoxy groups often have a higher capability of causing genetic damage in different test systems, and cancer in animals, than epoxy substances with 1 epoxy group (12, 13).

The clinical significance of the cross-reactions demonstrated to X, 2,6-MPTBP and PTBC in animals sensitized to IX is currently not known. However, the strong patch test responses in patients hypersensitive to PTBP-F-R, and the strong sensitizing capacity together with several induced cross reactions in sensitized guinea pigs, indicate that IX is a main allergen in PTBP-F-R.

Acknowledgements

The authors are grateful to Monica Andersson, Lena Persson and Östen Sörensen for skilful technical assistance. The study was supported by grants from the Swedish Council for Work Life Re-

search and the Swedish Foundation for Health Care and Allergy Research.

References

1. Tarvainen K. Analysis of patients with allergic patch test reaction to a plastics and glue series. *Contact Dermatitis* 1995; 32: 346–351.
2. Handley J, Todd D, Bingham A, Corbett R, Burrows D. Allergic contact dermatitis from para-tertiary-butylphenol-formaldehyde resin (PTBP-F-R) in Northern Ireland. *Contact Dermatitis* 1993; 29: 144–146.
3. Mancuso G, Reggiani M, Berdondini R M. Occupational dermatitis in shoemakers. *Contact Dermatitis* 1996; 34: 17–22.
4. Marques C, Goncalo M, Goncalo S. Sensitivity to para-tertiary- butylphenol-formaldehyde resin in Portugal. *Contact Dermatitis* 1994; 30: 300–301.
5. Zimerson E, Bruze M. Contact allergy to 5,5'-di-tert-butyl-2,2'-dihydroxy-(hydroxymethyl)-dibenzyl ethers, sensitizers in p-tert-butylphenol-formaldehyde resin. *Contact Dermatitis* 2000; 43: in press.
6. Magnusson B, Klingman A M. *Allergic contact dermatitis in the guinea pig*. Springfield, Ill.: CC Thomas, 1970.
7. Wahlberg J E, Boman A. Guinea pig maximization test. In: Andersen K E, Maibach H I (eds): *Contact allergy predictive test in guinea pigs*. Basel: S. Karger AG, 1985: 59–106.
8. Bruze M. Contact sensitizers in resins based on phenol and formaldehyde. *Acta Dermato-venereologica* 1985; suppl 119: 1–83.
9. Bruze M. Sensitizing capacity of 4,4'-dihydroxy-(hydroxymethyl)-diphenyl methanes in the guinea pig. *Acta Dermato-venereologica* 1986; 66: 110–116.
10. Bruze M. Sensitizing capacity of 2-methylol phenol, 4-methylol phenol and 2,4,6-trimethylol phenol in the guinea pig. *Contact Dermatitis* 1986; 14: 32–38.
11. Bruze M, Fregert S, Persson L, Zimerson E. Contact allergy to 4,4'-dihydroxy-(hydroxymethyl)-diphenyl methanes; sensitizers in a phenolformaldehyde resin. *J Invest Dermatol* 1986; 87: 617–623.
12. Weil C S, Condra N, Hann C, Steigel J A. Experimental carcinogenicity and acute toxicity of representative epoxides. *Industrial Hygiene Journal* 1963; juli-aug: 305–325.
13. Ehrenberg L, Hussain S. Genetic toxicity of some important epoxides. *Mutation Research* 1981; 86: 1–113.

Address:

Erik Zimerson
Department of Occupational and Environmental Dermatology
Malmö University Hospital
S-205 02 Malmö
Sweden