

Sensitizing capacity and cross-reactivity of phenyl glycidyl ether studied in the guinea-pig maximization test

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Background: Simultaneous contact allergies to the reactive diluent phenyl glycidyl ether (PGE) and epoxy resins based on diglycidyl ether of bisphenol A (DGEBA) or epoxy resins of the bisphenol F-type have been reported. The latter epoxy resins contain three isomers of diglycidyl ether of bisphenol F (DGEBF). PGE, DGEBA, and DGEBF are chemically similar with respect to the reactive glycidyl group and an aromatic ring, making cross-reactivity one of the possible explanations to simultaneous reactions.

Aim: To investigate the sensitizing capacity of PGE and the cross-reactivity of PGE versus the epoxy resin monomers DGEBA and the three DGEBF monomers and vice versa.

Method: The guinea-pig maximization test.

Results: PGE is a strong sensitizer. Induction with DGEBA resulted in a statistically significant number of animals reacting to PGE, but when induced with PGE, the animals did not react significantly to DGEBA.

Conclusions: When the results are applied to humans, it can be assumed that patients sensitized primarily to DGEBA react to PGE, but when sensitized exclusively to PGE, they do not react to DGEBA. Both reactive diluents and epoxy resins should be tested when contact allergy to epoxy resin systems are suspected.

Key words: animal study; contact allergy; cross-reactivity; DGEBA; DGEBF; diglycidyl ether of bisphenol A; diglycidyl ether of bisphenol F; epoxy resin; epoxy resin system; GPMT; guinea-pig maximization test; PGE; phenyl glycidyl ether; reactive diluent; sensitization. © Blackwell Munksgaard, 2009.

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Epoxy resin systems (ERSs) are often modified by the addition of ingredients such as reactive diluents. These are used mainly to reduce the viscosity of the epoxy resin. Reactive diluents are epoxy compounds and may be mono-functional, that is contain one reactive epoxy group, or poly-functional, and they may be aromatic or aliphatic (1). Several epoxy-based reactive diluents are known to cause occupational contact allergies (2). Phenyl glycidyl ether (PGE, Fig. 1) is an aromatic mono-functional reactive diluent, which is reported to cause occupational contact allergy in workers exposed to ERSs (3–8). Many cases with

contact allergy to PGE simultaneously have contact allergy to epoxy resins based on diglycidyl ether of bisphenol A (DGEBA-R) in the European baseline patch test series, but several cases without simultaneous contact allergy to DGEBA-R are also described (3–5, 9). Contact allergy to PGE is sometimes found in workers exposed to ERSs where no obvious source of PGE is found (10). The partially overlapping pattern of contact allergies to DGEBA-R and PGE might be explained by exposure to both the resin and the PGE or by cross-reactivity. Chemically, PGE is similar both to the monomers in DGEBA-R and to the epoxy resins

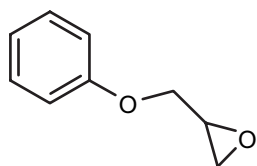


Fig. 1. Chemical structure of the reactive diluent phenyl glycidyl ether.

of the bisphenol F-type. The latter types of epoxy resin contain the diglycidyl ether of bisphenol F (DGEBF), which has three isomers: *p,p'*-DGEBF, *o,p'*-DGEBF, and *o,o'*-DGEBF (Fig. 2) (11, 12). The epoxy resins of the bisphenol F-type can be produced from reacting epichlorohydrin either with bisphenol F or with phenolic novolacs [DGEBA-R and DGEBA(n)-R, respectively]. DGEBA-R is manufactured so as to contain virtually only one isomer of the monomer diglycidyl ether of bisphenol A (*p,p'*-DGEBA) (11). PGE is obtained by substitution of the hydroxyl group in phenol with glycidyl ether. Phenol is used for the manufacture of both DGEBA-R and epoxy resins of the bisphenol F-type (11). Therefore, PGE could be suspected to be found as a contaminant in these resins. That could especially be the case for epoxy resins based on phenolic novolac because they have a smaller repetitive unit, consisting of one aromatic ring, than epoxy resins based on bisphenol A or F (11). However, chemical analyses of epoxy resins performed by us have shown that contamination of PGE in DGEBA-R and epoxy resins of the bisphenol F-type is a less likely

explanation of simultaneous contact allergies to this reactive diluent and these resins (13).

Because there are cases that seem not to be explained by the presence of PGE in the ERS, cross-reactivity can be an important explanation. This can only be investigated in animal studies. The aim of the present study was to investigate the sensitizing capacity of PGE and the cross-reactivity of PGE versus the epoxy resin monomers DGEBA and the three DGEBF monomers and vice versa in a guinea-pig maximization test (GPMT).

Materials and Methods

Substances

PGE was bought from Acros Organics, Geel, Belgium. According to the manufacturer, the concentration was 99%. The purity was investigated by high-performance liquid chromatography. PGE was not found to contain DGEBA or DGEBF (detection limits <0.0002% w/w DGEBA and <0.002% w/w DGEBF). DGEBA and the DGEBF isomers were isolated and purified as previously described (12, 14).

Guinea-pig maximization test

The GPMT was performed according to the original description (15). In order to standardize the test and objectify the evaluation of the patch test reactions, some modifications were made including statistical calculations, blind reading, and a positive control group (16, 17). Female albino guinea-pigs weighing 400 ($\pm$ 20) g of the Hartley–Dunkin strain (M & B A/S, Ry, Denmark) were used. The Malmö/Lund Ethics Committee on Animal Experiments approved the study.

Topical irritancy. Topical irritancy was determined by applying different concentrations of each substance used for induction as a closed patch test for 2 D on both the neck and the flank of 4–6 animals. One week before testing, the animals were pretreated with Freund's complete adjuvant (FCA) (Pierce, Rockford, IL, USA). Concentrations that did not cause irritation were chosen for topical induction and elicitation.

Concentrations. Equimolar concentrations were used for the epoxy resin monomers. Due to topical irritation, the concentration of PGE was reduced and therefore not equimolar to the epoxy resin monomers. The concentrations used for induction and challenge are given in Table 1. The animals induced with PGE were in addition to the epoxy resin monomers challenged with a lower concentration of PGE, i.e. 0.83% w/v.

Induction. Twenty-four test animals were used for induction according to the following procedure:

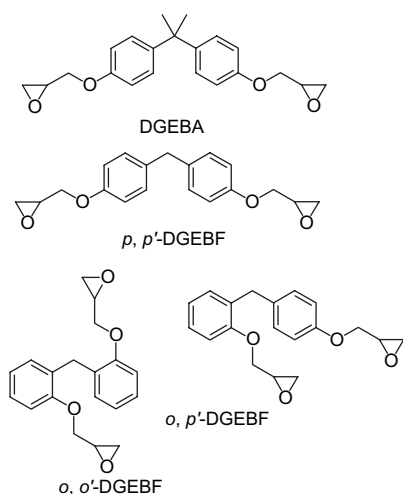


Fig. 2. The chemical structures of the monomer DGEBA and the three main constituents of epoxy resins based on DGEBA. DGEBA, diglycidyl ether of bisphenol A; DGEBF, diglycidyl ether of bisphenol F; *o,o'*-DGEBF, *o,o'*-diglycidyl ether of bisphenol F; *o,p'*-DGEBF, *o,p'*-diglycidyl ether of bisphenol F; *p,p'*-DGEBF, *p,p'*-diglycidyl ether of bisphenol F.

Table 1. Concentrations (% w/v mol/l) used for induction and challenge in the guinea-pig maximization test

Substance	Intradermal sensitization	Topical sensitization	Challenge I	Challenge II
PGE	0.55/0.037	0.83/0.055	1.7/0.11	1.7/0.11*
DGEBA	5.0/0.15	10/0.29	15/0.44	15/0.44
<i>p,p'</i> -DGEBF	4.6/0.15	9.2/0.29	14/0.44	14/0.44
<i>o,p'</i> -DGEBF	4.6/0.15	9.2/0.29	14/0.44	14/0.44
<i>o,o'</i> -DGEBF	4.6/0.15	9.2/0.29	14/0.44	14/0.44

DGEBA, diglycidyl ether of bisphenol A; *o,o'*-DGEBF, *o,o'*-diglycidyl ether of bisphenol F; *o,p'*-DGEBF, *o,p'*-diglycidyl ether of bisphenol F; PGE, phenyl glycidyl ether; *p,p'*-DGEBF, *p,p'*-diglycidyl ether of bisphenol F.

*The animals induced with PGE were additionally challenged (left flank) with a lower concentration of PGE, i.e. 0.83/0.055% w/v mol/l.

Day (D) 0: three intradermal injections in a row at each side of the shoulder were given: (i) 0.1 ml of FCA in water 40% w/v (FCA/water 50:50 v/v); (ii) 0.1 ml of a solution of induction substance (w/v) dissolved in acetone and diluted in propylene glycol. PGE was dissolved in propylene glycol only. (iii) 0.1 ml of mixture of 40% w/v FCA in acetone/propylene glycol and with the same concentration of induction substance as in (ii). For PGE, FCA in propylene glycol was used.

D6: pretreatment of a 2 × 4 cm area for topical induction with 0.2 ml sodium lauryl sulfate 10% w/v in dimethylacetamide/acetone/ethanol 99.5% 4:3:3 v/v/v 1 D before topical application of the induction substance.

D7: topical induction with 0.2 ml of the induction substance in acetone on 2 × 4 cm piece of filter paper (130 g/m²; Munktell[®] Filter AB, Grycksbo, Sweden) placed on Durapore (3M Health Care, St. Paul, MN, USA). The patches were covered with impermeable plastic adhesive tape (Acrylastic, Beiersdorf AG, Germany) and held in place by adhesive bandage (Durapore[®]). The patch test was left on for 2 D.

Controls. Twelve controls were given exactly the same treatment as described for the test animals, but with the induction substance excluded. In addition, six controls were given the known sensitizer 2-MP. These animals were used as a positive control in order to objectify the evaluation of test reactions and as an indication of the accuracy of the induction procedure (17).

Challenge. D21, Challenge I, sensitization rate (right flank, two patches): 12/24 test animals were challenged with the induction substance on both the cranial and the caudal patch. 6 + 6 test animals were challenged with the induction substance on either the cranial patch or the caudal patch with vehicle alone on the other. Al-test[®] (Imeco AB, Södertälje, Sweden) on Durapore was used for patch testing. Thirty microlitres of the substance

used for induction diluted in acetone was applied. Acrylastic and an outer layer of Durapore held the tests in place. The patches were removed after 1 D. Six of 12 control animals were tested with the induction substance on both patches, and 3 + 3 animals were patch tested with the induction substance on either the cranial or the caudal patch, with vehicle alone on the other patch (18).

Challenge II, cross-reactions (left flank, six patches): on the same occasion as challenge I, the same 24 test animals and 12 control animals were tested with putative cross-reacting substances. The animals induced with PGE were in addition to the epoxy resin monomers challenged with a lower concentration of PGE, i.e. 0.83% w/v. The distribution of the positions of test substances was based on a Latin square table. In this article, results of sensitization with PGE and the cross-reaction patterns of PGE vis-à-vis DGEBA and the isomers of DGEBF are described.

Evaluation. D23: the minimum criterion for an allergic (positive) reaction is a confluent erythema. All tests were evaluated blindly 1 D after the patch tests had been removed, i.e. after 2 D after the application. First, the right flanks (challenge I) were read and thereafter, still blindly and without knowledge of the readings of the right flanks, the left flanks (challenge II) were read. The procedure concerning the control group sensitized and challenged with 2-MP is described elsewhere (17).

Statistical calculation. The number of positive animals within the test group was compared with the number of positive animals in the control group. The number of positive test animals was also compared with the number of positive animals tested with vehicle only. Among the animals challenged with the induction substance on both cranial and caudal patches (12 test animals and 6 control animals), only one of the patches chosen in advance was included.

Statistical significance was calculated with one-sided Fisher's exact test. When a significant value ($P < 0.05$) was obtained both in comparison with the controls tested with allergen and the animals tested with vehicle alone, the compound (i.e. PGE) was judged as a sensitizer.

Results

Sensitizing capacity

PGE was found to be a strong sensitizer in the guinea-pig with 23/24 animals sensitized. No control animals had reactions to PGE ($P < 0.001$). Lowering the concentration for challenge (left flank) did not reduce the number of reacting animals. All 24 animals induced with PGE reacted

human skin (22). It can be assumed that the similar difference exists for PGE compared with DGEBA. The calculated $\log P_{o/w}$ for PGE and *o*-CGE is 1.48 and 1.98, respectively, and the $\log P_{o/w}$ of DGEBA is 3.84 (23).

Cross-reactivity

Simultaneous reactions to different contact allergens can be due to exposure to both allergens in question or to cross-reactivity. Exposure-related simultaneous contact allergies to PGE and epoxy resins can be explained by PGE being present as part of ERSs, but contamination by PGE in epoxy resins could also be suspected. However, chemical analyses of a selection of commercial epoxy resins containing DGEBA and/or DGEBF have shown that sensitization from the comparatively low content of PGE is less likely to occur (13).

PGE and DGEBA have similar chemical structures with respect to the reactive glycidyl group and the aromatic ring (Fig. 1), and thus cross-reactivity can be suspected. In the present study, induction with PGE did not result in a significant number of animals reacting to DGEBA, but when sensitized with DGEBA, the animals reacted to PGE (Tables 2 and 3). These results might reflect the difference in size of the molecules. When sensitized with a smaller molecule, the bigger but chemically similar molecule is not recognized, but when sensitized with the bigger molecule, the smaller one is recognized. On the other hand, it could perhaps be anticipated that the pattern of cross-reactivity for PGE and DGEBA depends on differences in skin penetration. In this experimental setting, the difference is however not explained by less penetration into the skin for PGE compared with DGEBA because (i) PGE sensitized all animals and (ii) it has been found that PGE most probably penetrates the skin easier than DGEBA (22). The easier penetration of PGE compared with DGEBA might reduce the effect of the comparatively lower induction concentration. The number of cross-reactions in the animals induced with PGE compared with the animals induced with *p,p'*-DGEBF or *o,p'*-DGEBF showed the same tendency as for DGEBA, and the mechanisms might be the same as hypothesized for DGEBA. The difference between DGEBA on one hand and *p,p'*-DGEBF or *o,p'*-DGEBF on the other hand when challenged with PGE is possibly explained by the fact that the structure of the latter molecules, with respect to the absence of the methyl groups, is more similar to PGE than DGEBA, but there are, of course, other possible explanations.

In our study, PGE generated a strong inflammatory response in the sensitized animals. Within the study of the sensitization rate of PGE, the animals challenged with PGE on both positions (12 animals) had the same number of reactions for the substances investigated for cross-reactions as the group of animals challenged with PGE on one position (12 animals). The total challenge dose of PGE and the inflammatory reaction created by this dose thus did not affect the number of cross-reactions.

Conclusion

PGE is a strong sensitizer in the GPMT. When the results from the investigation of cross-reactions are applied to humans, it can be assumed that persons sensitized primarily to DGEBA could react to PGE as well, but when exposed and sensitized exclusively to PGE, they would not react to DGEBA. Therefore, PGE and other reactive diluents to which the patient is exposed should be patch tested separately.

References

1. Shaw S J. Additives and modifiers for epoxy resins. In: *Chemistry and Technology of Epoxy Resins*, Ellis B (ed): Glasgow, Blackie Academic & Professional, 1993: 117–120.
2. Jolanki R, Kanerva L, Estlander T. Epoxy resins. In: *Handbook of Occupational Dermatology*, Kanerva L, Elsner P, Wahlberg J E, Maibach H I (eds): Berlin, Heidelberg, New York, Springer-Verlag, 2000: 574, 582.
3. Rudzki E, Rebandel P, Grzywa Z, Jakiminska B. Dermatitis from phenyl glycidyl ether. *Contact Dermatitis* 1983; 9: 90–91.
4. Jolanki R, Kanerva L, Estlander T, Tarvainen K, Keskinen H, Henriks-Eckerman M L. Occupational dermatoses from epoxy resin compounds. *Contact Dermatitis* 1990; 23: 172–183.
5. Angelini G, Rigano L, Foti C, Grandolfo M, Vena G A, Bonamonte D, Soleo L, Scorpiniti A A. Occupational sensitization to epoxy resin and reactive diluents in marble workers. *Contact Dermatitis* 1996; 35: 11–16.
6. Conde-Salazar L, Gonzalez de Domingo M A, Guimaraens D. Sensitization to epoxy resin systems in special flooring workers. *Contact Dermatitis* 1994; 31: 157–160.
7. Holness D L, Nethercott J R. The performance of specialized collections of bisphenol A epoxy resin system components in the evaluation of workers in an occupational health clinic population. *Contact Dermatitis* 1993; 28: 216–219.
8. Geier J, Lessmann H, Hillen U et al. An attempt to improve diagnostics of contact allergy due to epoxy resin systems. First results of the multicentre study EPOX 2002. *Contact Dermatitis* 2004; 51: 263–272.
9. Jolanki R, Estlander T, Kanerva L. Contact allergy to an epoxy reactive diluent: 1,4-butanediol diglycidyl ether. *Contact Dermatitis* 1987; 16: 87–92.
10. Pontén A, Carstensen O, Rasmussen K, Gruvberger B, Isaksson M, Bruze M. Epoxy-based production of wind turbine rotor blades: occupational contact allergies. *Dermatitis* 2004; 15: 33–40.
11. Ellis B. The synthesis and manufacture of epoxy resins. In: *Chemistry and Technology of Epoxy Resins*, Ellis B

- (ed): Glasgow, Blackie Academic & Professional, 1993: 16–36.
12. Pontén A, Zimerson E, Sörensen Ö, Bruze M. Chemical analysis of monomers in epoxy resins based on bisphenols F and A. *Contact Dermatitis* 2004; 50: 289–297.
 13. Pontén A, Zimerson E, Bruze M. Can simultaneous contact allergies to phenyl glycidyl ether and epoxy resins of the bisphenol A/F-types be explained by contamination of the epoxy resins? *Contact Dermatitis* (in press).
 14. Pontén A, Zimerson E, Sörensen Ö, Bruze M. Sensitizing capacity and cross-reaction pattern of the isomers of diglycidyl ether of bisphenol F in the guinea pig. *Contact Dermatitis* 2002; 47: 293–298.
 15. Magnusson B, Kligman A M. *Allergic Contact Dermatitis in the Guinea Pig. Identifications of Contact Allergens*. Springfield, IL, Charles C. Thomas, 1970.
 16. Wahlberg J E, Boman A. Guinea pig maximization test. In: *Contact Allergy Predictive Test in Guinea Pigs*, Andersen K E, Maibach H I (eds); Basel, S Karger AG, 1985: 59–106.
 17. Bruze M. Contact sensitizers in resins based on phenol and formaldehyde. *Acta Derm Venereol Suppl (Stockh)* 1985; 119: 1–83.
 18. Bruze M, Dahlquist I, Fregert S, Gruvberger B, Persson K. Contact allergy to the active ingredients of Kathon CG. *Contact Dermatitis* 1987; 16: 183–188.
 19. Thorgeirsson A, Fregert S. Allergenicity of epoxy resins in the guinea pig. *Acta Derm Venereol* 1977; 57: 253–256.
 20. Thorgeirsson A, Fregert S, Magnusson B. Allergenicity of epoxy-reactive diluents in the guinea pig. *Berufsdermatosen* 1975; 23: 178–183.
 21. Thorgeirsson A. Sensitization capacity of epoxy reactive diluents in the guinea pig. *Acta Derm Venereol* 1978; 58: 329–331.
 22. Boogaard P J, Denneman M A, Van Sittert N J. Dermal penetration and metabolism of five glycidyl ethers in human, rat and mouse skin. *Xenobiotica* 2000; 30: 469–483.
 23. *Chem Draw Ultra*. Cambridge, MA, Cambridge Soft Corporation, 2003.

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