

Identification of sensitizing diethyleneglycol maleate in a two-component polyester cement

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Unsaturated polyester (UP) cement caused allergic contact dermatitis in car repair work. The resin was a condensate of polyols and maleic anhydride with reactive solvent, auxiliary substances, and inorganic reinforcement substances. To identify the causative chemicals, the cement was tested on a sensitized patient. For analysis, samples of the resin were eluted with acetone and eluted with hexane to precipitate inorganic material and large polyester molecules. The eluate was evaporated. The remainder, dissolved in acetone, was separated into fractions on silica plates by thin layer chromatography (TLC). On the developed (hexane/chloroform, 15/85) plates, 20 bands were obtained under UV-light at 254 nm. Samples of the bands were scraped and used for patch testing. The scraping at a retention factor (R_f) of 0.24 caused a skin reaction. The bands at this retention were removed from six plates, combined, eluted with acetone and purified again by TLC. The purified fraction mixed in petrolatum in the dilution series was used for conclusive patch testing on the patient. An allergic reaction was induced at down to 0.003% wt/wt. According to MS and IR analyses, the isolated compound was diethyleneglycol maleate (DEGM, MW204). In addition to the resin part, the sanding dust also contained this monomer.

Key words: allergic contact dermatitis; car painting; chemical analysis; diethyleneglycol maleate; mould manufacture; occupational dermatitis; polyester cement; polyester resin. © Blackwell Munksgaard, 2002.

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Two-component (resin and hardener) unsaturated polyester (UP) cements have caused allergic contact dermatitis (ACD) in car repair workers (1, 2). The workers have been exposed to repair cements in the mixing of the components and spreading of the uncured mixture. The surface of the cured polyester is matt and spotty, and therefore an after-treatment by sanding and polishing is necessary to achieve an acceptable result before final painting of the surface. The sanding dust of the hardened cements has also caused symptoms of ACD.

Unsaturated polyesters (UP-resins) (3) are products consisting of straight-chain polyesters having reactive double bonds at intervals along the chain. They are generally supplied dissolved in styrene. UP is obtained by esterifying dicarboxylic acid with a diol. A common combination is maleic anhydride (MA) and ethylene glycol (EG). Phthalic anhydride can replace a part of MA, and diethylene glycol (DEG) or 1,2-propanediol EG, either wholly or in part. An inhibitor is needed to prevent premature polymerization. Two groups of different additives are needed before curing. The accelerator

(cobalt compound) is often added to the UP solution before delivering the product to the final user. Curing, i.e. the final polymerization, takes place after the addition of a hardener. Organic peroxides (benzoyl peroxide or methylethylketone peroxide) are the usual hardeners.

Thus, the products based on polyesters are two-component materials, and are delivered to users in two separate packages, the contents of which must be mixed together just before use. One package contains a solution of UP in styrene together with auxiliary substances and, in case of moulding resins (repair cement), also inorganic filling agents. The other package contains the hardener in the form of solution or dispersion in plasticizer.

The high-molecular weight polyester itself (1, 2) is seldom sensitizing or otherwise harmful to health, but traces of the starting agents, small-molecular weight polymerizing monomers or intermediates and auxiliary additives may sometimes be sensitizing and irritating.

UP cement used for car repair painting has caused allergic contact dermatitis to car painters

(1, 2). It was suspected that the allergy might be related to a small-molecular weight monomer of polyester. In our study, the allergen was isolated and confirmed to be the monomer of polyester, diethyleneglycol maleate (DEGM). The isolation and identification of the causative agent was based on chemical analysis and patch testing on a sensitized patient.

Materials and Methods

Polyester cement

The cement under study was a two-component product of a resin and a hardener used in mould-manufacturing. According to the manufacturer, the starting agents of the polyester were two polyols, EG and DEG, and the acid component was MA. Styrene was the reactive solvent for the polymer. The resin also contained common inorganic reinforcement substances and a small amount (ca. 3%) of a mixture for polymerizing important auxiliary additives. The manufacturer provided us with samples of the additives, but the information concerning these ingredients was stated to be strictly confidential. The hardener was organic peroxide.

Patch testing

The patient was first patch tested with the resin of a 'test cement' received from an earlier patient (1), brand, which he had not been previously exposed to. He was also tested with a pure polyester resin, which was dissolved in styrene but was without auxiliary additives. In addition, he was tested alone with the additives used in the resin and also those in the mixture with the pure polyester in styrene. The test was also performed with sanding dust. The test program is shown in Table 1.

The patient was also patch tested with his own car repair cement, which he had handled at work. The patch testing was also performed on him with the dilution series in pet., prepared from DEGM which was separated from his own car cement by chemical analysis (Table 1).

The tests were performed with Finn Chambers and Scanpor tape (1, 2). A small amount of dry cement was placed into the test chamber with a drop of water, and small scrapings of silica gel from thin layer chromatography (TLC) plates (see chemical analysis below) were moistened with a drop of acetone. Patch test reactions on the patient were observed on the 2nd, 3rd and 4th D after application, and were scored as suggested by the International Contact Dermatitis Research Group (ICDRG).

Chemical analysis

(Semi)quantitative analysis of the resin was done by weighing and/or measuring the amounts of analyte during the course of the analysis. Samples of the test substances, resin and sanding dust, were eluted with acetone, and the solutions were extracted three times with hexane. During extraction, inorganic filling material and high-weight polymer molecules were precipitated and discarded. The clear hexane extracts were evaporated to dryness. The dry residue was dissolved in acetone and applied onto TLC plates (4) (silica gel, thickness 0.25 mm, with fluorescein, available from Merck AG, Darmstadt, Germany). The plates were developed in a TLC chamber with a solvent mixture of hexane and chloroform (Fluka AG, Buchs, Switzerland) of 15% and 85%, respectively.

After developing the plates, 20 bands with different retention factors (R_f , the distance travelled by the compound divided by the distance travelled by the solvent) were detected under UV light at 254 nm on the plates. Small silica gel samples of all these 20 bands were scraped, placed in the patch test chambers, and applied onto the patient's skin as described above. Silica gel from the white area of a developed plate was abraded for two 'blank samples'.

According to the results of patch testing, the silica gel of whole bands at $R_f = 0.24 (\pm 0.3)$ on six plates were carefully scraped off, combined and eluted with acetone in an ultrasonic bath. The eluate was concentrated and purified again on a TLC plate. The repurified and scraped band (at $R_f = 0.24$) was eluted with acetone. The eluate was centrifuged and evaporated to dryness. The weighed residue was dissolved in a known volume of acetone. A certain part of this acetone solution was further mixed in petrolatum (g) to give dilution series of the analyte for (semi)quantitative patch testing on the patient (Table 1 and 2nd patch test session). The rest of the acetone solution was used for the sequel chemical analysis of the causative agent by mass spectrometry (MS, Hewlett Packard 59970 GC-MS/MSD, electron impact 75 eV; Palo Alto, CA, USA). The apparatus was connected to a gas chromatograph (GC, Hewlett Packard HP5790A) which was equipped with a splitless injection port and a capillary column (HP-5, 5% diphenyl- and 95% dimethyl polysiloxane, length 30 m, diameter 0.32 mm, phase thickness 0.25 μ m). The temperature program for the GC determination was the following: at 40°C for 2 min, an increase of 2°C/min, up to 260°C and held for 10 min. The temperature of the injection port was 230°C. The infrared spectroscopy was performed on KBr disks by Fourier Transform Infrared spec-

Table 1. Patch testing with components of polyester (UP) cements

		Reading days		
Vehicle*		D2	D3	D4
1st patch testing session				
<i>UP cement for mould-manufacturing, test material from an earlier case</i>				
UP test resin in styrene (no additives)	2.5% ac	+	++	++
UP test resin in styrene with auxiliary substances	1% pet	++	++	++
UP test resin in styrene with auxiliary s. and fillings	1% pet	++	++	++
Sanding dust of hardened test cement	as is (+ aq)	+	++	++
Three auxiliary substances	1% pet	—	—	—
Five filling materials	as is (+ aq)	—	—	—
Solvent (styrene)	1% pet	—	—	—
Peroxide hardener	1% pet	—	—	—
Fraction R _f = 0.24 from TLC plate, UP test resin	as is (+ ac)	+	++	++
Two blank scrapings from a developed TLC plate	as is (+ ac)	—	—	—
<i>Patient's own car repair cement</i>				
Sanding dust of hardened cement	as is (+ aq)	?+	?+	+
Resin with additives	10% pet	?+	?+	+
	3.2% pet	?+	?+	+
	1% pet	—	—	—
Peroxide hardener	1% pet	—	—	—
2nd patch testing session				
<i>Patient's own car repair cement</i>				
Fraction R _f = 0.24 from TLC plate, resin	as is (+ ac)	++	+++	+++
Dilution series prepared in petrolatum of the fraction R_f= 0.24 from TLC plate				
Diethyleneglycol maleate (DEGM)	0.01–0.003% pet	++	++	++
	0.001% pet	?+	?+	?+
	0.0003–0.0001% pet	—	—	—

*% wt/wt.

ac, acetone. aq, aqua. pet, petrolatum. R_f , retention factor. UP, unsaturated polyester. TLC, thin layer chromatography.

trometer (FT-IR) (Nic Nicolet 20SXC/60, Nicolet, Madison, WI, USA) equipped with a mercury–cadmium–tellurium detector.

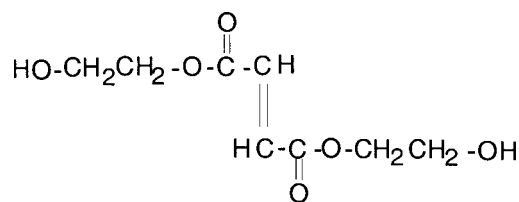
The samples of sanding dust were also analyzed as above, but the result was regarded as qualitative, because the dust was in particle form.

Results

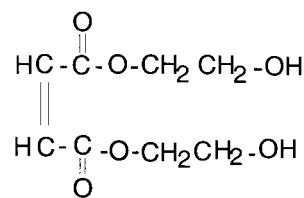
Based on MS (5), the small molecular ion confirmed the mass of the causative agent being 204. The base peak in the spectrum was 98, corresponding to the molecular mass of MA. Fragments of MA were the ions m/z 54 (after loss of CO_2) and m/z 82 (after loss of O). The fragment ion m/z 143 corresponded to the loss of one ethyleneglycol ($-\text{O}-\text{CH}_2\text{CH}_2\text{OH}$) of the DEGM ester molecule. The ions m/z 156 and m/z 126 were other fragments cleft from the glycol part of the molecule. Thus, the fragmentation of the compound proved that it might have been either a *cis*-isomer of the ester, i.e. diethyleneglycol maleate (DEGM) or a *trans*-isomer, i.e. diethyleneglycol fumarate (DEGF). These steric isomers cannot be distinguished by MS (Fig. 1).

The IR-signal at 1735cm^{-1} indicated that the compound was an ester ($-\text{CO}-\text{O}-$) (6). There was

also olefinic stretching at 1630cm^{-1} , which referred to the dipole moment of the molecule and thus the presence of the *cis*-isomer (7). The mol-



Diethyleneglycol fumarate (DEGF)



Diethyleneglycol maleate (DEGM)

Fig. 1. Chemical structures.

ecule had also a weak C-H out-of-plane vibration at 740cm^{-1} , confirming that the molecule was a *cis*-isomer (7), i.e. DEGM (Fig. 1).

Based on the analysis, the cement resins contained a monomer of the polyester, the concentration of which was about 1% in the UP resin component. Traces of DEGM were also found in the sanding dust, but the concentration was not quantified. The resin and the sanding dust contained the polymerized resin and thus also the monomer. The pure additives did not contain polymer, and were negative in patch testing. The lowest concentration to which the patient reacted was as low as 0.003% wt/wt pet.

Discussion

As far as we know, this study is the first to isolate and identify the causative monomer DEGM in UP polymer resin as an allergen. Additionally, the cases of ACD, which were reported from the FIOH (1, 2), were the first identified ACD cases due to DEGM. Based on the analysis, DEGM was also found in the polyester cement that had hardened more than 1 year earlier, suggesting that the sanding dust may also cause the symptoms.

Furthermore, one other compound has the same molecular mass 204 as DEGM or DEGF. This is the monoester of MA (MDEGM) with the higher glycol, diethyleneglycol (DEG). This asymmetric compound was more polar than DEGM, and moved faster on the TLC plate, and was thus well separated from DEGM. Its MS fragmentation was also different from that of DEGM.

Previous studies (1, 2) have shown that the starting agents of the polyester (the diols and MA) gave negative patch testing responses. MA is the known cause of type I but not type IV sensitization (8). On the other hand, MA was probably totally consumed in the polyester synthesis containing extra amounts of diols, which is the usual balance between the starting compounds in esterification (3).

According to the earlier patch tests (1, 2), the results of the two commercial UP resins used on a large-scale for the lamination of reinforced UP plastic products, such as boats, vehicles, construction items, and electrical engineering products, were negative. The cause may be that the primary starting diol for lamination resins was 1,2-propane glycol (information from the manufacturer). The

symmetric monomer of this compound with MA (MW 232) may not be sensitizing, as no cases (ACD) have been reported despite the widespread use of these resins. Because DEGM could also be a sensitizer in many other kinds of polyester products, an alternative to EG in the resins could be another alcohol, e.g. 1,2-propane glycol, which is used at present as a diol in most resins of reinforced polyester products (3).

Because DEGM is not available from test substance distributors, patients examined for suspected allergic contact dermatitis caused by two-component polyester cements should be patch tested with the cement components which they handle, i.e. with the resin at 5% concentration in petrolatum. TLC and instrumental analysis, as described above, may also be useful for isolating and identifying the allergen.

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