

An epidemic of occupational contact dermatitis from an immersion oil for microscopy in laboratory personnel

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Since November 1997, 14 successive cases of occupational contact dermatitis were observed in 13 laboratory technicians and 1 physician, working in a genetics laboratory in Leuven (Belgium) in 3 laboratories of bacteriology in Strasbourg, Montbéliard and Angers, and in the laboratory of hematology in Bordeaux (France). The dermatitis, located on the hands, forearms and face, relapsed after each exposure to an immersion oil for microscopy. Patch tests performed in 10 patients were positive to epoxy resin (ER) in the European standard series (10/10 patients) and to newly formulated Leica® immersion oil (7/7), 1 patient testing negatively with the former oil. A breakdown performed in 2 patients with the oil's ingredients confirmed sensitization to liquid modified ER components, contained at >80% concentration in the oil. The presence of DGEBA was demonstrated by HPLC analysis at a $\pm 30\%$ rate. Although the safety data sheet indicated a revision of the formula, nobody was alerted to the risk of sensitization and the need for skin protection. ERs, as a source of occupational allergy, can provoke epidemics of contact dermatitis in industry. This report of epidemic contact dermatitis from ERs, used for their optical properties in an immersion oil for microscopy, emphasizes the need for perpetual vigilance in occupational medicine and the usefulness of multicentre contacts in dermato-allergology.

Key words: epoxy resin; occupational; allergic contact dermatitis; immersion oil for microscopy; laboratory technicians; diglycidyl ether of bisphenol A. © Munksgaard, 1999.

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Epoxy resin (ER) compounds are a well-known source of contact dermatitis, occurring mainly in industry manufacturing or using ERs, in painters and in construction workers (1–4). We have recently alerted the medical community to the high risk of sensitization and subsequent occupational dermatitis from a newly formulated immersion oil for microscopy (5). We here relate the first 5 cases in detail, with 9 additional reports, all observed simultaneously in 5 European centres and successively occurring in 13 laboratory technicians and 1 physician working on microscopy with immersion procedure.

Case Reports

Case nos. 1–3 (Leuven, Belgium)

A 38-year-old female laboratory technician consulted on 4 November 1997 because of dyshidrotic eczema on the fingers, which had spread to the neck and thighs. The condition had been present for 2 weeks. For more than 10 years, her work had consisted of DNA typing amniotic fluid in a genetics laboratory. She examined microscopic preparations to which was added an immersion oil to enhance the optical characteristics. She had no dermatological antecedents. Patch testing was

done with the European standard series, a photographic series, as she had had sporadic contact with such materials, and with a recently (3 months) introduced immersion oil for microscopy (Leica®, Wetzlar, Germany). Reading after 2 and 4 days revealed an allergy to the immersion oil as is +/+/, as well as to ER 1% pet.: +/+. Testing with additional ER components revealed a positive reaction to n-butyl glycidyl ether (Chemotechnics 0.25% pet.: + at D3) (Table 1).

The 2nd patient, a 32-year old female colleague of the 1st patient, consulted for a similar problem on 18 November 1997. 3 days prior to the occurrence of the lesions in Patient no. 1, she manifested eczematous lesions on the face, including the eyelids, and also on the backs of the middle and index fingers of the right hand. The lesions had improved by replacing the PVC gloves she usually wore with nitrile gloves and the application of corticosteroid. She was not atopic and had no known history of contact allergy. She was tested with the European standard series, her own material, a photographic series, and the Leica® immersion oil for microscopy, with which she also came into contact. Reading after 2 and 4 days showed allergic reactions to the immersion oil as is +/+ and to ER +/+.

The 3rd patient, a 33-year old female, who also worked in the same laboratory, consulted on 2 December 1997. She had suffered from eczematous lesions on 2 different occasions, the 1st time on the face, the 2nd time on the face and the left hand when doing microscopy. The specific localization occurred because she would pick up the microscopic preparations with her right hand and apply and wipe off the oil with her left. We patch tested her only with ER, the former Leica® immersion oil (in error), and an immersion oil of another brand. Reading after 2 days showed a + reaction only to ER.

Case nos. 4–5 (Strasbourg, France)

A 51-year-old woman, without any history of allergy, consulted on 11 December 1997 for an itchy eruption on her forearms that had appeared 3 weeks previously. 3 days after its onset, itchy edema developed on the eyelids, ears, nose, and lips. Topical corticosteroids were applied and she took a week's holiday. The lesions promptly disappeared. She had been a laboratory technician at the University Laboratory of Bacteriology since 1967, and her present job included the examination of Gram-stained bacteriological slides under a microscope. Patch testing was administered with the European standard series and the topical agents and products with which she came in contact. The tests were positive at 2 and 3 days only to ER +/+, the Leica® immersion oil (1% pet. and 10% pet. in a semi-open test) +/+, and a hand towel impregnated with the oil +/+. As she could not avoid contact with the product at her work, the lesions recurred until the oil was definitively removed.

This 5th patient, a 45-year-old laboratory technician at the University Laboratory of Bacteriology, consulted on 12 January 1998 for an eczematous eruption on the forearms, with some patchy scaling erythematous lesions on her face. The lesions had first appeared some weeks previously, 8 days after she had replaced her colleague (patient no. 4). She had no history of allergy. Patch testing was performed with the European standard series and the 2 products that she came in contact with, the Leica® immersion oil and a diluted detergent. The tests were read at 2 and 3 days and showed positive reactions to ER (1% pet.) +/+, and to the Leica® immersion oil (1% pet. and 10% pet. in a semi-open test) +/+. They were negative for bisphenol A, epichlorhydrin and di-n-butyl phthalate.

Table 1. Results of patch tests in the first 6 patients

	Patient no. 1	Patient no. 2	Patient no. 3	Patient no. 4	Patient no. 5	Patient no. 6
Leica® immersion oil for microscopy*	++	+++	—	+	+	++
epoxy resin (European standard series) 1% pet.	+	++	+	+	+	++
bisphenol A 1% pet.	—	—	NT	NT	—	NT
epichlorhydrin 0.1% pet.	NT	NT	NT	—	—	NT
butyl glycidyl ether 0.25% pet.	+	—	NT	NT	NT	NT
allyl glycidyl ether 1% olive oil.	—	—	NT	NT	NT	NT
di-n-butyl phthalate 5% pet.	—	—	NT	NT	—	NT
dioctyl phthalate 1% pet.	—	—	NT	NT	NT	NT

*Patient nos. 1, 2, 6: immersion oil tested as is. Patient no. 3 tested with the former immersion oil. Patient nos. 4, 5: immersion oil tested 1% pet.

Case nos. 6–10 (Bordeaux, France)

5 cases of allergic contact dermatitis of the hands and face were observed from March 1998 onwards, in 4 technicians and in 1 doctor working in a hematology laboratory, using the same immersion oil. 1 technician (patient no. 6) was patch tested with the European series and the oil, as is, in a semi-open test. Positive ++ reactions were observed to ER and to the oil. The other patients declined patch testing, but their lesions cleared after the oil was removed.

Case nos. 11–12 (Montbéliard, France)

2 patients working in a laboratory of bacteriology were referred in January and April 1998 for contact dermatitis of hands and face, related to the use of the same immersion oil for microscopy. Patch tests were positive in both to ER in the standard series. The Leica® immersion oil was not tested, but the lesions rapidly cleared after it was withdrawn.

Case nos. 13–14 (Angers, France)

2 female technicians, aged 50 and 48, working at the University Laboratory of Bacteriology, were referred for contact dermatitis since January 1998. The lesions were first located on the eyelids, and then on the face, hands and forearms. They had associated the lesions with the use of a new supply of the Leica® immersion oil. Patch testing was positive to ER in the standard series (++) and to the immersion oil, as is, with a bullous +++ reaction. The lesions cleared after careful cleaning of microscopes and plans of work.

A subsequent patch test session was performed in the 2 patients from Strasbourg with the ingredients of the oil, provided by the Leica® Company to this centre only. The detailed results are reported in Table 2. This 2nd patch tests session confirmed sensitization to the liquid modified ER components of the immersion oil, each tested at a 1%. The patients reacted to 3,4-epoxycyclohexyl methyl 3',4'-epoxycyclohexyl carboxylate included

in the cycloaliphatic epoxy resin ERL-4221 (Brenntag Eurochem GMBH, Essen, Germany) and to bisphenol A epoxy MW<700 and/or to 1,4-butanediol diglycidylether, both contained in Araldit CY 223® (Ciba-Geigy GMBH, Wehr/Baden, Germany). There were no positive reactions to bis(2-ethylhexyl)phthalate, a phthalate compound contained in Palatinol® AH (BASF, Ludwigshafen, Germany), or to the fatty acid polyester compound from Ultramoll III® (Bayer, Dormagen, Germany).

The safety data sheet of the product was obtained by the patients, indicating that the Leica® immersion oil contained a high percentage of modified ER, at an average of 80%.

On the basis of the CAS registry numbers indicated on the safety data sheet, subsequent research into the chemical formulation was performed with a data base (CAPADOC, Boulogne, France), in order to obtain the chemical formulae of the substances used in the oil. Those are reported in the Fig. 1, indicating an original component of the "modified cyclohexyl-epoxyde resin", namely 3,4-epoxycyclohexylmethyl 3',4'-epoxycyclohexylcarboxylate.

According to information dispensed to some centres, the newly formulated immersion oil was eventually withdrawn by the firm.

Chemical Investigations

For chemical analysis, 2 samples of the immersion oil were sent to Malmö from Bordeaux and Leuven.

Thin-layer chromatography (TLC)

The method described by Fregert & Trulsson (6) was slightly modified. The solvent in the tank was chloroform/acetonitrile (Lab-Scan, Dublin, Ireland) (86/14 v/v). The thin-layer plate (silica gel 60 F₂₅₄) (Merck, Darmstadt, Germany) was 10×20 cm. The standard solution was 2% w/v epoxy resin based on bisphenol A in acetone (Apoteksbolaget, Gothenburg, Sweden).

Table 2. Results of 2nd patch test session in the patient nos. 4 and 5

Trade name	Composition	Patient no. 4(D2/D3)	Patient no. 5(D2/D3)
Ultramoll III® 1% pet.	fatty acid polyester	—	—
Palatinol AH® 5% pet.	bis (2-ethylhexyl)phthalate	—	—
Araldit 223® 1% pet.	bisphenol A epoxy MW<700 and 1,4-butanediol diglycidyl ether	+/+	+/+
Epoxy resin cycloaliphatic ERL 4221® 1% pet.	3,4-epoxycyclohexyl methyl 3', 4' epoxycyclohexyl carboxylate	+/+	+/+

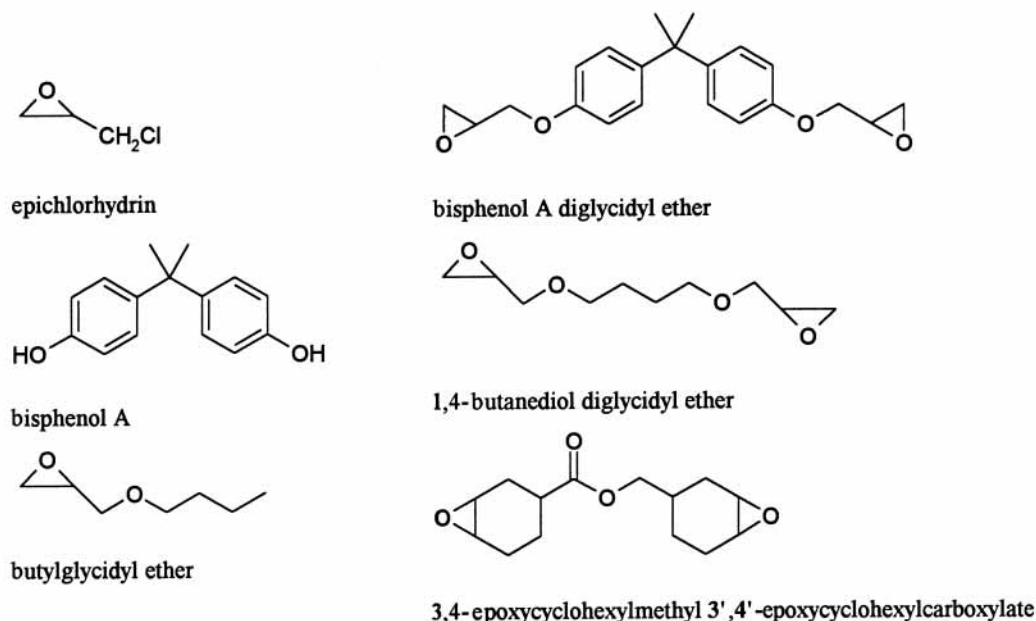


Fig. 1. Chemical structures of epichlorhydrin, bisphenol A, butylglycidylether, bisphenol A diglycidyl ether (monomer MW 340), 1,4-butanediol diglycidylether, and of the "modified cyclohexyl-epoxyde resin" (3,4-epoxycyclohexylmethyl) 3',4'-epoxycyclohexylcarboxylate.

Acetone was added to the immersion oil from Bordeaux 5% w/v). After development the chromatogram was air dried and visualized by spraying 1 mole/l sulfuric acid (Acros Organics, Geel, Belgium) and 2.5% v/v p-anisaldehyde (Acros Organics) in methanol (LabScan) (6).

High-performance liquid chromatography (HPLC)

A reversed phase and isocratic HPLC system was used, comprising an HP 1100 binary pump (Hewlett-Packard Sverige AB, Sweden), an HP 1100 variable wave-length detector, and an HP 3396 series III integrator. The system was equipped with a Rheodyne 7725i injector (Rheodyne, Cotati, USA) with a 20 μ l loop. The column (4.6 mm i.d. $\times$ 250 mm) was packed with Nucleosil C₁₈ 5 μ m particles (Macherey-Nagel, Düren, Germany). The mobile phase was methanol/water (79/21 v/v). The flow rate was 1 ml/min and the eluate monitored at 280 nm. To make the standard curves, the stock solution diglycidyl ether of bisphenol A (Koninklijke, Shell laboratorium, Duisburg, Germany) at 1.0% w/v in methanol was diluted with methanol to 0.0625–0.25%.

Methanol was added to the oils and the ingredients (0.5–1.0% w/v) and then injected into the HPLC system.

Results of chemical investigations

The only sample investigated by TLC was the immersion oil from Bordeaux, in which the presence of epoxy resin was indicated. Diglycidyl ether of bisphenol A (DGEBA) was demonstrated by HPLC in the oils from Bordeaux and Leuven at 30% (w/w) and 28%, respectively.

Discussion

Among the 14 patients with suspected occupational contact dermatitis from the Leica® immersion oil for microscopy, 10 were patch tested and found to be allergic to epoxy resin (ER) in the European standard series (10/10), and to the Leica® immersion oil for microscopy (7/7). 1 patient was tested in error with the former immersion oil, because the labelling was very similar, with a negative reaction.

The first cases observed in each centre were particularly surprising because of dermatitis apparently arising from an a priori very common and familiar product. In Strasbourg, the technicians even thought they were using cedar oil as the immersion oil. Looking at the safety data sheet of the Leica® immersion oil for microscopy, a revision date was given, 1 August 1997, indicating

that the ingredients had been changed. In Leuven, before August 1997, the patients had used the former Leica[®] immersion oil, which was based on chlorinated paraffins. Because of the presence of PCBs (chlorinated hydrocarbons), that are dangerous to the human health and environment, the Company substituted it for the new formula containing modified ER components. According to them, liquid modified ER was contained at a >80% concentration in the new oil, in order to achieve specific optical properties of refraction, dispersion, and low fluorescence, as so as to optimize microscopy application.

Skin manifestations occurred very rapidly after the introduction of the newly formulated oil and, since the first reports (5), other cases have been published (7, 8). When a technician became allergic in a laboratory, the colleague that replaced her rapidly became sensitized. ERs have previously been reported as responsible for "epidemics" of contact dermatitis (1, 9). In this case, however, the epidemic was only gradually noticed in the various European centres, because of the progressive utilization of the new oil's specimens.

ER compounds act as collective allergens at high concentrations and they are able to provoke sensitization after a single contact. The safety data sheet mentioned the risk of sensitization by skin contact, but this went unnoticed until several technicians became sensitized. It was also indicated that protective gloves and eye protection should be worn during manipulation of the immersion oil. Nevertheless, strict avoidance of contact with the product was very difficult, and even impossible, in this job despite the use of gloves, since the oil was handled and contaminated the microscope eyepieces and knobs, which accounts for the facial localizations. In other reported cases, the dermatitis was frequently located on the face and/or neck and the authors (7, 8) proposed a diagnosis of airborne contact dermatitis. Our data, however suggest a handling rather than an airborne contact dermatitis. Moreover, the plans of work were often contaminated by the oil and minimal amounts of allergen are often enough to provoke a relapse of the symptoms on exposed skin in patients highly sensitized to ER (3). Laminated multilayered plastic protective gloves impermeable to ER have been especially developed for such work (3), but the most commonly used are based on polyvinyl chloride (PVC) or nitrile-butadiene-rubber (NBR) (10). In Strasbourg, the technicians initially wore latex gloves during their procedure, which were effective protection, probably because of a contact duration being too short to allow skin contact, by glove penetration.

ER compounds contain several chemicals that

are well-known occupational sensitizers (1–4). The most frequent and the main sensitizing ERs are those based on diglycidyl ether of bisphenol A, and cycloaliphatic type ERs (3). ER bisphenol A type are synthesized from epichlorhydrin and bisphenol A, that lead to diglycidyl ether of bisphenol-A (DGEBA) CAS registry number [1675-54-3]. This is the monomer of bisphenol-A based epoxy resins. ER phenol-formaldehyde type (resol, novolac), which consist of condensation of phenol and formaldehyde and can contain bisphenol F or F', are thought to be weaker or less common sensitizers (11).

Bisphenol A (diphenylol propane) CAS registry number [80-05-7] is rarely reported as a contact sensitizer and such reports, particularly in workers at ER plants, are controversial (2, 12). Sensitization to epichlorhydrin (1-chloro 2,3-epoxypropane) CAS registry number [106-89-8] mainly occurs in workers in the ER industry. Sensitization in individuals not working in ER plants is rare (2, 13, 14). Delayed hypersensitivity to ER is mainly caused by the low molecular-weight monomer DGEBA (MW 340), the dimer having much lower sensitization potential. We suppose that monomers were present in the immersion oil, since the safety data sheet indicated the presence of "bisphenol A epoxy MW<700" and that subsequent patch testing in the 2 French patients was positive with the Araldit 223 compound that probably contained this monomer or oligomers. The presence of DGEBA in the oil was eventually proved by chemical analysis, and at high concentrations (28–30%).

Epoxy hardeners are also potent allergens (4, 15). They include low molecular-weight aliphatic polyamines such as ethylenediamine, diethylenetriamine and triethylenetetramine (3, 13, 16), cycloaliphatic polyamines such as isophoronediamine (17, 18), and aromatic polyamines, 4,4' diaminodiphenylmethane, for example. In our cases, the presence of epoxy hardeners was not mentioned and was improbable, since the ER was expected to stay liquid.

Diluents are added to ERs so as to decrease their viscosity. The reactive diluents derived from glycidyl ether are also strong sensitizers (3). They are numerous and include n-butyl glycidyl ether, hexanediol diglycidyl ether, phenyl glycidyl ether (3, 13, 19–22), allyl glycidyl ether (3, 20), 1,4-butanediol diglycidyl ether (3, 23), o-cresyl glycidyl ether (3, 13, 23–25), diethyleneglycol diglycidyl ether (26) or alpha-naphtyl glycidyl ether (27). 1,4-butanediol diglycidyl ether, CAS registry number [2425-79-8] (Fig. 1), has previously been reported to cause contact dermatitis in industry (3). It was included in the immersion oil with bisphenol A epoxy MW<700 in the Araldit 223[®] component,

which gave positive reactions in the 2 French patients. Because of a lack of breakdown of this mixture, we suspect both 1,4-butanediol diglycidyl ether and the ER oligomers of being the sensitizers and responsible for the positive reactions. The positive reaction to butyl glycidyl ether in the 1st patient could have resulted from cross-reaction with butanediol diglycidyl ether.

Bis (2-ethylhexyl)phthalate, CAS registry number [117-81-7], was included in the formulation, probably as a non-reactive epoxy diluent. It did not provoke any positive reaction in the 2 French patients when tested at 5%. Phthalates are plasticizers and dibutyl phthalate, CAS registry number [84-74-2], mainly used as a non-reactive epoxy diluent, has been reported as a sensitizer (25, 28). As dioctyl phthalate, another phthalic acid ester, it tested negatively in 2 Belgian patients. Although cross-reactivity is uncertain between phthalic acid esters, 1 patient reported by Sommer et al. (7) reacted to dibutyl phthalate and probably had co-sensitization both to ER and to the phthalate component of the oil.

The declared concentrations of ERs were 35% in the oil. Thus, the results of the HPLC analysis (28–30%) seem to be lower than expected. However the oil and the ingredient were declared to contain an ER with a mean molecular weight of ≤ 700 g/mole and, in the chemical analyses performed, only the monomer DGEBA was quantified, and not higher oligomers such as dimers and trimers of DGEBA. Higher oligomers were indicated in the TLC investigation of the oil from Bordeaux. Therefore, the demonstrated concentrations of DGEBA in the 2 samples of the oil are in agreement with the declarations of the oil and the ingredient.

The 12 other patients, although not tested with the ingredients, were certainly sensitized to ER compounds. On the basis of the results obtained in the French patients, they were probably sensitive both to the bisphenol A epoxy MW < 700 and to the cycloaliphatic ER. The main sensitizer was probably DGEBA, which explains the positive patch tests to ER in the European standard series. Standard ER test substances are prepared from low molecular diglycidylether-bisphenol A ER. They include bisphenol A diglycidylether (MW 340) and some higher molecular mass oligomers, by-product oligomers and some impurities (3). A 2nd ER was however implicated, at least in the 2 French patients. The cycloaliphatic ER 3,4-epoxycyclohexyl methyl 3',4'-epoxycyclohexyl carboxylate CAS registry number [2386-87-0], has not previously been reported as a sensitizer occupationally. According to the chemical formula, its sensitizing potential seems weaker than BADGE's.

ERs specifically used in a liquid phase have

sporadically been described as a cause of occupational allergic contact dermatitis from cutting fluids. Contact dermatitis from a cutting fluid was observed in 5 machine operatives, due to glycidyl ether of hexahydrophthalic acid used as a stabilizer of the chlorinated paraffin fraction (29). In 2 other machine tool setters, the sensitizer was the additive Epoxide 7, a monofunctional glycidyl ether epoxy compound used as a stabilizer and corrosion inhibitor in a neat grinding oil (30). In 12 patients working at a factory, ER compounds were included in the chlorinated paraffin fraction that formed a part of the cutting oil, as well as to inhibit corrosion or to confer stability by scavenging free chlorine radicals released by chlorinated paraffins (31). In the previously reported cases from liquid ERs added to grinding oils, a positive patch test reaction was observed to the ER in the European standard series in, respectively, 2/5, 0/2, and 6/12 patients (29–31). This was probably due to cross-reaction rather than to the presence of standard ER in the oil. Although the safety data sheet did not mention its presence, the 1st Belgian patient had a positive reaction to n-butyl glycidyl ether, CAS registry number [2426-08-8] (Fig. 2). This reactive diluent is used to reduce viscosity of epoxy resins of bisphenol A type and can provoke sensitization (3, 13). Cross-reactivity is possible between the standard ER and butyl glycidyl ether. A similar situation was previously reported by Fregert, who observed 3 positive reactions to butyl glycidyl ether among 20 ER-positive patients (4) and by Sommer et al., with 1 patient sensitized to the immersion oil and to both phenyl glycidyl ether and cresyl glycidyl ether (7).

These cases emphasize the necessity for permanent watchfulness for the presence of sensitizing components in materials assumed to be harmless. Because of persistent worldwide diffusion of this immersion oil, considered as innocuous by technicians, numerous other epidemic cases of contact sensitization have surely been observed.

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