

Triethylene glycol bis(2-ethylhexanoate) – a new contact allergen identified in a spectacle frame

Klaus E. Andersen¹, Malene E. Vestergaard¹ and Lars P. Christensen²

¹Department of Dermatology and Allergy Centre, Odense University Hospital, University of Southern Denmark, Sdr. Boulevard 29, DK-5000 Odense C, Denmark and ²Department of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

doi:10.1111/cod.12152

Summary

Background. Allergic reactions to spectacle frames are not unusual. A patient had a reproducible strong allergic patch test reaction to scrapings from the plastic material, and negative patch test results with available spectacle frame chemicals.

Objectives. To identify the culprit allergen in this patient's spectacle frame.

Materials and methods. An extract from the temple arms was analysed by gas chromatography mass spectrometry (GC-MS), and a major low molecular weight compound was detected. This compound was isolated by semi-preparative high-performance liquid chromatography and identified by GC-MS and nuclear magnetic resonance spectroscopy. The purified compound was diluted in ethanol, and a dilution series was prepared for patch testing.

Results. Triethylene glycol bis(2-ethylhexanoate) was identified as the major compound in the extract. Patch testing of the patient proved that triethylene glycol bis(2-ethylhexanoate) was the causative allergen in the spectacle frame. Ten consecutive eczema patients tested as controls were negative.

Conclusion. Triethylene glycol bis(2-ethylhexanoate) is a new, hitherto unreported contact allergen.

Key words: CAS no. 94-28-0; contact allergen; patch test; plastic; plasticizer; spectacle frame; triethylene glycol bis(2-ethylhexanoate).

Spectacle frame dermatitis is not unusual. Potential allergenic compounds in frames include metals, plastics, and rubbers, and various additives as plasticizers and ultraviolet stabilizers, dyes, composites, and polishes (1). A problem is the lack of information about the chemical content in the frames, partly because of the long chain between the retail shops, the distributors, and the manufacturers. Furthermore, the industry may

be reluctant to give detailed information about the chemistry involved (2). We recently examined a male patient with chronic temporal eczema, and suspected spectacle frames to be the cause. By chemical analysis of the plastic in the frames, we identified a new contact allergen: triethylene glycol bis(2-ethylhexanoate).

Materials and Methods

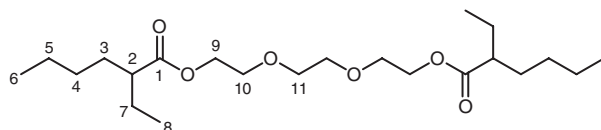
Case report

A 51-year-old non-atopic male, with no history of psoriasis, was referred with persistent bilateral temporal eczema. The temporal eczema, in which itching was not a prominent feature, had developed over a year, and was the site of two episodes of acute cellulitis around

Correspondence: Klaus E. Andersen, Department of Dermatology and Allergy Centre, Odense University Hospital, University of Southern Denmark, Sdr. Boulevard 29, 5000 Odense, Denmark. Tel: +45 65412700; Fax: +45 66123819. E-mail: keandersen@health.sdu.dk

Conflicts of interest: The authors declare no conflict of interests.

Accepted for publication 7 September 2013



Triethylene glycol bis(2-ethylhexanoate)

Fig. 1. Chemical structure of triethylene glycol bis(2-ethylhexanoate), a new allergen isolated and identified from a spectacle frame.

the ears. Recently, the patient had developed patches of dermatitis on the back of both hands and on the chest, and the temporal eczema had been exudative. When he was seen for clinical examination, the eczema on the temples was diffuse, dry, erythematous, infiltrated, and scaly. In addition to the dermatitis on the back of the hands, there was diffuse scaling on the scalp. The patient had no suspicion of a precipitating factor, except for the fact that he had bought new spectacles a few years previously.

Patch testing was performed with the department's baseline series, encompassing TRUE Test™ panel 1–3 supplemented with 23 petrolatum-based or water-based allergens in Finn Chambers® on Scanpor® tape (extra preservatives, Compositae allergens, Disperse Blue 106 and 124, and phenol formaldehyde resin). The tests were occluded for 2 days, and read twice on days 3 and 7. All tests were negative. The patient was further tested with scrapings from his black plastic spectacle frame, and showed a vesicular positive reaction (++) at both days 3 and 7. Supplementary patch tests with the plasticizers triphenyl phosphate and tricresyl phosphate gave negative results. The patient stopped wearing the spectacles, and the temporal eczema healed completely.

The patient was asked to contact his optician to obtain information about the composition of the frame material. Fortunately, the distributor in France revealed that the frames were made of Tenite™ propionate 380A4000018 Clear Trsp from the Eastman Chemical Company. The material safety data sheet showed that the plastic was composed of > 75% cellulose acetate propionate, < 1% proprietary additive colorants, and < 25% triethylene glycol bis(2-ethylhexanoate).

It was then suspected that the allergen was triethylene glycol bis(2-ethylhexanoate) (Fig. 1). Despite an extensive search, we were unable to obtain a sample of this chemical, which had not previously been reported as an allergen. Therefore, we performed extraction and identification of the chemical directly from the spectacle frame material.

Chemicals

High-performance liquid chromatography (HPLC)-grade solvents, ethyl acetate, methanol and ethanol were purchased from Rathburn Chemicals (Walkerburn, UK). Deionized water was prepared with a Siemens SG Ultra-Pure Water system (Siemens, Barsbüttel, Germany), and CDCl₃ with tetramethylsilane as internal standard was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA).

Isolation and identification of triethylene glycol bis(2-ethylhexanoate) from the spectacle frame

The temple arms from the spectacle frame were extracted (dissolved) with ethyl acetate (50 ml), yielding a brown oil-like extract after filter paper filtration. A small part of the extract (3 × 1.0 ml) was filtered through 0.2-μm organic nylon filters (Frisenette Aps, Knebel, Denmark) for further analysis. Gas chromatography mass spectrometry (GC-MS) analysis of the extract revealed one major low molecular weight (MW) compound with an MW of 258, constituting > 60% of the compounds detected by GC-MS, which, according to the mass spectrum, was triethylene glycol bis(2-ethylhexanoate), and was considered to be the main contact allergen in the spectacle frame. Isolation of this target compound from the extract was performed by semi-preparative HPLC on a Dionex UltiMate 3000 binary semi-preparative HPLC system from Thermo Fisher Scientific (Hvidovre, Denmark), consisting of a Dionex HPG-3200P HPLC pump and a Dionex Ultimate 3000 DAD operating from 190 to 800 nm, and equipped with a manual injection port and a 2-ml sample loop. Separations were performed on an Uptisphere 120-Å C18-ODB semi-preparative HPLC column (internal diameter, 250 × 21.2 mm; particle size, 5 μm; Interchim, Montluçon, France) at room temperature (22°C). The mobile phase consisted of water (solvent A) and methanol (solvent B), and the following linear-programmed solvent gradient was used to isolate the target compound: 0 min of 80% B, 10 min of 80% B, 20 min of 100% B, 30 min of 100% B, 32 min of 80% B, and 50 min of 80% B; flow rate of 5 ml/min; detection wavelength (λ), 200 and 254 nm; injection volume, 0.5 ml. Owing to low absorbance of the compounds in the extract of the spectacle frame, the collection of fractions was performed manually. Under these conditions, the retention time of the target compound was between 31 and 35 min, yielding 70 mg of crude triethylene glycol bis(2-ethylhexanoate), which was further purified by semi-preparative HPLC under the same conditions as described above, yielding 60 mg of triethylene glycol bis(2-ethylhexanoate) at a purity of ~92% as determined by GC-MS. The remaining

8% impurities were compounds structurally related to triethylene glycol bis(2-ethylhexanoate) according to their GC-MS spectra; however, it was not possible to make an unambiguous identification of these impurities solely on the basis of their GC-MS spectra. The amounts of crude extract and crude and/or purified triethylene glycol bis(2-ethylhexanoate) were determined by weighing, after evaporation of solvent, with an analytical balance (XS 205 Dual Range; Mettler Toledo, Glostrup, Denmark). Specific amounts of the crude extract and purified triethylene glycol bis(2-ethylhexanoate) were used to prepare 1%, 3% and 5% (wt/wt) and 1% (wt/wt) stock solutions, respectively, in 96% ethanol for patch testing. The identity of triethylene glycol bis(2-ethylhexanoate) was determined by one-dimensional (1D) nuclear magnetic resonance (NMR) [^1H , ^{13}C , and distortionless enhancement by polarization transfer (DEPT)] and two-dimensional (2D) NMR [correlation spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC)] experiments on a Bruker 400-MHz spectrometer and by GC-MS.

GC-MS was performed on a Thermo Scientific DSQ II mass spectrometer (Thermo Scientific, Waltham, MA, USA) operated at an ionization potential of 70 eV, coupled to a Trace GC Ultra gas chromatograph fitted with a Phenomenex ZB-WAX plus column (60 m $\times$ 0.25 mm $\times$ 0.25 μm ; Phenomenex, Værløse, Denmark). The oven temperature was programmed from 80 to 250°C at 5°C/min, and this was followed by an isothermal step at 250°C for 40 min. Helium was applied as carrier gas at a flow rate of 1.2 ml/min. The injection volume was 1 μl , with a split ratio of 50:1. Under these conditions, the retention time of triethylene glycol bis(2-ethylhexanoate) was 47.4 min. Identification of triethylene glycol bis(2-ethylhexanoate) was based on comparison with the mass spectra suggested by the National Institute of Standards and Technology (NIST) mass spectrometry database.

Triethylene glycol bis(2-ethylhexanoate). GC-MS (70 eV) m/z (relative interval): 258 [M] $^+$ (3), 189 (5), 171 (90), 143 (12), 127 (60), 114 (19), 99 (45), 57 (100). ^1H -NMR (CDCl_3 , 400 MHz) ^{a} : δ 0.88 (6H, overlapping triplets, J = 7.6 Hz, H_3 -6 and H_3 -8), 1.25–1.30 (4H, m, H_2 -4 and H_2 -5), 1.40–1.65 (4H, m, H_2 -3 and H_2 -7), 2.29 (1H, m, H-2), 3.63 (2H, s, H_2 -11), 3.69 (2H, t, J = 4.8 Hz, H_2 -10), 4.24 (2H, t, J = 4.8, H_2 -9). ^{13}C -NMR (CDCl_3 , 100 MHz) ^{a} : δ 11.9 (q, C-8), 14.1 (q, C-6), 22.8 (t, C-5), 25.6 (t, C-7), 29.8 (t, C-4), 31.9 (t, C-3), 47.3 (d, C-2), 63.2 (t, C-9), 69.5 (t, C-10), 70.7 (t, C-11), 176.5 (s, C-1). Because of symmetry in the molecule, all NMR signals appear twice. Assignments are based on DEPT, HSQC and COSY experiments.

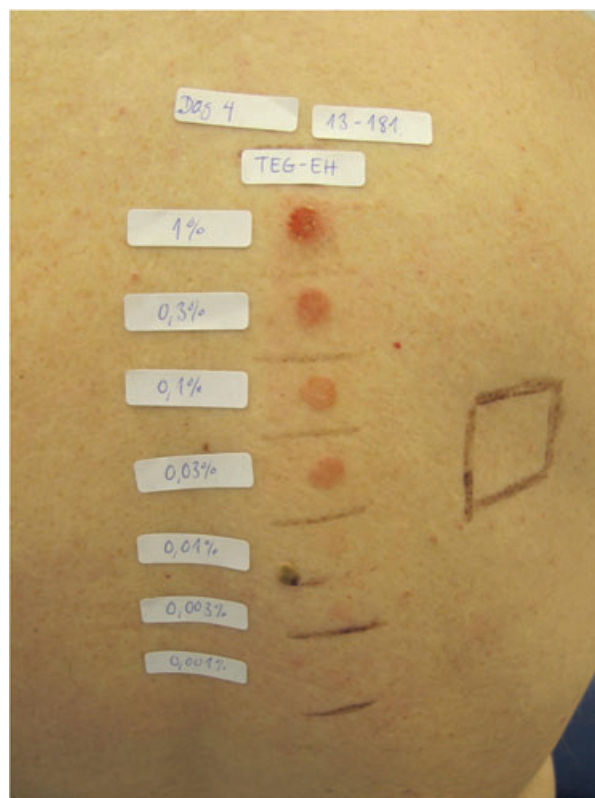
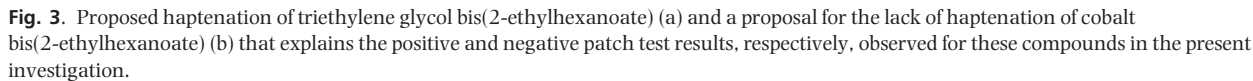


Fig. 2. Patch test results at the day 4 reading with a dilution series of triethylene glycol bis(2-ethylhexanoate).

Results

When the extract was obtained, further testing was performed. Scrapings from the frame material again showed a strongly positive reaction, and triethylene glycol bis(2-ethylhexanoate) 1%, 3% and 5% in ethanol were all strongly positive (+++). In the Allergen Bank (3), we had a polyester catalyst, cobalt-2-ethylhexanoate 2% pet. (3), for patch testing, and it was negative. Ten consecutive controls were tested with triethylene glycol 5% in ethanol, and showed negative test results. Testing of the patient with a dilution series of triethylene glycol bis(2-ethylhexanoate) 1%, 0.3%, 0.1%, 0.03%, 0.01%, 0.003% and 0.001% in ethanol showed a positive patch test reaction to 0.03%, which was the lowest concentration giving a positive reaction (Fig. 2).

The contact allergen triethylene glycol bis(2-ethylhexanoate) (Fig. 1) was isolated from an ethyl acetate extract of the temple arms from a spectacle frame by semi-preparative HPLC at a purity of ~92%. The chemical structure of the allergen was determined by GC-MS and 1D and 2D NMR spectroscopy. The GC-MS spectrum of the allergen with an MW of 258 was fully in accordance



with the NIST database mass spectrum of triethylene glycol bis(2-ethylhexanoate). The ^{13}C -NMR and ^1H -NMR data confirmed the identity of the allergen, showing the presence of 11 different carbons, including 2 methyl carbons, 7 methylene carbons, 1 methine carbon, and 1 quaternary carbon. The fact that the methylene group at C-11 gave a singlet at δ 3.69 in the ^1H -NMR spectrum indicated that these protons are magnetically equivalent and identical to the protons on the neighbouring methylene group, thus confirming the symmetry in the molecule (Fig. 1). The quaternary carbon signal at δ 176.5 (C-1) and the methylene carbon signals at δ 63.2 (C-9), 69.5 (C-10) and 70.7 (C-11) in the ^{13}C NMR spectrum clearly indicated the presence of an ester group and three methylene groups in proximity to electron-withdrawing groups, respectively, in accordance with the allergen being triethylene glycol bis(2-ethylhexanoate). The assignments of the methylene carbons were confirmed by the HSQC and the COSY experiments. The assignments of the remaining signals in the ^{13}C -NMR and ^1H -NMR spectra of triethylene glycol bis(2-ethylhexanoate) were based on DEPT, HSQC and COSY experiments (see Materials and Methods).

Triethylene glycol bis(2-ethylhexanoate) (CAS no. 94-28-0; EC no. 202-319-2) is a plasticizer. A synonym name is Eastman™ TEG-EH. It is a colourless liquid with negligible solubility in water. According to the material safety data sheet, it is a stable chemical at normal temperatures. Although it has been stated to be non-sensitizing in a mouse assay, in Toxnet (<http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~hlZSwq:1>) (June 2013), it has been reported to cause weak skin sensitization in guinea-pigs.

According to the material safety data sheet for Tenite™ Propionate 380A4000018 Clear Trsp, it contains < 25% of triethylene glycol bis(2-ethylhexanoate). Triethylene glycol bis(2-ethylhexanoate) contains two acyl groups that can be attacked by a nucleophile such as an amino (R-NH₂) or a thiol (R-SH) group in a protein, and thus may act as a hapten, being able to generate a protein–hapten complex in contact with the skin. In such a nucleophilic acyl substitution reaction, the nucleophile attacks the carbonyl carbon (addition mechanism), forming a tetrahedral intermediate, after which it collapses, recreating the carbonyl group and ejecting the leaving group in an elimination reaction. The leaving group of

the acyl derivative, which in this case is a highly stable leaving group, is the triethylene glycol part of triethylene glycol bis(2-ethylhexanoate), and the resulting product is a carbonyl-containing compound (hapten–protein complex) in which the protein nucleophile has taken the place of the leaving group present in the original acyl derivative, as shown in Fig. 3a. This haptation is likely to occur because the leaving group is stable, and thus may explain the strong positive reaction observed in our patient towards the allergen triethylene glycol bis(2-ethylhexanoate). However, patch testing with cobalt bis(2-ethylhexanoate) 2% pet., which is used as an accelerator in polyester resins, gave a negative result. This chemical was available from the Allergen Bank, where it was applied in an earlier case report (4). The negative result with cobalt bis(2-ethylhexanoate) in our patient makes sense from a chemical point of view, as the leaving

group would most likely be an oxide anion (O^{2-}) or a hydroxyl anion (HO^-), which are not very stable leaving groups, and therefore cobalt bis(2-ethylhexanoate) is not very susceptible to a nucleophilic acyl substitution reaction. Hence, cobalt bis(2-ethylhexanoate) cannot be considered to be a strong allergen, and it is unlikely that this compound will give rise to an allergenic reaction as observed for triethylene glycol bis(2-ethylhexanoate), or any reaction at all, in accordance with the observations of this study (Fig. 3b).

Acknowledgements

We would like to express our gratitude to Dorte Lillesø, the Department of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark, for her excellent technical assistance.

References

- Walsh G, Wilkinson M. Materials and allergens within spectacle frames a review. *Contact Dermatitis* 2006; **55**: 130–139.
- Nakada T, Maibach H I. Eyeglass allergic contact dermatitis. *Contact Dermatitis* 1998; **39**: 1–3.
- Andersen K E, Rastogi S C, Carlsen L. The Allergen Bank a source of extra contact allergens for the dermatologist in practice. *Acta Derm Venereol* 1996; **76**: 136–140.
- Cahill J, Andersen K E. Occupational cobalt-allergic contact dermatitis resulting from polyester resin. *Contact Dermatitis* 2010; **63**: 292–294.