Repeated open application test with methyldibromo glutaronitrile, a multicentre study within the EECDRG.

Gruvberger, Birgitta; Andersen, K E; Brandão, F M; Bruynzeel, D P; Bruze, M; Frosch, P J; Goossens, A; Lahti, A; Maibach, H I; Menné, T; Orton, D; Seidenari, S

Published in:
Contact Dermatitis

DOI:
10.1111/j.0105-1873.2005.00481.x

2005

Contact Dermatitis 2005: 52: 19–23
Printed in Denmark. All rights reserved

CONTACT DERMATITIS

Repeated open application test with methyldibromo glutaronitrile, a multicentre study within the EECDRG

B. Gruvberger[1]*, K. E. Andersen[2], F. M. Brandão[3], D. P. Bruynzeel[4], M. Bruze[1], P. J. Frosch[5], A. Goossens[6], A. Lahti[7], H. I. Maibach[8], T. Menné[9], D. Orton[10]* and S. Seidenari[11]

[1]Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, Sweden,[2]Department of Dermatology, Odense University Hospital, Odense, Denmark,[3]Department of Dermatology, Hospital Garcia de Orta, Almanda, Portugal,[4]Department of Dermatology, Free University Hospital, Amsterdam, The Netherlands,[5]Department of Dermatology, Klinikum Dortmund and University of Witten/Herdecke, Dortmund, Germany,[6]Department of Dermatology, University Hospital, K.U. Leuven, Belgium[7]Department of Dermatology, University of Oulu, Oulu, Finland,[8]Department of Dermatology, UCSF, School of Medicine, San Francisco, CA, USA,[9]Department of Dermatology, Gentofte Hospital, Copenhagen, Denmark,[10]Department of Dermatology, Amersham General Hospital, Bucks, UK, and[11]Department of Dermatology, University of Modena, Modena, Italy

Contact allergy to and allergic contact dermatitis from methyldibromo glutaronitrile (MDBGN) have frequently been reported. This study was initiated to help determine the optimal patch test preparation for MDBGN. In 51 patients with a doubtful or a positive patch test reaction to at least 1 of 4 test preparations with MDBGN in petrolatum at 1.0% w/w, 0.5%, 0.3% and 0.1%, a repeated open application test (ROAT) with moisturizers with and without MDBGN at 0.03% w/w was performed on the upper arms for 2 weeks. 18 of the 51 (35.3%) patients developed a positive ROAT. In all patients, there was a positive ROAT only to the moisturizer with MDBGN ($P < 0.001$). A statistically significant association was also found between the patch test reactivity (PTRL) and the outcome of the ROAT ($P < 0.001$). If only considering those with a PTRL above 0.3%, thus with negative or doubtful test reactions to 0.1% and 0.3%, there were still statistically significantly more patients with a positive ROAT to the moisturizer with MDBGN than to the moisturizer without MDBGN. The study demonstrates that patch testing with MDBGN at 0.3% and 0.1% will miss clinically relevant patch test reactions to MDBGN.

Key words: 1,2–dibromo–2,4–dicyanobutane; allergic contact dermatitis; CAS 35691–65–7; clinical relevance; Euxyl K400; methyldibromo glutaronitrile; moisturizer; patch testing; preservative; ROAT; Tektamer 38; usage test.

Accepted for publication 22 October 2004

Contact allergy to the preservative methyldibromo glutaronitrile (MDBGN) (1,2–dibromo–2,4–dicyanobutane) has frequently been reported (1–8). Furthermore, allergic contact dermatitis from MDBGN in products, such as lotions, moist toilet paper, ultrasonic gels, natural products, eye gels, hair mousse, hair conditioners, sunscreen creams, protective creams, abrasive cleansers, work cream, detergents and glues, is likewise frequently reported (1, 9–24). Thus, MDBGN is a strong candidate to be introduced in the European standard patch test series (25). However, there has been no agreement on which test preparation to use to define contact allergy to MDBGN. As the purpose of patch testing with a standard test series is to find individuals with a clinically relevant contact allergy (25), 2 studies were initiated within the European Environmental and Contact Dermatitis Research Group (EECDRG) to help determine the optimal patch test preparation for MDBGN. The study with patch testing with various concentrations of MDBGN in petrolatum (pet.) is also published in this issue of *Contact Dermatitis* (26), while the study presented here concerns repeated open application testing (ROAT) with moisturizers with and without MDBGN.

*Not members of the EECDRG.

Materials and Methods

Patients

From 10 European and 1 American patch test clinics during the period January 2002–June 2002, 51 patients with doubtful or positive reactions to at least 1 of the 4 simultaneously patch tested preparations with MDBGN in pet. at 1.0% w/w, 0.5%, 0.3% and 0.1% participated as a part of their individual investigation on the clinical relevance of the demonstrated or possible (doubtful reaction) contact allergy to MDBGN. 18 were males (mean age 53.4 year and range 27–71 year) and 33 were females (mean age 45.5 year and range 21–75 year).

Chemicals

MDBGN (Schülke and Mayr, Hamburg, Germany) from the same batch was used both for the patch testing with the 4 MDBGN preparations (26) and the ROAT. The pharmacy at the University Hospital in Malmö prepared 2 moisturizers which were identical (pet., sorbitan oleate and water) but for the preservative. 1 moisturizer was preserved with MDBGN at 0.03% w/w and the other with methyl paraben at 0.1% w/w and propyl paraben at 0.2% w/w.

Repeated open application test

The patients were instructed to use the moisturizers $\times 2$ daily on the ventral aspects of the upper arms where a square measuring 5×5 cm was marked. Each patient received a pair of moisturizers with and without MDBGN. The pairs were numbered and the allocation of the moisturizer to be applied to the respective arm was done in a randomized way. The moisturizer to be applied to the right upper arm was marked with a blue tape, while a red tape was used for the moisturizer to be applied to the left arm. The patients were instructed to use an approximately 0.5-cm long string of the cream for each application (35 mg on $25\,cm^2 = 1.4\,mg/cm^2$). The study period was 2 weeks unless terminated earlier because of a positive ROAT or at the patient's request. Inspection of the upper arms was done before the ROAT on the first day and then after 1 week and 2 weeks or at the request of the patient. An eczematous reaction was sought and considered to be positive if there was at least an erythematous infiltration with/without papules and/or vesicles covering at least 25% of the marked area (27). The patients were encouraged to continue the applications if there was only an erythematous reaction without infiltration or an eczematous reaction covering less than 25% of the area.

The code was broken after the individual termination, and the result of the ROAT could thus be used for the individual assessment of clinical relevance and as a basis for individual advice and preventive measures.

Statistical calculations

McNemar's test was used to compare the number of patient arms with a positive ROAT for the moisturizers with and without MDBGN. This comparison was also done in those with a negative and/or doubtful patch test reaction to 0.1% and 0.3%, but a positive and/or doubtful reaction to 0.5% and 1.0%. The Spearman rank correlation test was used to investigate any association between the individual patch test reactivity (PTRL), defined as the lowest patch test concentration of MDBGN giving a positive reaction, i.e. at least a positive reaction according to ICDRG guidelines (28), and ROAT outcome, defined as the number of days until a positive ROAT appeared.

Results

18 of the 51 (35.3%) patients developed a positive response. Within the first week, 12 patients tested positively and 6 more patients tested positively the second week. In all patients, there was a positive ROAT only on 1 arm, and for all patients, this was the arm to which the MDBGN-containing moisturizer had been applied ($P < 0.001$). When the same comparison was made for those with a negative or doubtful patch test reaction to 0.1% and 0.3%, but a positive or doubtful reaction to 0.5% and 1.0%, there were statistically significantly more patients with a positive ROAT to the moisturizer with MDBGN than to the moisturizer without MDBGN ($P < 0.05$, McNemar's test, one sided). 6 of those participating had at patch testing doubtful reactions to MDBGN at 0.3%, and in 1/3 a positive ROAT developed on the arm to which the moisturizer with MDBGN had been applied. Figure 1 shows the relationship between the outcome of ROAT with regard to the number of days until a positive ROAT appeared and the respective PTRL including those with doubtful reactions ($P < 0.001$), and Fig. 2 gives the distribution of positive and negative ROATs for the respective PTRLs including doubtful reactions.

Discussion

ROAT and other types of use tests can be used both in individual cases to help assess the clinical relevance of a positive or doubtful reaction to a sensitizer present in a product used by the patient

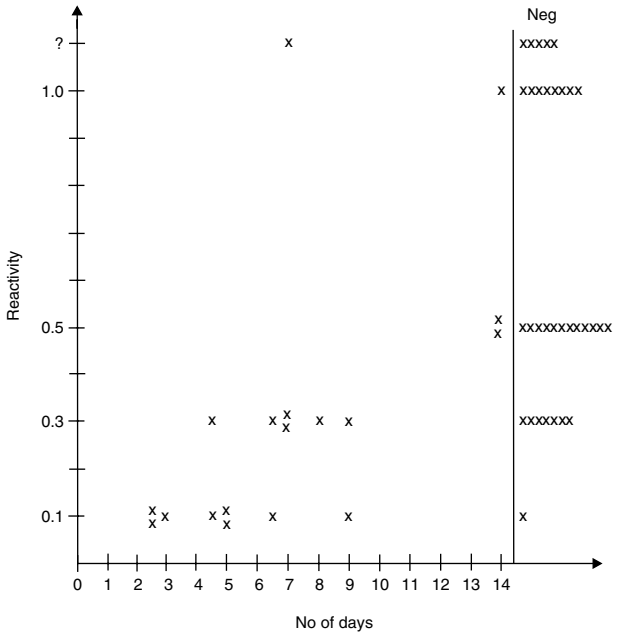

Fig. 1. Relationship between the outcome of repeated open application test (ROAT) with regard to the number of days until a positive ROAT appeared and the respective patch test reactivity level (0.1%–1.0%) in 45 patients with allergic reaction to methyldibromo glutaronitrile and 6 patients with doubtful (?) reactions.

but with a negative product patch test and in groups of hypersensitive patients undergoing ROAT with a product containing the sensitizer. For the individual ROAT, a positive test does not say anything about the nature of the reaction, i.e. the eczematous response can be either allergic or irritant. On the other hand, when a ROAT is scientifically designed and conducted, information can be gained on both the general clinical relevance and the nature of the ROAT reactions.

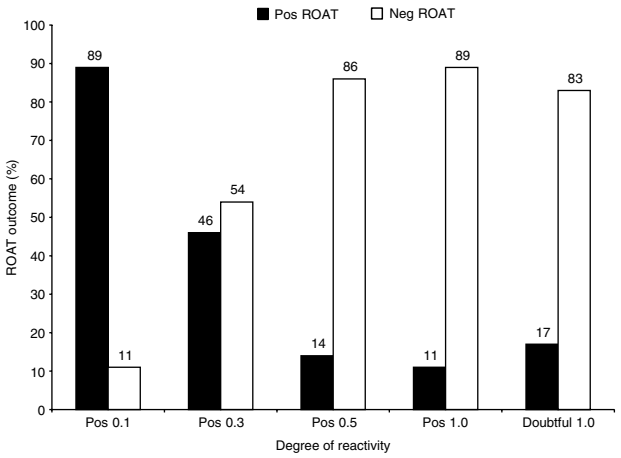

Fig. 2. Distribution in percentage of positive and negative repeated open application tests (ROATs) for the respective patch test reactivity level in 45 patients with allergic and 6 with doubtful reactions to methyldibromo glutaronitrile.

The present study gives information on the individual clinical relevance in those testing positively to ROAT and information on the general clinical relevance of MDBGN positivity on patch testing, as an instrument to help determine the optimal patch test preparation for MDBGN. This study demonstrates the clinical relevance of contact allergy to MDBGN traced by patch testing with pet. preparations with MDBGN at 0.1%–0.5%. A dose–response relationship for PTRL and ROAT outcomes (Fig. 1) was also demonstrated, which gives further support to the significance of the demonstrated positive patch test reactions.

The use of many different test preparations with regard to vehicle and MDBGN concentrations (1–3, 6, 7, 11, 15, 18, 23, 24, 29–34) and the results of the EECDRG patch test study (26) strongly indicate that patch test reactions to MDBGN may be difficult to read. Therefore, some dermatologists claim that MDBGN, besides giving irritant reactions with an irritant morphology, can give irritant reactions which are misinterpreted as allergic reactions, i.e. false-positive reactions. In the case that a patch test reaction representing a single application of a chemical is false-positive, it is likely that multiple applications of the same chemical at a lower concentration over an extended period of time would result in a positive reaction of the same nature, i.e. a false-positive reaction. When performing ROAT in groups of individuals with contact allergy, false-positive reactions and other irritant reactions can be recognized by using appropriate controls. As there was no control group in this study, the positive ROATs could therefore be false-positive. This interpretation, however, seems highly unlikely and, furthermore, what is more important, of only minute significance for individual diagnostic and preventive measures concerning allergic contact dermatitis/contact dermatitis from MDBGN.

Actually, critical to the present possible incapacity always to unambiguously determine the nature of positive patch tests (26) and ROATs to MDBGN, besides legislative aspects, is how to advise the positive reactors on which substances to avoid. For allergenicity, but not for irritancy, cross-reactivity is a phenomenon to consider, which means that allergic persons should be advised on potentially cross-reacting substances to avoid relapses of allergic contact dermatitis. However, cross-reactivity is not of major concern for MDBGN, as there currently are virtually no known chemically related substances in the environment. Considering the fact that higher MDBGN concentration can be present in leave-on products, the possible significance of the use of

MDBGN-preserved leave-on products on damaged skin instead of healthy skin, and the significance of ROAT study periods exceeding 2 weeks (35, 36), makes it highly likely that the positive reactors to 1.0% at patch testing in this study would also have tested positively (statistically significant) with another ROAT study design, taking into account the above-mentioned factors.

This study demonstrates that a person who tests positively, independent of whether the positive patch test reactions to MDBGN at concentrations equal to or lower than 0.5% represent positive allergic or false-positive reactions, should avoid use of moisturizers preserved with MDBGN to avoid contact dermatitis relapses. This statement is supported by the fact that the used MDBGN concentration of 0.03%, chosen as chemical analysis of several hundred leave-on products on the Swedish market at the Department of Occupational and Environmental Dermatology in Malmö had shown 0.03% to be the mean concentration of MDBGN in those products preserved with this preservative (unpublished observation), is below the highest concentration that has been allowed in leave-on products (0.1% except for sunscreen products with 0.025%). Although a previous use test with a rinse-off product in MDBGN-hypersensitive individuals was negative (37), some of the authors of this article have for many year advised our MDBGN-hypersensitive patients also to avoid rinse-off products preserved with MDBGN. This advice is supported by the result of a recent study (38).

In summary, this study documents that patch testing with MDBGN at 0.3% and 0.1% will miss clinically relevant patch test reactions to MDBGN. With the present knowledge of contact allergy rates to MDBGN and clinical relevance, patients with positive patch tests should avoid MDBGN exposure, at least prolonged exposure occurring, for example, when using leave-on products. Furthermore, the circumstances of high and increasing European contact allergy rates to MDBGN, the reports on anecdotal cases with allergic contact dermatitis from MDBGN, as well as the ROAT results of this study merit legislative measures to confine or ban non-occupational, as well as occupational, exposure to MDBGN, particularly in leave-on products, to prevent sensitization to and elicitation from MDBGN in already sensitized persons. And, from July 2004, MDBGN has been banned in cosmetics of leave-on type, though patients may still now be exposed to MDBGN in leave-on products manufactured before that date, at concentrations up to 0.1%.

Acknowledgements

The study was financially supported by a grant from the National Skin Allergy Research Centre at Gentofte Hospital, Copenhagen, Denmark.

References

1. Senff H, Exner M, Görtz J, Goos M. Kontaktallergie auf einen neuen Konservierungsstoff. *Dermatosen* 1989: 37: 45–46.
2. Tosti A, Guerra L, Bardazzi F, Gasparri F. Euxyl K 400: a new sensitizer in cosmetics. *Contact Dermatitis* 1991: 25: 89–93.
3. Van Ginkel C J W, Rundervoort G J. Increasing incidence of contact allergy to the new preservative 1,2-dibromo-2, 4-dicyanobutane (methyldibromoglutaronitrile). *Br J Dermatol* 1995: 132: 918–920.
4. De Groot A C, van Ginkel C J W, Weijland J W. Methyldibromoglutaronitrile (Euxyl K 400): an important ''new'' allergen in cosmetics. *J Am Acad Dermatol* 1996: 35: 743–747.
5. Vigan M, Brechat N, Girardin P, Adessi B, Meyer J P, Vuitton D, Laurent R. Un nouvel allergène: Le dibromodicyanobutane. Compte rendu d'une étude portant sur 310 malades de janvier à décembre 1994. *Ann Dermatol Venereol* 1996: 123: 322–324.
6. Jackson J M, Fowler J F. Methyldibromoglutaronitrile (Euxyl K400): a new and important sensitizer in the United States? *J Am Acad Dermatol* 1998: 38: 934–937.
7. McFadden J P, Ross J S, Jones A B, Rycroft R J G, Smith H R, White I R. Increased rate of patch test reactivity to methyldibromoglutaronitrile. *Contact Dermatitis* 2000: 42: 54–55.
8. Guimaraens D, Hernández M I, Gonzalez M A, Conde-Salazar L. Contact allergy to Euxyl K 400 in consecutively patch-tested patients. *Contact Dermatitis* 2000: 43: 55–56.
9. Hillen U, Franckson T, Goos M. Allergic contact dermatitis due to deer-fat cream (Hirschtalgcreme). *Contact Dermatitis* 2001: 44: 58.
10. Fernández E, Navarro J A, Del Pozo L, Fernándes de Corrès L. Allergic contact dermatitis due to dibromodicyanobutane in cosmetics. *Contact Dermatitis* 1995: 32: 109–110.
11. Torres V, Soares A P. Contact allergy to dibromodicyanobutane in a cosmetic cream. *Contact Dermatitis* 1992: 27: 114–115.
12. de Groot A C, Weyland J W. Contact allergy to methyldibromoglutaronitrile in the cosmetics preservative Euxyl K 400. *Am J Contact Dermat* 1991: 2: 31–32.
13. Erdmann S M, Sachs B, Merk H F. Allergic contact dermatitis due to methyldibromo glutaronitrile in Euxyl K 400 in an ultrasonic gel. *Contact Dermatitis* 2001: 44: 39–40.
14. Gebhart M, Stuhlert A, Knopf B. Allergic contact dermatitis due to Euxyl® K 400 in an ultrasonic gel. *Contact Dermatitis* 1993: 29: 272.
15. O'Donnell B F, Foulds I S. Contact dermatitis due to dibromodicyanobutane in cucumber eye gel. *Contact Dermatitis* 1993: 29: 99–100.
16. Ross J S, Cronin E, White I R, Rycroft R J G. Contact dermatitis from Euxyl K 400 in cucumber eye gel. *Contact Dermatitis* 1992: 26: 60.
17. Armstrong D K B, Smith H R, Rycroft R J G. Contact allergy to methyldibromo glutaronitrile presenting as severe scalp seborrhoeic eczema. *Contact Dermatitis* 1999: 40: 335.
18. Silvestre J F, Rodriguez-Serna N, Miquel J F, Gauchia R, Aliaga A. Allergic contact dermatitis from Euxyl K 400 in a sunscreen cream. *Contact Dermatitis* 1996: 35: 315.
19. Kelterer D, Kaatz M, Bauer H I, Thiele J, Elsner P. Contact allergy to methyldibromo glutaronitrile in Euxyl K 400 in a

cosmetic cream for protection against a permanent wave solution. *Contact Dermatitis* 2002: 46: 250.
20. Wong C S M, Beck M H. Occupational contact allergy to methyldibromo glutaronitrile in abrasive cleansers and work creams. *Contact Dermatitis* 2001: 44: 311–312.
21. Aalto-Korte K, Jolanki R, Estlander T, Alanko K, Kanerva L. Occupational allergic contact dermatitis caused by Euxyl K 400. *Contact Dermatitis* 1996: 35: 193–194.
22. Pigatto P D, Bigardi A, Legori A, Altomare G F, Carminati G. Allergic contact dermatitis from Tektamer 38® (dibromocyanobutane). *Contact Dermatitis* 1991: 25: 138–139.
23. Diba V C, Adisesh A, Statham B N. Occupational allergic contact dermatitis in hospital workers caused by methyldibromoglutaronitrile in a work soap. *Contact Dermatitis* 2003: 48: 118–119.
24. Mathias C G T. Contact dermatitis to a new biocide (Tektamer 38®) used in a paste glue formulation. *Contact Dermatitis* 1983: 9: 418–435.
25. Bruze M, Conde-Salazar L, Goossens A, Kanerva L, White I R. Thoughts on sensitizers in a standard patch test series. The European Society of Contact Dermatitis. *Contact Dermatitis* 1999: 41: 241–250.
26. Gruvberger B, Andersen K E, Brandao F M et al. Patch testing with methyldibromo glutaronitrile, a multicentre study within the EECDRG. *Contact Dermatitis* 2005: 52: 14–18.
27. Johansen J D, Bruze M, Andersen K E et al. The repeated open application test: suggestions for a scale of evaluation. *Contact Dermatitis* 1997: 39: 95–96.
28. Wilkinson D S, Fregert S, Magnusson B et al. Terminology of contact dermatitis. *Acta Derm Venereol* 1970: 50: 287–292.
29. De Groot A C, De Cock P A J J M, Coenraads P J et al. Methyldibromoglutaronitrile is an important contact allergen in The Netherlands. *Contact Dermatitis* 1996: 34: 118–120.
30. Tosti A, Vincenzi C, Trevisi P, Guerra L. Euxyl K 400: incidence of sensitization, patch test concentration and vehicle. *Contact Dermatitis* 1995: 33: 193–195.
31. Corazza M, Mantovani L, Roveggio C, Virgili A. Frequency of sensitization to Euxyl K 400 in 889 cases. *Contact Dermatitis* 1993: 28: 298–299.
32. De Groot A C, Bruynzeel D P, Coenraads P J et al. Frequency of allergic reactions to methyldibromoglutaronitrile (1,2-dibromo-2,4-dicyanobutane) in The Netherlands. *Contact Dermatitis* 1991: 25: 270–271.
33. Motolese A, Seidenari S, Truzzi M, Giannetti A. Frequency of contact sensitization to Euxyl K 400. *Contact Dermatitis* 1991: 25: 128.
34. Fuchs Th, Enders F, Przybilla B et al. Contact allergy to Euxyl K 400. *Dermatosen* 1991: 39: 151–153.
35. Bruze M, Johansen J D, Andersen K E et al. Deodorants: an experimental provocation study with cinnamic aldehyde. *J Am Acad Dermatol* 2003: 48: 194–200.
36. Andersen K E, Johansen J D, Bruze M et al. The time-dose–response relationship for elicitation of contact dermatitis in isoeugenol allergic individuals. *Toxicol Appl Pharmacol* 2001: 170: 166–171.
37. Tosti A, Vincenzi C, Smith K A. Provocative use testing of methyldibromoglutaronitrile in a cosmetic shampoo. *Contact Dermatitis* 2000: 42: 64–67.
38. Jensen C D, Johansen J D, Menné T, Andersen K E. Methyldibromoglutaronitrile in rinse-off products causes allergic contact dermatitis: an experimental study. *Br J Dermatol* 2004: 150: 90–95.

Address:
Birgitta Gruvberger
Department of Occupational and Environmental Dermatology
University Hospital
S-205 02 Malmö
Sweden
Tel: +46 40 33 17 59
Fax: +46 40 33 62 13
e-mail: birgitta.gruvberger@derm.mas.lu.se