



# LUND UNIVERSITY

## 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](https://doi.org/10.1111/j.0105-1873.2005.00481.x)

2005

[Link to publication](#)

### Citation for published version (APA):

Gruvberger, B., 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. (2005). Repeated open application test with methyldibromo glutaronitrile, a multicentre study within the EECDRG. *Contact Dermatitis*, 52(1), 19-23. <https://doi.org/10.1111/j.0105-1873.2005.00481.x>

Total number of authors:  
12

### General rights

Unless other specific re-use rights are stated the following general rights apply:  
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117  
221 00 Lund  
+46 46-222 00 00

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

B. GRUVBERGER<sup>1\*</sup>, K. E. ANDERSEN<sup>2</sup>, F. M. BRANDÃO<sup>3</sup>, D. P. BRUYNZEEL<sup>4</sup>, M. BRUZE<sup>1</sup>, P. J. FROSCH<sup>5</sup>, A. GOOSSENS<sup>6</sup>, A. LAHTI<sup>7</sup>, H. I. MAIBACH<sup>8</sup>, T. MENNÉ<sup>9</sup>, D. ORTON<sup>10\*</sup> AND S. SEIDENARI<sup>11</sup>

<sup>1</sup>Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, Sweden, <sup>2</sup>Department of Dermatology, Odense University Hospital, Odense, Denmark, <sup>3</sup>Department of Dermatology, Hospital Garcia de Orta, Alameda, Portugal, <sup>4</sup>Department of Dermatology, Free University Hospital, Amsterdam, The Netherlands, <sup>5</sup>Department of Dermatology, Klinikum Dortmund and University of Witten/Herdecke, Dortmund, Germany, <sup>6</sup>Department of Dermatology, University Hospital, K.U. Leuven, Belgium <sup>7</sup>Department of Dermatology, University of Oulu, Oulu, Finland, <sup>8</sup>Department of Dermatology, UCSF, School of Medicine, San Francisco, CA, USA, <sup>9</sup>Department of Dermatology, Gentofte Hospital, Copenhagen, Denmark, <sup>10</sup>Department of Dermatology, Amersham General Hospital, Bucks, UK, and <sup>11</sup>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 PTSL 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. © Blackwell Munksgaard, 2005.

*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  $5 \times 5$  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<sup>2</sup> =  $1.4$  mg/cm<sup>2</sup>). 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



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 years 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 (methylidibromoglutaronitrile). *Br J Dermatol* 1995; 132: 918–920.
4. De Groot A C, van Ginkel C J W, Weijland J W. Methylidibromoglutaronitrile (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 Venerol* 1996; 123: 322–324.
6. Jackson J M, Fowler J F. Methylidibromoglutaronitrile (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 methylidibromoglutaronitrile. *Contact Dermatitis* 2000; 42: 54–55.
8. Guimarães 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 (Hirschtalcreme). *Contact Dermatitis* 2001; 44: 58.
10. Fernández E, Navarro J A, Del Pozo L, Fernández de Corrés 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 methylidibromoglutaronitrile 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 methylidibromo glutaronitrile in Euxyl K 400 in an ultrasonic gel. *Contact Dermatitis* 2001; 44: 39–40.
14. Gebhart M, Stuhler A, Knopf B. Allergic contact dermatitis due to Euxyl<sup>®</sup> 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 methylidibromo 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 methylidibromo 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<sup>®</sup> (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<sup>®</sup>) 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, Roveglio 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