

Methyldibromoglutaronitrile allergy: relationship between patch test and repeated open application test thresholds

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Summary

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Conflicts of interest

None declared.

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Background Methyldibromoglutaronitrile (MDBGN) is a preservative, which was approved for use in cosmetics in the mid-1980s. The incidence of allergy to MDBGN rose during the 1990s, but is now decreasing due to regulatory intervention. Experimental studies with other allergens have shown a significant relationship between the patch test and the repeated open application test (ROAT) reactivity.

Objectives To study the relationship between elicitation threshold doses at single occluded exposure and repeated open application, using MDBGN as the allergen.

Methods Eighteen subjects allergic to MDBGN were tested with a dilution series of MDBGN in a patch test and a ROAT (duration up to 21 days). Seventeen people with no MDBGN allergy were included as a control group for the ROAT.

Results The response frequency for the ROAT (in μg MDBGN cm^{-2} per application) was significantly higher than the response frequency for the patch test, while the response frequency for the accumulated ROAT dose, at 1, 2 and 3 weeks was very similar to the patch test response frequency; indeed there was no statistical significant difference.

Conclusions For elicitation of MDBGN allergy the response frequency for the patch test is lower than the response frequency (per application) for the ROAT, but approximately the same as the response frequency for the accumulated ROAT doses. This is important for risk assessment in general.

Methyldibromoglutaronitrile (MDBGN) is a preservative, which was approved for use in cosmetics in the mid-1980s at a maximum concentration of 0.1% in both leave-on and rinse-off products and a maximum of 0.025% in sunscreen products.¹ The incidence of sensitization to MDBGN increased in Europe from 0.7% positive patch tests among patients in 1991 to 3.5% in 2000.² This increasing incidence of MDBGN allergy led to prohibition of MDBGN in leave-on cosmetic products in 2002,³ and in 2006 it was decided that MDBGN should also be prohibited in rinse-off cosmetic products.⁴ In Denmark the incidence of MDBGN allergy among patients with eczema in 2003 was 4.6% and decreased to 2.6% in 2007 due to regulatory intervention.⁵ Several animal and human studies failed to demonstrate the sensitizing potential of MDBGN,^{6,7} and Menné and Wahlberg⁸ suggested that 'new chemicals in cosmetics should not be permanently approved until a sufficient database on human clinical endpoint parameters for the final risk assessment and risk management has been established'. In this context, dose-response elicitation data play a central role, and a standardization of protocols as

well as interpretation of data is desirable. A core issue is the transformation of patch test data into more realistic exposure situations. Several dose-response studies⁹⁻¹⁶ performed with different allergens in patients with contact allergies have shown a significant relationship between the patch test elicitation threshold and the repeated open application test (ROAT) elicitation threshold.

The aim of the present study was to investigate further the relationship between the elicitation threshold doses at a single occluded exposure and repeated open applications, using MDBGN as the allergen.

Materials and methods

Test subjects

The inclusion criteria were: age over 18 years and a previous positive standard patch test to MDBGN, with a positive reaction defined by erythema and homogeneous infiltration in the whole test area. The exclusion criteria were: active eczema,

Table 1 The dilution series in the patch test and the equivalent exposure using the repeated open application test (ROAT)

Patch test dilution series (μg MDBGN cm^{-2})	Equivalent ROAT exposure (μg MDBGN cm^{-2})
150^a	Accumulated 3-week dose (3.57 per app.) in the ROAT
100	Accumulated 2-week dose (3.57 per app.) in the ROAT
50	Accumulated 1-week dose (3.57 per app.) in the ROAT
25	
15	Accumulated 3-week dose (0.357 per app.) in the ROAT
10	Accumulated 2-week dose (0.357 per app.) in the ROAT
5	Accumulated 1-week dose (0.357 per app.) in the ROAT
3.57	Highest dose per application in the ROAT (3.57)
1.5	Accumulated 3-week dose (0.0357 per app.) in the ROAT
1	Accumulated 2-week dose (0.0357 per app.) in the ROAT
0.5	Accumulated 1-week dose (0.0357 per app.) in the ROAT
0.357	Middle dose per application in the ROAT (0.357)
0.1786	
0.0893	
0.0357	Lowest dose per application in the ROAT (0.0357)
0.0179	
0.0089	
0.0045	
0.0022	

^aOnly patients 1–5 were tested with this dose. Bold values: corresponding to the dose per application, the accumulated 1-week, 2-week and 3-week doses in the ROAT. MDBGN, methyldibromoglutaronitrile; app., application.

systemic immunosuppressive therapy, ultraviolet light on the test area within 3 weeks of the study, pregnancy, breastfeeding and not being able to cooperate. Eighteen patients with contact allergy to MDBGN, demonstrated by a positive standard patch test (Finn Chamber) to MDBGN within the past 6 years at the Department of Dermatology, Gentofte Hospital, University of Copenhagen, Denmark, were enrolled in the study after oral and written consent. The age range of the test subjects was 24–63 years with a mean age of 44 years. Fifteen women and three men participated in the study.

Control subjects

Inclusion and exclusion criteria were the same as for the test subjects, except for the required negative patch test to MDBGN. Seventeen control subjects with no MDBGN allergy were enrolled in the study after oral and written consent. They were respondents to an advertisement displayed in the medical faculty, University of Copenhagen, Denmark. The control subjects were aged 19–69 years with a mean age of 27 years. Eleven women and six men participated as control subjects.

Study design and materials

The study was performed using the same methodology as a nickel dose–response study.¹⁷ The patch test material was MDBGN in 20% ethanol with 80% water and the control was 20% ethanol with 80% water. The MDBGN was purchased from Bie & Berntsen, Rødovre, Denmark. The two highest concentrations were heated in order to dissolve the MDBGN. Patch testing and ROAT were performed simultaneously on

the test subjects. The doses were chosen so that the dose per application in the ROAT was represented in the patch test. The accumulated 1-week, 2-week and 3-week doses from the ROAT were also represented in the patch test in order to facilitate a comparison of the threshold for the two test methods (Table 1). The placement of the different test concentrations in both tests were randomized and blinded for the investigator and the subjects. After termination of the study the randomization code was broken. The study was performed according to the Helsinki II declaration and the study was approved by the local Ethics Committee (the capital region of Denmark, KA04032).

Patch test

Each test subject was tested with the following dilution series (of MDBGN in 20% ethanol with 80% water; in μg MDBGN cm^{-2} skin): 100, 50, 25, 15, 10, 5, 3.57, 1.5, 1, 0.5, 0.357, 0.1786, 0.0893, 0.0357, 0.0179, 0.0089, 0.0045, 0.0022, and one blank containing 20% ethanol with 80% water. Subjects 1–5 were also tested with 150 μg MDBGN cm^{-2} . Fifteen microlitres of each dilution was applied on a filter paper in a small Finn Chamber of 0.5 cm^2 on Scanpore (Epitest Ltd, Oy, Finland).

The patch test was applied on day 0, occluded for 2 days and the reactions were read on day 2, 3 or 4 and day 7, but only the second reading was used for the statistical calculations. The reading scale for the patch test was based on the International Contact Dermatitis Research Group reading scale, further developed by Hindsén and Bruze¹⁸ in order to recognize smaller differences in the allergic reactions. The scale used was a modification of this scale to include weaker

reactions. The scale was as follows: 0 = no reaction; 1 = few papules with no erythema, no infiltration; 2 = faint erythema with no infiltration or papules; 3 = faint erythema with few papules and no homogeneous infiltration; 4 = erythema, homogeneous infiltration; 5 = erythema, infiltration and a few papules; 6 = erythema, infiltration and papules; 7 = erythema, infiltration, papules and a few vesicles; 8 = intensive erythema, infiltration and vesicles. The threshold concentration was defined as the weakest concentration giving a visible reaction on day 3 or 4 in a continuous line of patch test reactions, starting from the highest test concentration (150 or 100 $\mu\text{g MDBGN cm}^{-2}$).

Repeated open application test

Each test subject was tested on four areas on the volar aspect of the forearms on areas 3×3 cm. Twenty microlitres was applied twice daily using a micropipette (Acura 815, 20 μL ; Buch & Holm, Herlev, Denmark) with a fixed volume and four numbered bottles. Each number referred to a specific area. The solutions were spread out on the area with the tip of the pipette and allowed to dry by evaporation. The subjects were instructed thoroughly orally and manually in using the pipette and also received a written instruction. The areas were tested with a dose per application of 3.57, 0.357 or 0.0357 $\mu\text{g MDBGN cm}^{-2}$. These doses per application correspond to an accumulated 1-week dose of 50, 5 and 0.5 $\mu\text{g MDBGN cm}^{-2}$, respectively, an accumulated 2-week dose of 100, 10 and 1 $\mu\text{g MDBGN cm}^{-2}$ and an accumulated 3-week dose of 150, 15 and 1.5 $\mu\text{g MDBGN cm}^{-2}$, respectively. The fourth area was a control and was exposed to 20% ethanol with 80% water. The control subjects were tested on two areas with 0.0357 $\mu\text{g MDBGN cm}^{-2}$ and 20% ethanol, respectively. The reactions were read in all subjects at day 2, 3 or 4, 7, 14 and day 21 of exposure routinely and additionally if a reaction occurred. In the case of a score of 5 or above (based on the involved area, erythema, number of papules and number of vesicles), the exposure to that area was terminated, and if no reaction occurred, or in the case of a score below 5, the exposure was terminated on day 21. The same investigator carried out the reading of the reactions in all the subjects using a ROAT reading scale developed previously.¹⁹ The threshold concentration was defined as the weakest concentration giving a visible reaction, which was also present at the end of week 3 if the exposure had not been terminated, or the weakest concentration giving a reaction with a score of 5 or above.

Statistics

Standard logistic regression analysis was used to estimate the dose-response relationship in the patch test. The elicitation dose (ED_{xx}) which predicts a dose that will elicit a reaction in 1%, 5%, 10%, 25% and 50% of cases was calculated and a fitted dose-response curve for the patch test was drawn.

A comparison of elicitation reactions to the dose per application in the ROAT ($\mu\text{g MDBGN cm}^{-2}$) and the elicitation

reactions to the corresponding dose in the patch test ($\mu\text{g MDBGN cm}^{-2}$) was performed using McNemar's test. A fitted dose-response curve for the patch test and the dose-response curve for the ROAT, in dose per application, were calculated and drawn.

Furthermore, the elicitation reactions to the accumulated 1-week, 2-week and 3-week doses in the ROAT ($\mu\text{g MDBGN cm}^{-2}$) were compared with the elicitation reactions to the corresponding doses in the patch test ($\mu\text{g MDBGN cm}^{-2}$) also using McNemar's test. A fitted dose-response curve for the patch test and the 1-week accumulated, 2-week accumulated and 3-week accumulated dose-response curve for the ROAT was drawn.

When the patch test and the ROAT dose-response reactions were compared, only the results from the 16 subjects who participated in both tests were used.

Results

The patch test

Eighteen subjects allergic to MDBGN participated in the patch test. All the test subjects had a reaction to at least one of the patch test MDBGN concentrations (Table 2). The lowest threshold concentration observed was 0.0357 $\mu\text{g MDBGN cm}^{-2}$. One person had a weak reaction (score 1) to the ethanol control.

Table 2 Results of patch testing with methyl dibromoglutaronitrile (MDBGN) in all 18 test subjects (A) and in the 16 test subject who also performed the repeated open application test (ROAT; B)

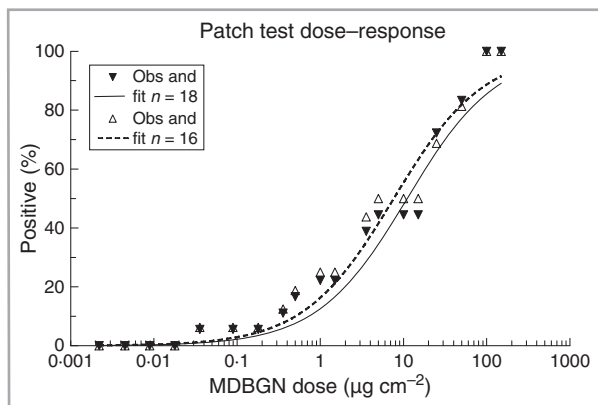
Patch test concentration ($\mu\text{g MDBGN cm}^{-2}$)	No. of test subjects reacting (A)	%	No. of test subjects reacting (B)	%
150^a	5	100	5	100
100^b	17	100	15	100
50	15	83.3	11	81.3
25	13	72.2	11	68.8
15	8	44.4	8	50
10	8	44.4	8	50
5	8	44.4	8	50
3.57	7	38.9	7	43.8
1.5	4	22.2	4	25
1	4	22.2	4	25
0.5	3	16.7	3	18.8
0.3570	2	11.1	2	12.5
0.1786	1	5.6	1	6.3
0.0893	1	5.6	1	6.3
0.0357	1	5.6	1	6.3
0.0179	0	0	0	0
0.0089	0	0	0	0
0.0045	0	0	0	0
0.0022	0	0	0	0

^aOnly five subjects were tested with the highest dose; ^bone person was not tested with this dose. Bold values: corresponding to the dose per application, the accumulated 1-week, 2-week and 3-week doses in the ROAT.

Table 3 Predicted doses (ED_{xx}) (μg MDBGN cm^{-2}) from the patch test with 95% confidence limits

Probability (%)	Patch test calculated dose-response		
	ED_{xx}	Upper limit	Lower limit
50	7.68	22.1	2.44
25	1.96	5.52	0.4
10	0.5	1.69	0.052
5	0.2	0.8	0.0125
1	0.025	0.164	0.00049

ED_{xx} , elicitation dose that predicts a dose that will elicit a reaction in 1%, 5%, 10%, 25% and 50% of cases; MDBGN, methyldibromoglutaronitrile.

**Fig 1.** Observed (Obs; black dots) and calculated logistic dose-response curve from the patch test for all 18 subjects participating in the patch test (solid line) and for the 16 subjects who also participated in the repeated open application test (broken line). MDBGN, methyldibromoglutaronitrile.

The threshold concentration for the patch test for this person was $25 \mu\text{g}$ MDBGN cm^{-2} . In one subject the patch test was removed after 24 h due to intense itching; the threshold concentration for this person was $3.57 \mu\text{g}$ MDBGN cm^{-2} .

The calculated dose with a response frequency of 50% (ED_{50}) was $7.68 \mu\text{g}$ MDBGN cm^{-2} , the ED_{10} was $0.5 \mu\text{g}$ MDBGN cm^{-2} and the ED_1 was $0.025 \mu\text{g}$ MDBGN cm^{-2} (Table 3). The fitted dose-response curve for all 18 test subjects and for the 16 test subjects who also participated in the ROAT is shown in Figure 1.

The repeated open application test

Eighteen subjects allergic to MDBGN were included in the ROAT, but within the first week two test subjects were excluded from the ROAT and only participated in the patch test part of the study because of relapse or worsening of eczema. Neither of the two subjects had a reaction to the ROAT prior to the

exclusion. Fourteen of the 16 test subjects had a reaction to the highest concentration within the 3 weeks of exposure, 10 out of 16 had a reaction to the middle concentration and five of 16 had a reaction to the lowest concentration. The test subject who had the patch test removed after 24 h had a severe allergic reaction to the highest concentration after 5 days, and stopped participating in the ROAT after 8 days. Another of the test subjects had a reaction to the ethanol control site (score 2) with four papules on day 14 and two papules on day 21. One of the control subjects had a reaction (score 3) with erythema on day 16 at the site exposed to MDBGN in the ROAT and the exposure was terminated.

Comparison of the patch test and the repeated open application test

The response frequencies of the dose per application in the ROAT were compared with the response frequencies to the same dose in the patch test (Table 4). When $3.57 \mu\text{g}$ MDBGN cm^{-2} was applied per application in the ROAT, 14 out of 16 (88%) had a reaction within the 3 weeks of exposure. In the patch test only seven of 16 (44%) had a reaction to this dose. This difference in response frequencies was statistically significant ($P = 0.016$). When $0.357 \mu\text{g}$ MDBGN cm^{-2} was applied per application in the ROAT 10 of 16 (62%) had a response within the 3 weeks of exposure, whereas in the patch test only two of 16 (12%) had a reaction; this difference was also statistically significant ($P = 0.0078$). When $0.0357 \mu\text{g}$ MDBGN cm^{-2} was applied per application in the ROAT five of 15 (33%) had a reaction, and in the patch test one of 15 (7%) had a reaction, this difference was not statistically significant. These results are illustrated by Figure 2, where the ROAT dose-response curve is displaced to the left compared with the patch test dose-response curve, which means that the response frequency within 3 weeks was higher in the ROAT (to the dose per application).

For each week a curve for the ROAT response was drawn using the response frequency from the accumulated weekly doses for the three different concentrations. These dose responses were compared with the equivalent doses in the patch test and no significant differences between the response frequencies for the ROAT and the patch test were found.

Table 4 The response frequency in the patch test compared with the response status in the repeated open application test (ROAT) (the dose per application) after 3 weeks

Dose (μg MDBGN cm^{-2})	Patch test:		P-value (McNemar's test)
	response frequency (%)	ROAT: response frequency after 3 weeks (%)	
3.57	43.8	87.5	0.016
0.357	12.5	62.5	0.0078
0.0357	6.7	33.3	0.13

MDBGN, methyldibromoglutaronitrile.

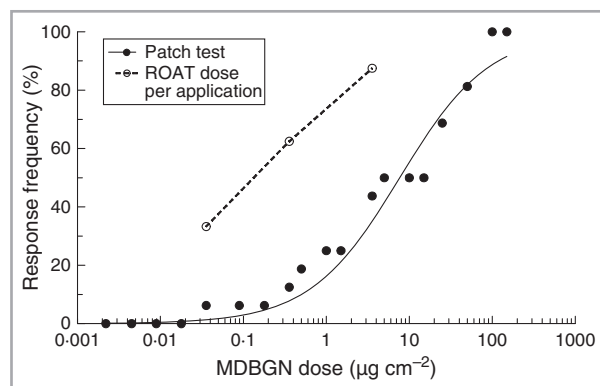


Fig 2. The fitted dose-response curve for the patch test ($n = 16$; solid line) and the observed response after 3 weeks of exposure to the dose per application in the repeated open application test (ROAT; broken line). MDBGN, methyl dibromoglutaronitrile.

Table 5 The response frequency in the patch test compared with the response status in the repeated open application test (ROAT) (the accumulated dose after 1, 2 and 3 weeks)

Dose (μg MDBGN cm^{-2})	Patch test: response frequency (%)	ROAT: response frequency (%)	P-value (McNemar's test)
0.5	20	20 ^a	0.5
1	26.6	33.3 ^b	0.5
1.5	26.6	33.3 ^c	0.5
5	50	37.5 ^a	0.5
10	50	50 ^b	0.5
15	50	62.5 ^c	0.5
50	81.3	56.3 ^a	0.13
100	100	87.5 ^b	0.5
150	100	87.5 ^c	0.5

^aAccumulated 1-week dose; ^baccumulated 2-week dose; ^caccumulated 3-week dose. MDBGN, methyl dibromoglutaronitrile.

(Table 5). This is well illustrated by Figure 3 where it is seen that the ROAT curves (accumulated dose weeks 1, 2 and 3) lie close to the patch test curve, meaning that the response frequency to the accumulated 1-, 2- and 3-week doses in the ROAT are similar to the patch test dose responses.

Discussion

Under standardized conditions it was shown that the ROAT response rate in terms of dose per application was significantly higher for the two highest concentrations compared with the same dose in the patch test. This means that even though a certain dose in a patch test is negative, an allergic person might react to this dose (per unit area per application) in a ROAT, as illustrated by Figure 2. Furthermore, we found that the dose response for the patch test and the dose response for the accumulated doses in the ROAT were not significantly

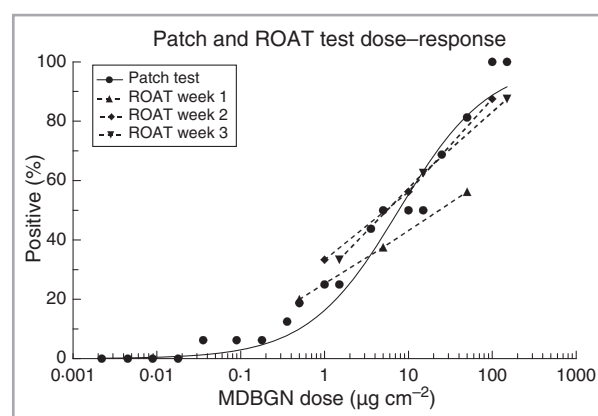


Fig 3. The fitted dose-response curve for the patch test ($n = 16$) and the accumulated 1-week, 2-week and 3-week repeated open application test (ROAT) doses. MDBGN, methyl dibromoglutaronitrile.

different, as illustrated in Figure 3. This means that the dose response for the patch test and for the ROAT (in accumulated dose per unit area) was approximately the same. These results correspond well with the results from another MDBGN study, which found approximately the same response frequency if the test subjects were exposed to a ROAT of 0.01% MDBGN four times a day or 0.04% MDBGN once a day.²⁰ Therefore, the accumulated dose was the same and so was the eliciting response. Another study with MDBGN²¹ investigating the patch test response to 1%, 0.5%, 0.3% and 0.1% showed that some of the subjects who had a positive ROAT to a moisturizer containing 0.03% MDBGN had a doubtful or positive patch test reaction to only the highest doses, which means that the allergic individuals had a reaction to a ROAT concentration (%) which would have been negative in the patch test (%), which is also similar to the results from the present study (although in our study we focused on the concentration in $\mu\text{g cm}^{-2}$).

Studies with *para*-phenylenediamine (PPD) also investigated the effect of accumulated exposure. One study²² investigated the sensitization rate to PPD after hair dyeing with 0.5% PPD for 5 min once a week for 6 months or 1.5% PPD for 30–40 min once a month for 6 months. The group exposed more often for a shorter period of time (5 min) to the low concentration of PPD had a significantly higher sensitization rate to PPD than the group which was exposed fewer times for a longer period (30 min) with the high concentration. Thus, the more frequent stimulation to low concentrations of PPD gave a stronger immunological response than the higher concentration applied less frequently. In another study with PPD,²³ the percentage of subjects responding in a ROAT after 6 days to 1% PPD applied 5 min a day, was comparable to the percentage responding to a 30-min single exposure patch test with 1% PPD. Thus, the accumulated exposure over time was important for the response frequency. The same result was found in another study²⁴ showing that intermittent exposure to a low concentration of PPD was equivalent to single, higher-dose exposure in most cases. In that same

study, a skin-binding experiment in rats was performed using ^{14}C -PPD, which showed that PPD accumulated in the skin when repeated exposures were performed.

Further, the current study was performed with the same methodology as a nickel dose-response study,¹⁷ and the results from the two studies are comparable.

From the present investigation, the nickel dose-response study and the above-mentioned studies with PPD, evidence is provided that elicitation is not only dependent on the allergen dose per application, but also on the frequency of exposure.

The ROAT threshold in dose per area per application of MDBGN is significantly lower than the patch test threshold for MDBGN. However, the patch test threshold and the accumulated ROAT thresholds are closely related. The patch test is an easy test to perform whereas the ROAT (which mimics real-life exposure) is much more time-consuming. If a parallel could be drawn between these two test methods, it would give us an instrument that could be used to translate the thresholds found in a patch test to ROAT thresholds. This instrument would make it possible to use patch test data from presensitized individuals and translate these data into noneliciting doses in real-life exposure situations, taking the frequency of exposure into account. This could be used as basis of preventive strategies.

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