

Quantitative patch and repeated open application testing in methyldibromo glutaronitrile-sensitive patients

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Contact allergy to methyldibromo glutaronitrile (MDBGN), often combined with phenoxyethanol (PE) (e.g., Euxyl K 400[®]), increased throughout the 1990s in Europe. Consequently, in 2003, the European Commission banned its use in leave-on products, where its use concentration was considered too high and the non-sensitizing use concentration as yet unknown. The 2 objectives of the study are (a) to find a maximum non-eliciting concentration in a leave-on product in MDBGN/PE-sensitized patients, which could possibly also be considered safe regarding induction and (b) to find the best patch test concentration for MDBGN. We, therefore, performed a use-related test (ROAT) in patients sensitized to MDBGN/PE ($n = 39$) with 3 concentrations of MDBGN/PE (50, 100 and 250 p.p.m. MDBGN, respectively). A subset of these patients ($n = 24$) was later patch-tested with various concentrations (0.1, 0.2, 0.3 and 0.5% MDBGN, respectively). 15 patients (38%, 95% confidence interval (CI) = 23–55%) had a negative and 24 (62%; 95% CI = 45–77%) a positive overall repeated open application test (ROAT) result. 13 reacted to the lowest (50 p.p.m.), 8 to the middle (100 p.p.m.) and 3 to the highest concentration (250 p.p.m.) only. In those 13 reacting to the lowest ROAT concentration, dermatitis developed within a few days (1–7). The strength of the initial and the confirmatory patch test result, respectively, and the outcome of the ROAT were positively associated. Of the 24 patients with a use and confirmatory patch test, 15 reacted to 0.1% MDBGN, 16 to 0.2%, 17 to 0.3% and 22 to 0.5%. With the patch test concentration of 0.5%, the number of ROAT-negative patients but patch-test-positive patients increases considerably, particularly due to + reactions. A maximum sensitivity of 94% (95% CI = 70–100%) is reached with a patch test concentration of 0.2%, and is not further improved by increasing the concentration. However, the specificity decreases dramatically from 88 (95% CI = 47–100%) with 0.2% to a mere 12.5% (95% CI = 0–53%) with 0.5%. It can be concluded (a) that for MDBGN 0.2% is very likely the best patch test concentration and (b) that 50 p.p.m. in a leave-on product can elicit contact dermatitis in sensitized persons. We were, therefore, unable to find a safe, still microbicidal, concentration for leave-on products. By contrast, with other contact allergens, dose-response use tests may be able to identify a non-eliciting concentration, which could give valuable clues to a non-inducing (i.e., safe) concentration in products.

Key words: leave-on cosmetics; methyldibromo glutaronitrile; patch testing technique; ROAT; use test. © Blackwell Munksgaard, 2005.

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Methyldibromo glutaronitrile (MDBGN) was introduced in the 1980s, often used in combination with phenoxyethanol (PE) (1 : 4), as a preservative in cosmetics, household products and industrial products. Phenoxyethanol is con-

sidered to be a very infrequent contact allergen (1). However, although first predictive tests with MDBGN in animals were negative (2, 3), contact allergies to the biocide combination (e.g., Euxyl K 400[®]) or to MDBGN alone were reported to

increase throughout the 1990s in Germany (4, 5) and in other parts of Europe (6–8), culminating in frequencies of sensitization of more than 3% by the end of the decade (9–11). Based on a model derived from clinical epidemiology and drug utilization research, we estimated the total number of people sensitized during the 90s at more than half a million in Germany alone (12). Consequently, in 2003, the European Commission banned the use of MDBGN in leave-on products, limiting its use to rinse-off products (13). The decision was founded on the epidemiological figures quoted, and was supported by risk-assessment calculations (14), finding that the approved (0.1%) and actually recommended (15) concentrations (up to 0.04%) of MDBGN were too high (leaving apart the possibility that recommendations were not always respected) and that the level of a safe, non-inducing, albeit sufficiently microbicidal use, concentration was as yet unknown.

From this background, the present study addresses 2 objectives:

- To find a maximum non-eliciting concentration used in a leave-on product in MDBGN/PE-sensitized patients. Such a safe, in terms of non-eliciting, concentration in leave-on cosmetics would protect people already sensitized. Moreover, this concentration might be a starting point, from which to derive a non-inducing concentration in humans. We, therefore, conceived a use-related test (ROAT) with 3 concentrations of MDBGN/PE (50, 100 and 250 p.p.m. MDBGN, respectively). The concentration of 50 p.p.m. (0.005%) MDBGN in MDBGN/PE was chosen because (a) it is the lowest active biocidal concentration according to one manufacturer and (b) we were unaware of patients elicited by such a low concentration.
- To find the best patch test concentration for MDBGN. Although MDBGN (alone or in combination with PE) has been patch-tested for over 15 years, there is still controversy among dermatologists with regard to the most suitable concentration (9, 16, 17). Therefore, we performed patch testing with various concentrations (0.1, 0.2, 0.3 and 0.5% MDBGN, respectively) in patients who underwent a ROAT with MDBGN/PE. The ROAT outcome served as an external criterion (gold standard) for evaluating the different patch test preparations.

Patients and Methods

Patients

Study patients ($n=40$; 30 women and 10 men; median age: 49.9 years; range: 19–72) were enrolled

at 10 centres of the IVDK (18). Inclusion criteria were at least a + reaction to MDBGN/PE at reading D3 on routine patch testing, age >18 years and informed consent. Exclusion criteria were systemic or local immunosuppressant treatment, immunocompromising disease, florid eczema or recent UV exposure in the test area and pregnancy.

The study was approved by the Ethics Committee of the University of Göttingen, Göttingen, Germany.

Use test

Ointments containing 10–15% glyceryl stearate in water as vehicle, and the test substance Euxyl K 400[®] (MDBGN/PE) in 3 concentrations, namely 0.025, 0.05 and 0.125%, equivalent to 0.005, 0.01 and 0.025% (50, 100 and 250 p.p.m.) of MDBGN. The vehicle without Euxyl K 400[®] served as a negative control. The products were prepared by Schülke & Mayr, Norderstedt, Germany, and were supplied in 10-cm³ syringes.

A repeated open application test (ROAT) (19) was performed on 5×5 -cm² areas on the volar side of each forearm. Each test area was assigned to one of the test preparations (marked in red) or the control preparation (blue). The participants were equipped with 2 preparations (one with, one without Euxyl K 400[®]) and were instructed to apply 2 times daily (morning and evening) each preparation in a suitable dose (approximately 0.5 ml) for at least 1 min. Patients were advised to avoid contact with water for the following 2 hr. If a reaction had occurred in a test area, further exposure was to be stopped.

The application of the same preparations was continued for 2 weeks. If there was no reaction after 2 weeks, the participants were supplied with preparations at the next higher concentration to be used for another 2 weeks. The preparation with the highest concentration was applied for the last 2 weeks, if there had been no reaction until the end of the preceding 4 weeks (Fig. 1).

The ROAT was evaluated at least weekly (Fig. 1), or earlier at the request of the patient, with the assessment of the following morphological features: erythema, papules/infiltration, vesicles and extent of area involved. The readings were classified according to the recommendations of Johansen et al. (20, 21). The ROAT was considered as positive, if there was (spotty) erythema of at least 25% of the test area and homogeneous infiltration or papules regardless of the number (21, 22).

Patch testing

At the end of the ROAT, patients were patch-tested. Patch tests were performed in accordance

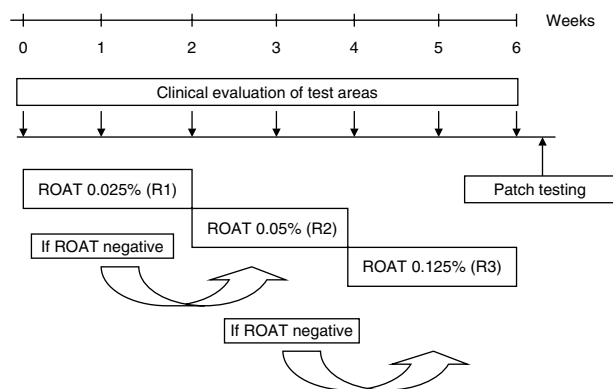


Fig. 1. Study design. R1–R3: preparations with MDBGN/PE (Euxyl K 400[®]), containing 50, 100 and 250 p.p.m. MDBGN. Patch testing is performed with 4 patch test preparations containing MDBGN alone in various concentrations (0.1, 0.2, 0.3 and 0.5%). MDBGN, methyldibromo glutaronitrile; PE, phenoxyethanol; ROAT, repeated open application test.

with the guidelines of the German Contact Dermatitis Research Group (DKG) (23) and current international recommendations (24). The 4 patch test preparations with different concentrations of MDBGN (0.1, 0.2, 0.3 and 0.5%, each in petrolatum) were kindly supplied by Hermal/Trolab, Reinbek, Germany, and were applied to the back for 1 day (2 departments) or 2 days (8 departments). Readings were performed until at least 3 days by using the following grading: negative, ?, +, ++, +++, irritant and follicular.

Statistical calculations

The sample size was calculated in advance based on the binomial test with the following premises: a use preparation was defined as 'safe', if not more than 10% reacted to the product. With this one-sided testing, a probability of error $\alpha = 0.05$ and an expected rate set at 4%, 107 cases (with ≤ 4 events) would be necessary to detect this difference as statistically significant with a power of 80%. In addition to this one confirmative analysis, 2 interim analyses regarding safety (i.e., a possible excess of positive ROAT reactions $>10\%$), as well as further explorative analyses, were scheduled.

For the descriptive presentation and statistical analysis of the data, current guidelines (25) were used. Exact confidence intervals (CI) to proportions, based on the binomial distribution, were calculated. The associations between ROAT outcome and initial and confirmatory patch test results of different concentrations have been presented as odds ratios (OR) accompanied by exact 95% CI.

The diagnostic quality of the different patch test preparations was assessed by calculating sensitivity, specificity and positive predictive value (PPV), with the ROAT outcome as gold standard (25).

In order to compare the pattern of allergic reactions to the different patch test concentrations, we calculated the positivity ratio (PR), which relates the proportion of + reactions to the total number of allergic reactions, + to +++ (26).

For this study, we adopted the design used by Svedman et al. (27). Data analysis was performed with the statistical software package SASTM (version 8.2, SAS Institute, Cary, NC, USA).

Results

After the inclusion of 40 patients, the first interim analysis showed a frequency of 13/39 reactions to the lowest ROAT concentration (33.3%; 95% CI = 19.1–50.2%). As this unexpectedly exceeded the 10% acceptability threshold, and as this excess was already significant (10% not included in the above CI), no further patients were included and the study was terminated. One patient was lost in the course of the ROAT because of a surgical intervention unrelated to the study. Therefore, the following analyses were based on 39 patients.

Threshold ROAT results

Regarding the main objective of the study – to establish a use concentration in leave-on products, which is 'safe' for the MDBGN-sensitized – it is evident that this is impossible even with the lowest concentration with sufficient microbicidal activity (50 p.p.m. MDBGN).

Of the 39 patients with a positive MDBGN/PE pre-trial patch test result (for reaction strength, see Table 2), 15 patients (38%, 95% CI = 23–55%) had a negative and 24 patients (62%; 95% CI = 45–77%) a positive overall ROAT result. 13 patients reacted to the lowest concentration R1 (50 p.p.m.), 8 to the middle concentration R2 (100 p.p.m.) after not being positive to the lowest concentration and 3 to the highest concentration R3 (250 p.p.m.) only. Reactions to the control preparation were not observed. The area involved was 90–100% in 8, 50–89% in 12 and 25–49% in 4. Infiltration was homogeneous in 3, many (>10) papules were observed in 14, some (5–10) papules in 5 and few (<5) papules in 2. One patient produced a few vesicles. From those 13 reacting to the lowest concentration, dermatitis developed after a very short exposure: within 1 day in 2 patients, 2

days (3 times), 3 days (1 time), 4 days (1 time), 6 days (1 time) and 7 days (4 times). In only 1 case was the dermatitis noticed after the full exposure duration of 14 days.

The strength of the initial patch test result and the outcome of the ROAT (Tables 1 and 2) were associated: with stronger patch test reactions (+++/+++), the odds of a positive ROAT to any concentration are increased (OR = 2.9; 95% CI = 0.54–19.4), compared to initial + reactions. This association is most marked regarding a positive reaction to the lowest ROAT concentration (OR = 3.9; 95% CI = 0.75–20.3). In all, an initial + reaction was confirmed by a positive ROAT result in 54% (95% CI = 33–77%), and the stronger reactions in 77% (95% CI = 46–95%).

Threshold patch test results

Confirmatory patch testing was performed in 24 patients, as in 3 departments patients were not available for this purpose. The patients who were not patch-tested after ROAT did not differ significantly from the patients tested with regard to sex, strength of the initial patch test and ROAT outcome (data not shown), while age was somewhat higher. Therefore, confounding of the relationship between confirmatory patch test and ROAT results seems unlikely. Further analysis is based on these 24 patients. 15 patients reacted positively to 0.1, 16–0.2, 17–0.3 and 22–0.5% (Table 3). The reaction pattern is presented in Table 3. The PR decreases with increasing patch test concentration, i.e., the proportion of + reactions decreases to a minimum of 24% with 0.3% MDBGN patch test concentration (Table 3, Fig. 2).

Of the 24 patients, 16 exhibited a positive ROAT (1 of these 16 ROAT-positive patients was patch-test-negative), 7 had a negative ROAT, but a positive patch test, and 1 had a doubtful patch test and a negative ROAT. The association between the outcome of the ROAT and the reactions to the single patch test concen-

Table 2. Association between the strength of patch test reaction to screening Euxyl K 400[®] and positive response to any ROAT (R1–R3, 50, 100 and 250 p.p.m.) (OR = 2.9; exact 95% CI = 0.54–19.4), i.e., non-significant

		ROAT-positive	ROAT-negative	Total
Initial patch test	+	14	12	26
	++/+++	10	3	13
		24	15	39

ROAT, repeated open application test.

trations is presented in Fig. 2 and in Table 4. It is clear that, with the patch test concentration of 0.5%, the number of ROAT-negative but patch-test-positive patients increases considerably, particularly due to + reactions.

The visual impression of differences in the diagnostic validity of the different patch test concentrations is also expressed numerically by calculating the sensitivity and specificity (Table 5). A maximum sensitivity of 94% (95% CI = 70–100%) is reached with a concentration of 0.2%, and is not further improved by increasing the concentration. However, the specificity decreases dramatically from 88% (95% CI = 47–100%) with 0.2% to a mere 12.5% (95% CI = 0–53%) with 0.5%. From these figures, it can be concluded that for MDBGN 0.2% is very probably the best patch test concentration.

The PPV of the initial screening test with MDBGN/PE 1% with regard to the ROAT outcome (any concentration) was 24/39 (62%, 95% CI = 45–77%), whereas the PPV of a subsequent patch test with 0.2% MDBGN, i.e., the very concentration of MDBGN in the initial test preparation with PE, with regard to the ROAT outcome was 16/24 (67%, 95% CI = 45–84%). However, these values are not directly comparable because of a much lower *a priori* likelihood of a positive test and, accordingly, a higher proportion of initially false-positive reactions with the initial (standard series screening) test, compared to the later confirmative patch test, according to Bayes' formula (25). The notion of false-positive reactions is supported by a comparison of test/retest results in ROAT-positive versus ROAT-negative patients: while in ROAT-negative patients all 6 initial + reactions were not confirmed with 0.2% MDBGN upon retesting, and only 1 of 2 initial stronger positive reactions, all but one initial + reaction was confirmed in the 16 ROAT-positive patients retested. The strength of reactions was the same in 12/16 patients, whereas it increased in 2 and decreased in 1 (non-significant, exact McNemar test).

Table 1. Association between the strength of patch test reaction to screening Euxyl K 400[®] and positive response to ROAT R1 (lowest concentration 50 p.p.m.) (OR = 3.9; exact 95% CI = 0.75–20.3), i.e., non-significant

		ROAT R1-positive	ROAT R1-negative	Total
Initial patch test	+	6	20	26
	++/+++	7	6	13
		13	26	39

ROAT, repeated open application test.

Table 3. Pattern of the strength of allergic reactions in various patch test concentrations and their positivity ratio (PR) as the proportion of the number of + reactions to total allergic reactions

Patch test	Number of patients tested	Negative	?	+	++	+++	PR (%)
Euxyl K 400 [®] pre-test	40	0	0	26	13	1	67
MDBGN 0.1	24	8	1	8	6	1	53
MDBGN 0.2	24	7	1	7	5	4	44
MDBGN 0.3	24	5	2	4	6	7	24
MDBGN 0.5	24	1	1	7	7	8	32

MDBGN, methyldibromo glutaronitrile.

Finally, there is a strong association between the strength of patch test reactions to MDBGN 0.2% and a positive response to a ROAT with the lowest concentration (50 p.p.m.): while only 1/7 with a + reaction to the 0.2% patch test had a positive ROAT reaction to 50 p.p.m., this proportion was 8/9 in patients with ++ or +++ patch test reactions (OR = 48, 95% CI = 1.8–2469).

Discussion

With the present study, we attempted to identify a use concentration for MDBGN in leave-on products, which was safe in terms of rarely eliciting contact dermatitis in sensitized subjects. This attempt definitely failed, because there is no such concentration, which, at the same time, still offers sufficient microbicidal activity. However, the present study (a) does provide a starting point for discussing the issue of a safe use con-

centration of MDBGN and (b) allows evaluation of the method of diagnostic patch testing with MDBGN. One drawback of the premature termination of the study, which we considered mandatory for ethical reasons, is that the sample size remained at a relatively small number of 39 evaluable subjects, rendering estimates of proportions or effects somewhat imprecise.

The issues to be discussed refer, therefore, to the questions of (a) a safe concentration in leave-on products and (b) the diagnosis of sensitization to MDBGN.

Use-related test and the question of a safe concentration in leave-on products

Use tests have been done with rinse-off products containing MDBGN (shampoos and soaps) in patients sensitized to MDBGN. It was shown that shampoos with 0.02% (200 p.p.m.) MDBGN were tolerated by such patients ($n = 12$), when used for a period of 9–13 weeks (28). In another study, a double-blind use test (ROAT) with 2 liquid soaps was performed, one containing MDBGN with the maximum permitted level in rinse-off products (0.1%, i.e., 1000 p.p.m.). 19 MDBGN-allergic patients and 9 controls washed an area of 50 cm² on their lower arm 2 times daily for 28 days. 7 pre-sensitized individuals (37%) developed allergic contact dermatitis, but none of the controls (22). In another study, a causal role of MDBGN-containing rinse-off products was assumed from the fact that MDBGN-positive patients with allergic contact dermatitis had used such products (liquid soaps and shampoos). However, shampoo exposure containing 78–79 p.p.m. was not considered relevant (14). Further cases of allergic contact dermatitis associated with the use of rinse-off products have been reported (6, 29). It can be concluded that, with regard to elicitation, the safe concentration of MDBGN in rinse-off products may lie between 200 and 1000 p.p.m., and probably closer to 200 p.p.m. The actual permitted maximum concentration of

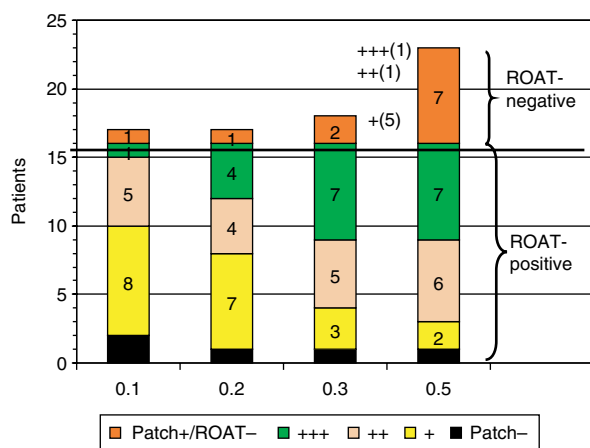


Fig. 2. Patch test reaction pattern of MDBGN preparations with various concentrations (0.1, 0.2, 0.3 and 0.5%) in ROAT-positive patients and patch test positives in ROAT-negative patients. The positivity ratio (PR), i.e., the proportion of + reactions to all allergic reactions, decreases with increasing concentration (Table 3). MDBGN, methyldibromo glutaronitrile; ROAT, repeated open application test.

Table 4. Association between the ROAT outcome and the reaction pattern of patch testing with various concentrations of MDBGN

	<i>n</i>	Patch test concentration			
		0.1%	0.2%	0.3%	0.5%
ROAT (50 p.p.m.)-positive	1	+++	+++	+++	+++
	3	++	+++	+++	+++
	2	++	++	+++	+++
	1	+	++	+++	+++
	1	+	++	++	++
	1	+	+	+	+
ROAT (100 p.p.m.)-positive	3	+	+	++	++
	1	+	+	+	++
	1	?	+	+	+
	1	Negative	Negative	Negative	Negative
ROAT (250 p.p.m.)-positive	1	+	+	++	++
ROAT (250 p.p.m.)-negative	1	++	++	++	+++
	1	Negative	?	?	?
	1	Negative	Negative	+	+
	1	Negative	Negative	?	+
	1	Negative	Negative	Negative	++
	3	Negative	Negative	Negative	+
Total	24				

15 patients reacted to 0.1%, 16 to 0.2%, 17 to 0.3% and 22 to 0.5%.

MDBGN, methyldibromo glutaronitrile; ROAT, repeated open application test.

1000 p.p.m. is definitely too high. A dose-finding use-test study should be envisaged.

By contrast, use tests with MDBGN-containing leave-on products have rarely been reported. Tosti et al. performed a use test with MDBGN/PE (0.1%, equivalent to 200 p.p.m. MDBGN) – containing skin care lotions in 11 patients sensitized to MDBGN/PE. 5 of these 11 patients reacted to the provocative use test (6). Analyses of leave-on products, which were considered to be relevant in MDBGN-allergic patients, contained 87 (1 product) to 399 p.p.m., with 8 products clearly >100 p.p.m. (14). Hence, a concentration of 50 p.p.m., as chosen in our study, seemed to offer a chance of being tolerable to sensitized persons. Only after the start of this study, did we learn from a presentation given at the ESCD Congress in Copenhagen, Denmark, June 2004, that a use test had been performed in 18 MDBGN-allergic volunteers over 14 days with moisturizers containing MDBGN in a concentration of 50 and 100 p.p.m. 10/18 developed a positive reaction to 50 p.p.m. moisturizers and 1 further individual reacted to a preparation with 100 p.p.m. (30). Our results confirm this study, showing that 50 p.p.m. MDBGN in a leave-on product can elicit an allergic reaction in a considerable number of sensitized individuals. However, as in other studies, e.g., with isoeugenol (31), cinnamic aldehyde (32) and chloroatranol (33), the notion of variable individual susceptibility is confirmed, as in the subgroup of ROAT-positive patients ($n = 24$), 54% (13/24) reacting already to

50 p.p.m., the majority after a very short exposure of few days, 33% (8/33) only to 100 p.p.m., and 13% (3/24) only to 250 p.p.m. and after a prolonged exposure time (4–6 weeks), which may additionally be a critical factor for elicitation in less susceptible individuals (34). Furthermore, there was a strong association between the reaction to the low (50 p.p.m.) ROAT preparation and the strength of the initial patch test reaction (OR = 3.9, 95% CI = 0.8–20) or the confirmatory patch test (OR = 48, 95% CI = 1.8–2469). Such associations between patch test results and ROAT results were also reported in other use-test studies (31).

Concentration of MDBGN in diagnostic patch testing

There is still controversy about the best patch test concentration of MDBGN, which has often been tested in petrolatum. Some centres used 0.5% MDBGN/PE (i.e., 0.1% MDBGN), and later 1.0% (0.2% MDBGN) (9), others 2% (i.e., 0.4% MDBGN) (8, 35) or 2.5% (6, 36). Some prefer testing MDBGN alone with 0.3% to the combination MDBGN/PE 0.5% (0.1% MDBGN) (37, 38). Whereas the German Contact Dermatitis Research Group (DKG) continued to favour 1% MDBGN/PE, based on a comparative study (9), many centres currently use 0.3% MDBGN (37, 38), 0.4% (17) and even 0.5% (39). This long-lasting uncertainty regarding the optimum screening patch test preparation

Table 5. Sensitivity and specificity of patch test preparations with various concentrations by using the ROAT result as gold standard

Patch test	0.1%		0.2%		0.3%		0.5%	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
ROAT-positive (<i>n</i> = 16)	14	2	15	1	15	1	15	1
ROAT-negative (<i>n</i> = 8)	1	7	1	7	2	6	7	1
Total	15	9	16	8	17	7	22	2
Sensitivity (%)	87.5 (95% CI = 61.6–98.4)		93.8 (95% CI = 69.8–99.8)		93.8 (95% CI = 69.8–99.8)		93.8 (95% CI = 69.8–99.8)	
Specificity (%)	87.5 (95% CI = 47.3–99.7)		87.5 (95% CI = 47.3–99.7)		75.0 (95% CI = 34.9–96.8)		12.5 (95% CI = 0.3–52.7)	

certainly had unwanted effects for the individual patient: concentrations too low yield partly false-negative results and products containing the allergen are not then avoided. Concentrations too high partly yield false-positive results, inflicting on the patient unnecessary preventive measures. From an epidemiological point of view, increasing the concentration will increase the number of diagnosed cases, which was reported from the IVDK (10), from the North American Contact Dermatitis Group (NACDG) (17, 40), from Dutch dermatologists (37) and from St John's/London (38). Both on an individual and on a public health level, the question arises as to whether sensitivity is more important (i.e., false-positive patients are more acceptable) or specificity is more important (i.e., false-negative patients are regarded as more acceptable).

Many dermatologists emphasize the need to diagnose contact allergy sensitively (albeit critically evaluating the positive patch test result in terms of clinical relevance), because while a (possibly false) positive patch test result is the starting point of further investigation (validation tests, patient history and product analysis), a negative test result will rarely prompt further action. Based on their clinical experience with single patients in whom the patch test concentration had been too low, dermatologists recommended an increase in the concentration (16). De Groot et al. having tested various patch test concentrations concluded that the commercial patch test preparation (MDBGN/PE 0.5%) and low concentrations of MDBGN alone (0.1 and 0.05%) produced false-negative reactions (no quantitative details have been given) (37). However, the criterion for 'true positive' was the reaction to 0.3% (and not clinical contact dermatitis). Hence, this study only indicates that, by increasing the concentration of a patch test, the number of positives increases, and probably the number of negatives decreases. Rarely are patch test concentrations examined against a use test. Tosti et al. have found, in a very small study with 5 use-test-positive patients, that 3/5 reacted positively to 0.5% MDBGN/PE on patch testing, whereas 2 were patch-test-positive only to 2.5% (6). However, the question remains as to how many false-positive cases are acceptable when increasing the concentration up to unreasonable ranges.

Until the availability of formal validation studies, such as this one, we used, in a former study, an internal validation approach (9), namely, evaluating the relation between doubtful and allergic reactions by using the reaction index (RI) proposed by Brasch and Henseler in 1992 (41), and the relation between + reactions and all

allergic reactions (+/++/+++) later described by the concept of the positivity ratio (PR) by Geier et al. (26). MDBGN/PE 0.5% and 1.0% were tested in 4615 patients, and MDBGN alone 0.3% (in 4343 patients) and 0.1% (in 988 patients) (9). The higher concentration of MDBGN/PE yielded an increase in positive patch test reactions from 2.3 to 3.5% ($P < 0.001$). More importantly, the RI remained stable with 0.54 (which indicates a 'good' patch test preparation relative to other allergens of the standard series (26, 42)). Comparing MDBGN 0.1% and 0.3%, the proportion of allergic reactions increased from 1.4 to 3.3% ($P < 0.001$). However, the RI decreased from 0.33 to -0.10 , the latter indicating more doubtful/irritant than allergic reactions, and from this pattern a higher probability of false-positive reactions with the higher concentration (MDBGN 0.3) was assumed. Comparing all 4 patch test preparations, the authors recommended the use of MDBGN/PE 1% (equivalent to MDBGN 0.2%), supported by Fransway (17).

In this study, we used an external validation of patch test results by relating the results of patch testing, with different concentrations of MDBGN (0.1, 0.2, 0.3 and 0.5%), to the external criterion of a positive ROAT. There is no difference between 0.2, 0.3 and 0.5% with regard to sensitivity (93.8), keeping in mind the relatively small sample size; only with the lowest concentration (0.1%) did sensitivity decline. This means that at least 70% (which is the lower limit of the 95% CI) of ROAT-positive patients were detected with 0.2% and higher. Hence, an increase in the concentration does not improve sensitivity. However, with regard to false-positive reactions, specificity decreases dramatically to 12.5% (upper 95% confidence limit: 53%) with the highest concentration (0.5%) (Table 5). Taking these results together with those of a previous study (9), and without any evidence of a boosting effect of positive ROAT results possibly lowering the elicitation threshold on subsequent patch testing, we conclude that for MDBGN 0.2% would be the best patch test concentration.

However, this recommendation is based on ROAT concentrations, which remained, in an attempt to identify a tolerable use concentration, in low ranges, namely, considerably lower than the recommended (0.04%) and the maximum permitted (0.1%) use concentration. We may, therefore, have missed a probably small number of very weakly sensitized persons, who would have required a use concentration higher than 250 p.p.m., which can still be encountered in consumer products (14). However, for (the majority

of) patients with a 'strong' or 'medium' sensitization, our conclusions seem justified. Only in those patients suspected of being false-negative to a patch test concentration of 0.2% or 0.3%, based on compelling exposure information, could higher concentrations be usefully patch-tested, as with other allergens (43), because in these patients the *a priori* likelihood of sensitization is high, and the probability of false-positive reactions to these higher concentrations sufficiently low, compared to the standard screening setting.

Nevertheless, it is still open for discussion whether MDBGN/PE 1% (with 0.2% MDBGN) or MDBGN alone 0.2% should be patch-tested. There was one case in our study not reacting even to the highest patch test concentration, despite a positive reaction to the middle ROAT concentration. This may be a rare case of 'compound allergy' (44), as discussed by Vigan et al. in 2 cases of sensitization to Euxyl K 400[®] (45).

Perspectives

Beyond the scope and purpose of use tests based on a dose-response design, such as our study, we wonder whether a non-eliciting concentration could possibly give indirect clues to the non-inducing concentration, which is even more important in terms of public health (46). However, it was shown in dose-response studies that there is an inverse relationship between induction dose and elicitation dose: Decreasing the induction dose requires an increase in the challenge dose for elicitation (47–50). In view of the lack of human induction experiments for ethical reasons, and in view of risk-assessment models being hampered by a number of assumptions and extrapolations (51, 52), 2 assumptions could be further examined and discussed, based on human morbidity data and available exposure data:

- In dose-response ROAT studies, a highest effect level (HEL) (e.g., a concentration eliciting at least 90%) and a no observed effect level (NOEL) (e.g., <10% reacting) could be determined. As in other risk-assessment models (51, 53), the (medium) concentration in a representative set of products can be determined. This average exposure, which obviously had sensitized people, could, therefore, be taken as the induction dose. The safe, non-inducing concentration would be the actual exposure (induction) concentration divided by the factor resulting from the quotient of HEL and NOEL. Alas this suggestion lacks the consideration of the

pitfall of non-linearity between induction and elicitation (46).

- A straightforward suggestion would be to take the NOEL (with regard to elicitation) as the safe concentration with regard to induction. As there is an inverse relationship between induction and elicitation, induction by a low dose in a product cannot be excluded entirely, but this would require an extremely high elicitation dose (e.g., a high patch test), which would never be reached in products, as they would be restricted by the NOEL.

Nevertheless, the results of such studies should be related to other risk-assessment approaches (21, 51, 53). However, whatever risk-assessment approach is chosen, the ultimate confirmation (or gold standard) will be the lack of newly sensitized persons (3). This is the issue of clinical epidemiology and post-marketing surveillance.

Conclusion

It is evident from this study, corroborated by another study from Denmark (30), that MDBGN should not be used in leave-on products in concentrations of ≥ 50 p.p.m., supporting current EU regulation (13). One manufacturer of MDBGN has, in the meantime, decided to advise against the use of MDBGN in leave-on cosmetics worldwide. Our recommendation to patch test MDBGN at 0.2% is based on a previous comparative patch test study (9) and the current validation study, employing threshold patch test and ROAT, i.e., 2 different and independent approaches and findings. There is little doubt that, by further increasing the concentration, the proportion of irritant/doubtful reactions and the probability of false-positive reactions will increase and will, at 0.5% MDBGN and beyond, not be balanced by a presumably small gain in true positives.

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