

Quantitative repeated open application testing with a rinse-off product in methyldibromo glutaronitrile-sensitive patients: results of the IVDK

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Background: While the use of methyldibromo glutaronitrile (MDBGN) in leave-on products is clearly associated with high sensitization or elicitation risk, such a clear-cut relation could be questioned with regard to rinse-off products.

Objective: The objective of this study was to find a maximum non-eliciting concentration for rinse-off products in MDBGN patch test-positive patients.

Patients and methods: We performed a use-related test [repeated open application test (ROAT)] in patients sensitized to MDBGN with a liquid soap containing three concentrations of MDBGN (50, 200, and 400 p.p.m. MDBGN, respectively). The soap at 50 p.p.m. was used twice daily for 4 weeks. If no reaction of the skin was observed, the product with the next higher concentration was used for another 4 weeks, etc.

Results: In total, 32/37 evaluated cases [86.5%; lower exact one-sided 95% confidence limit (CL): 73.7%] did not react to any of the preparations. The remaining reacted as follows: 1/37 reacted to 50 p.p.m., 3/37 to 200 p.p.m., and 1/37 to 400 p.p.m. The cumulative non-response to 50 p.p.m. was 97.3% (lower CL: 87.8%).

Conclusions: The majority of subjects sensitized to MDBGN-tolerated rinse-off products containing a maximum concentration of 400 p.p.m. A concentration in rinse-off products in the range of 50 p.p.m. could be regarded as safe for most individuals already sensitized. These concentrations will presumably prevent induction (sensitization) also.

Key words: CAS 35691-65-7; consumer safety; methyldibromo glutaronitrile; patch testing technique; rinse-off cosmetics; ROAT; use test. © John Wiley & Sons A/S, 2010.

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Conflict of interests: All participating centres are members of the Information Network of Departments of Dermatology. Schülke & Mayr, the producer of MDBGN, is one of the sponsors of the IVDK network. The study itself was funded by Schülke & Mayr.

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Methyldibromo glutaronitrile (MDBGN) was introduced in the 1980s and frequently used as a preservative in cosmetics, household products, and industrial products. It was often used in combination with phenoxyethanol (1:4), which is known as a very infrequent contact allergen (1). Throughout the 1990s, MDBGN was identified more and more as an important contact allergen when used alone or combined with phenoxyethanol for the preservation of cosmetics and cleansing products [e.g. Euxyl K 400® (Schülke&Mayr®)] (2–6). Based on several studies of clinical epidemiology and national sales data on patch test material (CE-DUR method), we estimated the total number of people sensitized during the 1990s at more than half a million in Germany (7). The increasing incidence of allergy to MDBGN prompted the European Commission in 2003 to restrict its use to rinse-off cosmetic products from March 2005 (8). This decision was later supported by two use tests [repeated open application test (ROATS)] showing that a relatively high number of individuals sensitized to MDBGN reacted to a leave-on preparation even at a concentration of 50 p.p.m. (9, 10). Furthermore, the prohibition of MDBGN in leave-on products was followed by a drop of the sensitization rate from more than 4% in 2002 to 2.5% in 2005 according to the surveillance data of the Information Network of Departments of Dermatology (IVDK) (11). A similar decrease was noted in Denmark (12).

While these data indicate an unequivocal relation between the use of MDBGN in leave-on products and high sensitization rates, such a clear-cut relation could generally be questioned with regard to rinse-off products as their use is associated with a lower allergen exposure because of the type of application (rinse-off). These products may differ with regard to sensitization/elicitation. Liquid soaps were occasionally found to be associated with sensitization/elicitation (13, 14), whereas shampoos seem to be less involved. Twelve subjects sensitized to MDBGN used a shampoo containing 0.02% MDBGN for a period of 9–13 weeks, with no reactions indicative of contact allergy (15). A European multicentre study has found that prolonged use of shampoos containing 15 p.p.m. of the potent allergen methylchloroisothiazolinone/methylisothiazolinone was tolerated by the majority of study patients in spite of sensitization to this allergen (16). However, a higher prevalence of sensitization to MDBGN was observed among hairdressers and the exposure to rinse-off products such as shampoos was suspected as a source of sensitization (17).

Taken together, data on the safe use concentration of MDBGN in rinse-off products were scarce. In this situation, the Scientific Committee for

Consumer Products of the European Commission recommended to ban the use of MDBGN in rinse-off products as well (18), which was taken up in terms of a ban for such product types from March 2008 (19). From this background, the present study addresses the objective to find a maximum tolerable concentration for rinse-off products in MDBGN-sensitized patients. ‘Tolerable’ in this context would mean that at least 90% of those sensitized will not be elicited under use conditions. The 10% threshold of response was used formerly by us (10, 20) and others (21, 22). Still, the 10% threshold is an arbitrary limit, even if supported by conventional use, and could be discussed. For the purpose of the study, we conceived a use-related test (ROAT) with three concentrations of MDBGN (0.005%, 0.02% and 0.04%). The concentration of 0.005% (50 p.p.m.) was chosen because (i) it is the lowest active biocidal concentration in combination with other active ingredients according to one manufacturer and (ii) we were unaware of cases elicited by such a low concentration in rinse-off products. The highest concentration was chosen as it appeared probable that almost all sensitized patients would react to such a concentration. A standard commercial liquid soap was chosen as vehicle because this application form is mainly used for MDBGN-containing rinse-off products.

Patients and Methods

Patients

Study patients ($n = 43$; 30 women and 13 men; median age: 53 years; range: 22–67 years) were enrolled at nine centres of the IVDK (23). They were identified through routine diagnostic patch testing, the ROAT starting some weeks later.

Inclusion criteria for the participation were at least a positive reaction to MDBGN/phenoxyethanol at reading D3 on routine patch testing, age more than 18 years, and informed consent. Exclusion criteria were systemic or local immunosuppressant treatment, florid eczema or recent ultraviolet exposure in the test area, pregnancy, and lactation. The study was approved by the Ethics Committee of the University of Göttingen, Göttingen, Germany.

Repeated open application test

The test preparation base was a commercial liquid soap (Esemtan Waschlotion®), consisting of aqua, sodium laureth sulfate, lauryl glucoside, sodium laureth sulfate, glycol distearate, stearamide MEA, cocamide DEA, sodium chloride, sodium hydroxide, citric acid, lactic acid, allantoin, undecylenic acid, benzyl salicylate, hexyl cinnamal, lauric acid, C.I.

42090, and C.I. 47005 (International Nomenclature Cosmetic Ingredients declaration). This product served as a negative control (marked in blue). For the test preparations, R1, R2, and R3 (red) MDBGN in three increasing concentrations 50, 200, and 400 p.p.m. was added to the formula named above. The products were prepared by Schülke & Mayr®, Norderstedt, Germany, and were supplied in containers dispensing 0.14 ml per press.

A ROAT (24) was performed on $5 \times 10 \text{ cm}^2$ areas on the volar side of each forearm. Each test area was randomly assigned to the test preparation or the control preparation. The participants were equipped with the two preparations (one with, one without MDBGN) and were instructed to apply twice daily (morning and evening) one press of each liquid soap to the areas on their forearms after moistening with water. Subsequently, these regions were washed with a wet soft sponge by moving the sponge about 10 times in both directions. After 30 seconds at the latest, the preparations had to be removed by running water. Patients were advised to avoid application of soaps and creams in the treated regions. In particular, MDBGN-containing shower gels, shampoos, or creams were not allowed.

If a reaction occurred in a test area, further exposure was terminated. Otherwise the application of the same preparations was continued for 28 days. If there was no reaction after this period, the participants were supplied with preparations at the next higher concentration to be used for another

4 weeks. The preparation with the highest concentration was applied for the last 4 weeks, if there had been no reaction until the end of the preceding 8 weeks (Fig. 1). During the whole study period, the skin was examined 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. (22, 25). 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 (22, 26).

Patch testing

At the end of the ROAT, patients were patch tested. Patch tests were performed in accordance with the guidelines of the German Contact Dermatitis Research Group (DKG) (27) and current international recommendations (28). The three patch test preparations with different concentrations of MDBGN (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 (one department) or 2 days (eight departments). Readings were performed until at least 3 days by using the following grading: negative, ?, +, ++, +++, irritant, and follicular.

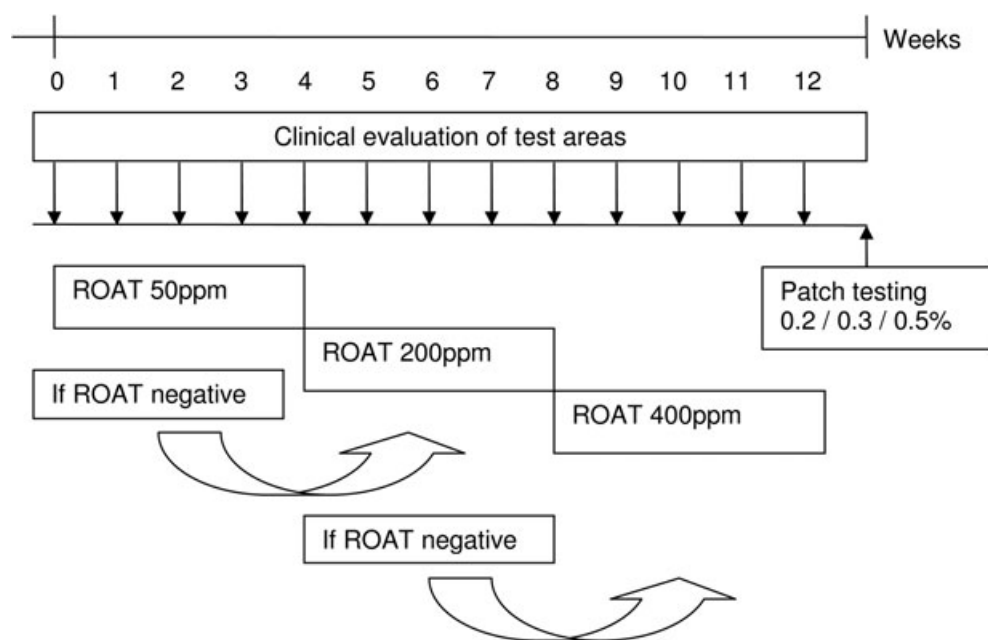


Fig. 1. Study design. R1–R3: preparations with methyldibromo glutaronitrile (MDBGN), containing 50, 200, and 400 p.p.m. MDBGN, were applied for 4 weeks at the volar side of one forearm. At the contralateral forearm, the control preparation was applied. If a reaction occurred, further application was terminated and patch testing was performed with three patch test preparations containing MDBGN alone in various concentrations (0.2%, 0.3%, and 0.5%). ROAT, repeated open application test.

Statistics

For the descriptive presentation and statistical analysis of the data, current guidelines (29) were considered. Exact confidence limits (CLs) to proportions, based on the binomial distribution, were calculated. As error, affecting the precision of the point estimates (proportions) was of interest only regarding one side, namely the lower limit in case of non-reactors and the upper limit in case of positive reactions, both in terms of a 'safety margin' to the study's point estimates, one-sided 95% CLs were calculated. Data analysis was performed with the statistical software package SASTM (version 9.2, SAS Institute, Cary, NC, USA).

Results

After exclusion of four cases (all with underlying atopic dermatitis) reacting to the control soap preparation and two cases from one department (because of insufficient documentation), 37 cases were suitable for analysis. In total, 32 of 37 patients did not react to any of the preparations after 12 weeks of continued application (86.5%; lower exact one-sided 95% CL: 73.7%), that is tolerated products with up to 400 p.p.m. MDBGN.

Threshold ROAT results

Of the 37 patients with a positive MDBGN/ phenoxyethanol pre-trial patch test result one patient elicited a positive reaction to the lowest concentration R1 (50 p.p.m.), three to the medium concentration R2 (200 p.p.m.), and one to the highest concentration R3 (400 p.p.m.) (Table 1). In order to assess a maximum non-eliciting concentration for rinse-off products in MDBGN-sensitized patients, the cumulative response data were interpolated with a spline curve. Spline as implemented in SAS produces a cubic spline that minimizes a linear combination of the sum of squares of the residuals of fit and the integral of the square of the second derivative (30). From this, an approximate point estimate

of a 10% threshold for no response can be identified at a concentration of 180 p.p.m. MDBGN (Fig. 2). Because of the small number of positive responders, this is, however, a rather imprecise estimate: even at the concentration of 50 p.p.m., with 97.3% non-response, the lower one-sided 95% CL is 87.8%. The fitting of a dose–response curve, for example a logistic curve, on the basis of five cases and with a mere 13.5% observed cumulative response was considered questionable and thus avoided.

Threshold patch test results

At the end of the ROAT, in all 37 patients a confirmatory patch test was performed with MDBGN 0.2%, 0.3%, and 0.5% in petrolatum. All five ROAT-positive cases reacted to the patch test concentration of 0.3%. In all, 9/37 originally patch test positives were patch test negative on second retesting. In agreement with (9), we refrained from declaring these nine patients 'not sensitized' and excluding them from further analyses for the following general reasons:

- (1) The method of patch testing is subject to limited reproducibility (31).
- (2) Patients have been found previously to react positively in a ROAT in spite of a negative second patch test result (20).
- (3) The nine 'positive' reactions on first patch testing (and negative reaction on retesting) may indicate a lower degree of sensitization. These borderline cases should also be considered with regard to the ROAT outcome (as in the two studies cited above).

Nevertheless, we calculated additionally the reactions on the basis of 5/28 ROAT positive: 17.9%, or in other words: $23/28 = 82.1\%$ (exact lower one-sided 95% CL: 66.1%), tolerated the rinse-off product up to the highest concentration of 400 p.p.m.

As there were only five individuals reacting to different concentrations in patch testing and in 23 the ROAT was negative and could not be used as 'external criterion', we refrained from calculating the sensitivity and specificity and draw any conclusions with regard to the optimal patch test preparation from the data of the present study (10).

Discussion

The aim of the present study was to identify the maximum use concentration of MDBGN in rinse-off products that is tolerated by the majority of individuals with proven sensitization to MDBGN. For this purpose, a stepwise ROAT with increasing concentrations of MDBGN was performed (until a

Table 1. Summary of response to threshold ROAT with an MDBGN-containing liquid soap (twice daily application on clinically healthy skin) in 37 patients with previously diagnosed sensitization to MDBGN

Concentration	Patients reacting <i>n</i>	Cumulative			
		Response		Non-response	
		<i>N</i>	% (upper CL)	<i>n</i>	% (lower CL)
50 p.p.m.	1	1	2.7 (12.2)	36	97.3 (87.8)
200 p.p.m.	3	4	10.8 (23.0)	33	89.2 (77.0)
400 p.p.m.	1	5	13.5 (26.3)	32	86.5 (73.7)

ROAT, repeated open application test; MDBGN, methylidibromoglutaronitrile; CL, exact one-sided confidence limit.

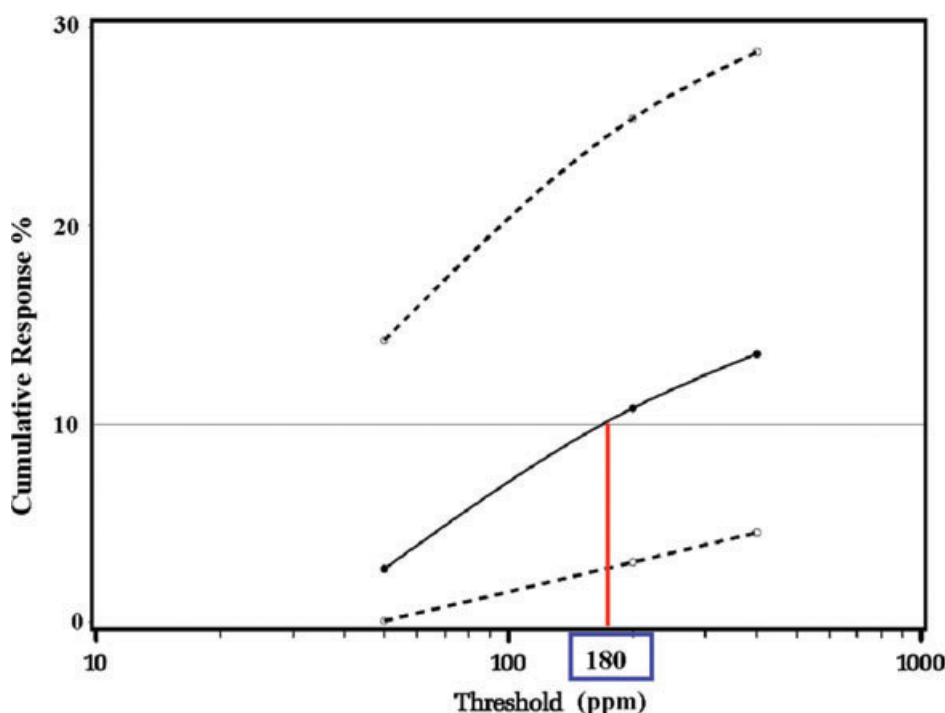


Fig. 2. Spline interpolated relationship between dose and cumulative response (in %) with exact upper one-sided 95% confidence limit.

positive ROAT reaction was obtained or the respective maximum concentration of the preparation was reached). The present study adds new evidence regarding the relevance of rinse-off products containing MDBGN for the development of allergic contact dermatitis.

The issues to be discussed refer to the questions of (i) a non-eliciting concentration in rinse-off products and (ii) the diagnosis of sensitization to MDBGN.

Use-related test and the question of the maximum non-eliciting concentration in rinse-off products

Use tests have been carried out with leave-on products containing MDBGN in pre-sensitized patients. In 18 and 39 individuals allergic to MDBGN, a ROAT with a leave-on product was performed (9, 10). Contact dermatitis was elicited at a concentration of 50 p.p.m. MDBGN in more than 50% and 30%, respectively, of the sensitized subjects. It was therefore concluded that a concentration of 50 p.p.m. MDBGN in leave-on products cannot be considered as safe.

Our present data showed that (under the particular conditions of the study, as described above), the same concentration will be tolerated by the vast majority of sensitized patients ($36/37 = 97.3\%$), even if a 87.8% (the lower one-sided 95% CL to the observed proportion) is conservatively considered.

Previous studies initially did not find a potential risk of sensitization to MDBGN because of the use

of shampoos. In a provocative use testing study, none of 12 pre-sensitized patients who used a shampoo containing 0.02% (200 p.p.m.) MDBGN over a period of 9–12 weeks developed any skin symptoms because of exposure (15). In a further study, an evaluation of clinical data followed by an exposure-based risk assessment focused on patients suffering from hand eczema (32). From the results, it was concluded that predominantly MDBGN-containing lotions, but not shampoos, might represent a relevant source of sensitization. For two shampoo preparations, a concentration of 78 and 79 p.p.m. MDBGN was calculated but not considered as causative for the development of hand eczema in this study. In contrast, liquid hand soaps were suspected to play a role for the development of hand eczema in a subgroup of study patients. In liquid hand soaps used by two patients and suspected as source of sensitization, a concentration of 144 and 399 p.p.m. MDBGN was found. Diba et al. (13) also identified MDBGN-containing hand wash products as a relevant trigger in a case report of three hospital workers with allergic contact dermatitis.

Later studies attempted to identify the concentration of MDBGN in rinse-off products capable of eliciting eczematous reactions in sensitized individuals. Jensen et al. (26) investigated the relevance of the use of a liquid hand soap preparation containing the maximum permitted level of 0.1% (1000 p.p.m.) MDBGN. In a ROAT, 19 MDBGN-allergic patients and 9 controls washed an area of 50 cm² on their

lower arm twice daily over 28 days. An allergic response was elicited in 37% of the pre-sensitized subjects but not in the controls. The results of this small study appear to be largely in line with our findings, if our data were extrapolated beyond the 400 p.p.m., although an extrapolation may be arguable. The authors recommended a re-evaluation of the permitted MDBGN level in rinse-off products. Two retrospective studies assessed the frequency of MDBGN allergy, clinical characteristics of patients with a positive patch test result to MDBGN, and their use of MDBGN-containing products. In the first study, a subgroup of 24/766 clinical cases with a relevant positive patch test reaction to MDBGN suffered from hand eczema and also reported a predominant exposure to liquid soaps or shampoos (33). Generalized dermatitis was observed in nine cases and was suspected to be because of the use of shampoos. The authors calculated concentrations ranging from 11 to 174 p.p.m. MDBGN for liquid hand soaps (11, 19, 22, 66, and 174 p.p.m.) and from 32 to 473 p.p.m. for shampoos (32, 44, 103, and 473 p.p.m.) used by the patients and suspected to be relevant for the hand eczema (33). In a further retrospective analysis the data of 2146 patients, who had undergone patch testing, were evaluated (14). In a subgroup of 110 patients, a significant association between hand eczema and MDBGN allergy was observed. Moreover, a distribution analysis of the causative products showed that a relative proportion of 23% accounted for liquid soaps in a subgroup of 53 patients with a currently relevant patch test reaction to MDBGN (14).

Taken together, these studies identified the use of MDBGN-containing rinse-off products as a potential risk for the development of allergic contact dermatitis and mainly addressed the question which concentration of MDBGN probably elicits eczematous symptoms in sensitized individuals.

However, the question which use concentration of MDBGN in rinse-off products might be considered as safe remained unsolved. The major outcome of the present study is that (i) the majority of the included subjects (86.5%, lower one-sided 95% CL: 73.7%) sensitized to MDBGN tolerated the rinse-off product containing the maximum concentration of 400 p.p.m. and (ii) a concentration of 50 p.p.m. can probably be regarded as safe under the circumstances of this study. Our ROAT was applied on uncompromised skin twice daily. The elicitation threshold may probably be lower when (i) there is repeated occupational use (e.g. hand soaps), (ii) application on areas of former allergic reaction to MDBGN (34), or (iii) application on areas of pre-damaged or irritated skin, for example because of repeated exposure to washing/wet work (35). Jensen et al. (36) have shown, for instance, that

0.04% MDBGN once daily or 0.01% MDBGN four times daily had approximately equal capabilities of provoking allergic contact dermatitis. In contrast, a further aggravating influence on elicitation relating to the used product itself, namely concomitant exposure to potentially irritating detergents (37), was considered in this study, as the preparations used were based on commercial washing liquids. Zachariae et al. described that the hand eczema 'improved or cleared within 6 months' following identification of the contact allergen. This observation indicates that the avoidance of MDBGN-containing rinse-off products may have had an effect on eczema, but in view of the long delay of improvement, other aspects than 'allergen avoidance' like reduction of skin irritation by improvement of skin care and protection regimes might probably have contributed to a decline of clinical symptoms as well. With regard to the very low concentrations (11–174 p.p.m., mean: 58.9 p.p.m.) calculated as relevant in eight of nine cases, it may be doubted that MDBGN was indeed the (sole) trigger of eczema in this study. Although the designs are not directly comparable, the present study yielded a cumulative response even to ($\leq$) 200 p.p.m. significantly less often (4/37 versus 8/9; $P < 0.0001$, Fishers exact test). From the previous studies cited above, and in particular the results of this study, it may be concluded that, with regard to elicitation, the safe concentration of MDBGN in rinse-off products may lie below 200 p.p.m. and probably close to 50 p.p.m.

While normal use of liquid soaps commonly entails several daily applications to the (possibly pre-irritated or damaged) hands, application of shampoo as a consumer is mostly once daily. Hence, in view of the importance of repetition of challenging doses (see above), it could be argued that regarding this application once daily to head and shoulders, 50 p.p.m. MDBGN are most probably tolerable even for sensitized persons. Moreover, all present results with ROAT (or patch test, for that matter) threshold testing are based on a population of patients who got sensitized by the relatively high doses previously used. It is well known, however, that sensitizing and elicitation doses are inversely related (38). Thus, if a further, marked restriction would effectively be set into force, it should be expected that the threshold dose capable of eliciting a given percentage of sensitized persons would be higher, that is the safety margin would much increase.

More importantly, induction by shampoos containing 50 p.p.m. would very probably be prevented, according to the quantitative risk assessment approach, as it was shown for 1000 p.p.m. cinnamal in a shampoo (39). Cinnamal and MDBGN are of comparable sensitizing potency. Therefore, the same

assessment can be used for MDBGN. The margin of safety (MOS) for cinnamal 1000 p.p.m. in a product was calculated as 12.5, which means that the sensitization reference dose is 12.5 times higher than the consumer exposure. The MOS for MDBGN 50 p.p.m. would then be 250. Thus the model predicts that concentrations of 1.2% and higher in a shampoo would lead to sensitization. This view was supported by others (32). Furthermore, sensitizing doses are normally higher than doses needed to elicit at least 1% in experimental sensitization (40). Nevertheless, subclinical sensitization through lower doses cannot be excluded, but then the eliciting dose would be much higher than found in products (38).

Conclusions

The main finding of our study is that the majority of participants (97.3%; exact lower one-sided 95% CL: 87.8%) tolerated a liquid soap containing 50 p.p.m. MDBGN. The findings indicate that elicitation through concentrations higher than 50 p.p.m. are to be expected, and thus MDBGN should not be used in rinse-off products exceeding this threshold. Single cases may react to lower, even to very low concentrations, which may be explained by a (very) high induction dose (initially exposed to products with very high concentrations) (38) or by presumably rare high individual susceptibility (41). Furthermore, frequent (occupational) exposure to liquid soaps or application to damaged or inflamed skin will lower the elicitation threshold, as with any allergen, so that application under these conditions should be precluded. In contrast, one can assume that induction of new cases can be avoided by concentrations in the range of 50 p.p.m. in rinse-off products. Future studies addressing the safe concentration of MDBGN could possibly employ (threshold) provocative use testing with shampoo, similar to the studies with MDBGN and methylchloroisothiazolinone/methylisothiazolinone previously described.

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