

An evaluation of dose/unit area and time as key factors influencing the elicitation capacity of methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) in MCI/MI-allergic patients

CLAUS ZACHARIAE¹, ANNE LERBÆK¹, PAULINE M. MCNAMEE², JOHN E. GRAY², MIKE WOODER³ AND TORKIL MENNÉ¹

¹Department of Dermatology, Gentofte Hospital, University of Copenhagen, 2900 Hellerup, Denmark,

²Procter & Gamble Technical Centres Limited, Rusham Park Technical Centre, Whitehall Lane, Egham, Surrey TW20 9NW, UK, and ³Rohm & Haas, Herald Way Coventry CV3 2RQ, UK

Methylchloroisothiazolinone and methylisothiazolinone (MCI/MI) contact allergy affects 1–3% of patch-tested patients in European centres. The aim of the present study was to evaluate the importance of the factors – time and concentration (dose/per unit area) – in the elicitation capacity by means of a repeated open application test (ROAT) experimental design. The study was designed as a double-blind, placebo-controlled, dose–response ROAT preceded by a diagnostic patch testing. 25 subjects with confirmed MCI/MI allergy and 10 healthy, non-MCI/MI allergic control subjects were exposed to 0.025 µg/cm² (2 p.p.m.) of MCI/MI/unit area of the skin for 4 weeks. After a wash-out period of at least 4 weeks, the subjects were exposed to 0.094 µg/cm² (7.5 p.p.m.) of MCI/MI/unit area of the skin for 4 weeks. The study showed the importance of both time and exposure in the elicitation process. It demonstrated that the elicitation threshold for MCI/MI is expected to be in the proximity of 0.025 µg/cm² although it was not possible to establish a definitive elicitation threshold for MCI/MI in this study.

Key words: concentration; dose/unit area; elicitation; methylchloroisothiazolinone and methylisothiazolinone (MCI/MI) contact allergy; repeated open application test (ROAT); time.

© Blackwell Munksgaard, 2006.

Accepted for publication 24 April 2006

Introduction

Methylchloroisothiazolinone and methylisothiazolinone (MCI/MI) are the active ingredients of a preservative system that has broad-spectrum antimicrobial activity against fungi, yeasts and both Gram-positive and Gram-negative bacteria. MCI/MI has been used extensively for more than 20 years as a preservative system for general consumer products (e.g. carpet cleaners, fabric softeners, dishwashing liquids), cosmetics (e.g. shampoos, skin creams) and diverse ranges of industrial product applications (e.g. inks, metal-working fluids, industrial cooling towers, adhesives). The usefulness of MCI/MI lies not only in its broad-spectrum activity but also in its achievement of preservative efficacy at very low concentrations in product. MCI/MI is one of the most important preservatives used in both cosmetic and industrial products (1).

MCI/MI is provided as a active ingredients to the cosmetic industry for use in cosmetic products in the ratio of 3:1. This preservative system is listed in Annex VI of the European Union Cosmetics Directive as approved for use in cosmetic products in Europe at up to 15 p.p.m. (2). However, the manufacturer of MCI/MI, trade-marked as Kathon CG[®], has recommended a maximum concentration of 7.5 p.p.m. for use in leave-on applications in line with the US Cosmetic, Toiletry, Fragrance Association (US CTFA) Cosmetic Ingredient Review Panel recommendations (1).

MCI/MI is a potent contact allergen as established in preclinical studies (1). Studies on guinea pigs have established MCI and MI as contact allergens and elucidated their potency – the former being a strong allergen and the latter a weak allergen (3). More recently, the local lymph node assay has confirmed MCI/MI as a potent contact allergen (4).

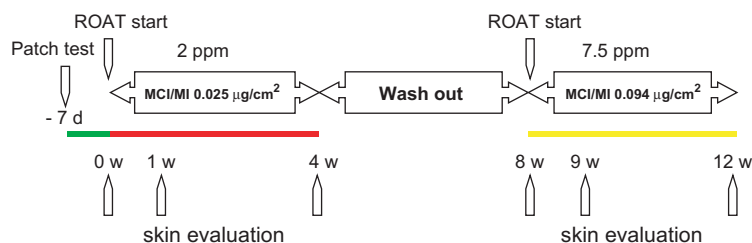


Fig. 1. Overall study design.

Following the introduction of this preservative in the early 1980s, a sharp increase in contact allergy rates has been observed by the specialist dermatology centres in Europe, with a prevalence rate of 3–8% among the clinical population across different countries (5, 6). It has been strongly opined that these levels of contact allergy were the result of the use of leave-on cosmetic products formulated with levels of MCI/MI that were, for the most part, well within the regulatory guideline/limit in force at that time (30 p.p.m.) but which were higher than those currently in effect. Many of these cases of contact allergy were observed in patients with pre-existing skin disease, such as hand eczema, where there is known to be an increased risk of contact allergy (5). More recent data indicate that MCI/MI contact allergy has reduced significantly, is stable and affects 1–3% of patch-tested patients in European centres (7).

It is well recognized that the ability of a contact allergen to induce contact allergy and lead to the manifestation of a clinically relevant allergic contact dermatitis (ACD) is dependent on several factors. Potency and ability to penetrate into the viable epidermis are examples of such factors (8). However, one of the most important factors is exposure (5, 9, 10). A recent update on the risk assessment for the use of MCI/MI in rinse-off products demonstrated very effectively the importance of understanding exposure as a part of the risk-assessment process for safe use of contact allergens (5). From an understanding of the consumer exposure to MCI/MI in rinse-off products, under normal-use conditions, it was concluded that the risk of primary sensitization from such exposure in rinse-off products is negligible and that elicitation of ACD in MCI/MI-allergic individuals is rare.

An important step in the risk-assessment process is to determine the dose of MCI/MI/unit area of skin that is relevant for the induction of contact allergy in naive individuals (5, 8–13). Also important for risk management is an understanding of the exposure (dose/unit area) that can elicit ACD in already sensitized individuals. In several studies on human volunteers, it has been demonstrated

that approximately 50% of the exposed, presensitized individuals will react with clinically relevant skin reactions in 1 week to twice-daily application of a leave-on product containing between 10 and 15 p.p.m. MCI/MI (14–16). Experimental elicitation studies at exposures levels of 1–2 p.p.m. MCI/MI or studies lasting longer than 1 week have not previously been reported.

The aim of this study was to investigate, using the repeated open application test (ROAT) (17), two key parameters of exposure – allergen concentration and time – in terms of the elicitation capacity of MCI/MI in MCI/MI-sensitized individuals and to explore the interrelationship between these two key factors.

The results of this study may be useful in risk management for the use of MCI/MI in cosmetic and other products.

Materials and Methods

Test subjects

29 eczema patients (male and female), 18 years of age or older, who on a prior occasion had experienced at least a positive reaction to the standard MCI/MI patch-test concentration of 100 p.p.m. aq.

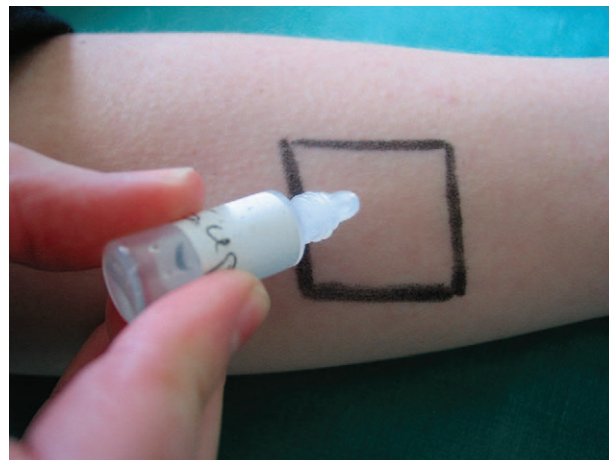


Fig. 2. Application technique for the delivery of methylchloroisothiazolinone/methylisothiazolinone solutions and the vehicle control in repeated open application test 1 and 2.

Table 1. Calculation of dose/unit area for concentrations of methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) used in repeated open application test (ROAT) 1 and 2

ROAT	Size of a drop (g)	Number of drops applied	Volume applied (μl)	MCI/MI concentration (p.p.m.)	Patch size (cm ²)	Dose/unit area per application (μg/cm ²)	Total dose/unit area per day (μg/cm ²)
ROAT 1	0.02834 [coefficient of variation (CV) of 4.4%]	2	0.05668	2	9	0.0125	0.025
ROAT 2	0.02834 (CV of 4.4%)	2	0.05668	7.5	9	0.047	0.094

were enrolled into the study. Each subject was patch tested with 100 p.p.m. aq. MCI/MI prior to the study to confirm his/her pre-existing allergy to MCI/MI. 10 healthy volunteers who tested negative to 100 p.p.m. aq. MCI/MI were recruited as control subjects. Pregnant or lactating women and subjects who had a current acute or chronic widespread eczema at any site were excluded from the study. The local ethics committees approved the study, and all participants were included after informed consent was obtained from them. The study was performed in accordance with Good Clinical Practice Guidelines (18).

Diagnostic patch testing

Diagnostic patch testing (DPT) to MCI/MI was performed at a dose/unit area of 3.0 μg/cm² MCI/MI (15 μl of 100 p.p.m. MCI/MI on a 0.5 cm² patch). The subjects were also patch tested with the vehicle control (deionized water with 10% ethanol) used in the subsequent ROAT. Patches were applied for 2D to the upper back, using Finn Chambers[®] (Epitest OY, Helsinki, Finland) and Scanpor[®] tape (Norges plaster A/S, Oslo, Norway). Readings of skin responses were recorded at D2, D3–4 and D7. International Contact Dermatitis Research Group recommendations for grading of skin responses were followed (19).

Study design

This study was designed as a double-blind, placebo-controlled, dose–response ROAT preceded by a DPT. The overall study design is detailed in

Fig. 1. All subjects, in accordance with a randomization schedule, were instructed to apply 2 drops of test material twice a day for 4 weeks on an area measuring 3 × 3 cm on the volar part of either the left or right forearm (Fig. 2). The test material and vehicle control were supplied in small plastic bottles in a blinded manner. Test material and vehicle control were spread evenly over the application site and allowed to dry before being covered with clothing. The dose metric used to define exposure to the MCI/MI solutions in ROATs 1 and 2 (and in the pre-ROAT DPT to confirm MCI/MI sensitivity) is dose/unit area expressed as μg/cm². In earlier studies, exposure to a material in both the clinical setting and through normal consumer use of products has been expressed as weight/volume (%) dose irrespective of the surface area over which the material was applied. This led to the assumption that across different patch/use test and consumer exposure scenarios the same per cent of material would result in equal exposure. However, as is well established at present, the expression of exposure across, for example, patch test systems as dose/unit area demonstrates significantly different levels of exposure although the per cent dose of a test material remains constant (9). It is therefore important to express exposure using the dose metric of dose/unit area (μg/cm²) to enable direct comparison of exposure to the same contact allergen in different scenarios. Per cent dose can be converted to a dose/unit area if, in addition to the per cent dose (converted to μg/g), the volume applied (μl) and surface area (cm²) over which the material is spread are known. The data required to calculate the dose/unit area/day exposure to MCI/MI

Table 2. Repeated open application test skin grading scale: morphology and strength of skin responses (20)

Overall clinical impression	Negative	Doubtful	Weakly positive	Moderately positive	Strongly positive
Involved area of application	0% (0)	1–24% (1)	25–49% (2)	50–89% (3)	90–100% (4)
Erythema					
Involvement	None (0)	Spotty (1)	Homogeneous (2)		
Strength	Weak (1)	Medium (2)	Strong (3)		
Papules/infiltration	None (0)	Few: <10 (1)	Some: 10–15 (2)	Many: >25(3)	Homogeneous infiltration (4)
Vesicles	None (0)	Few: <10(1)	Some: 10–25 (2)	Many: >25(3)	Confluent (4)
Total score					

Table 3. Results of the 29 patients diagnostically patch tested to methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) [$3 \mu\text{g}/\text{cm}^2$ aq. (100 p.p.m.)]

Patch test results	MCI/MI ($3 \mu\text{g}/\text{cm}^2$) (aq.) (100 p.p.m)
+++	1
++	8
+	16
Negative or doubtful	4

in this study are detailed in Table 1. The MCI/MI exposures expressed in ROATs 1 and 2 as $\mu\text{g}/\text{cm}^2$ are per diem values.

In the first ROAT period (ROAT 1), test material containing $0.025 \mu\text{g}/\text{cm}^2$ MCI/MI and the vehicle control was applied to the designated skin sites. This test period of up to 4 weeks was followed by a wash-out period of 4 weeks when no test material or vehicle control was applied. The wash-out period was followed by application of $0.094 \mu\text{g}/\text{cm}^2$ MCI/MI test material and the vehicle control to the designated skin sites under the same exposure conditions employed in ROAT 1 for a period of up to 4 weeks (ROAT 2). The development of a positive skin response at any time during the ROAT precluded further participation by the patient in the ROAT.

ROAT skin grading

Readings of the skin responses at the designated test area were made according to a grading scale as given in Table 2 (20). The strength of the overall positive reactions was judged, as weak, moderate or strong, based on the clinical signs – erythema, papules and vesicles. A positive response was defined as eczema in the test area. Skin response sites were photographed for documentation. Skin responses in the ROATs were evaluated: prior to the start of each ROAT; at the end of week 1 and at the end of week 4. In case of any signs of positive reaction in the test area, study participants were instructed to contact the dermatology department for an unscheduled visit and skin evaluation. The test bottles were weighed before and after use. The applied dose of MCI/MI in the test material was expressed in $\mu\text{g}/\text{cm}^2$ of skin.

Statistics

All tests were performed at the 5% significance level. The proportion of subjects showing a positive reaction in the MCI/MI-allergic group was compared with the proportion in the control group using Fisher's exact test (21). When comparing the proportion of positive reactions in the MCI/MI allergy group in ROAT 1 and ROAT 2, logistic regression was used, taking the subject effects into account. Simple summary statistics were also applied to look at average time to positive reaction and the amount of MCI/MI used.

The rate of positive reaction over time was assessed using Kaplan–Meier survival estimates. It should be noted that this is an approximate technique to apply here as the two sets of data being compared are nonindependents – they are linked by subject.

Results

Patch testing

29 subjects with a previously determined MCI/MI allergy were patch tested. In 25 subjects, the positive MCI/MI patch test could be reproduced (86%). The 4 subjects whose reactions were negative or doubtful on DPT were not recruited into the study. The results for the patch test are given in Table 3.

ROAT results

As shown in Table 4, the average amount of MCI/MI used expressed as $\mu\text{g}/\text{day}$ was comparable in the patients who reacted and the patients who did not react in ROAT 1 and ROAT 2 as well as the nonallergic control subjects. The amount of control material used was comparable between groups and comparable to the amount of test material used (data not shown).

Figure 3 shows the representative examples of positive reactions from ROATs 1 and 2. All positive reactions started with a follicular reaction. Significant adverse reactions were not observed during the course of the study. Patient drop-out

Table 4. The average amount of methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) used ($\mu\text{g}/\text{day}$) for subjects either reacting or not reacting in repeated open application test (ROAT) 1 and ROAT 2 as well as the non-allergic control persons

Skin response	Average amount of MCI/MI applied per day ($\mu\text{g}/\text{day}$)			
	MCI/MI-allergic individuals		Control subjects	
	ROAT 1 ($0.025 \mu\text{g}/\text{cm}^2$)	ROAT 2 ($0.094 \mu\text{g}/\text{cm}^2$)	ROAT 1 ($0.025 \mu\text{g}/\text{cm}^2$)	ROAT 2 ($0.094 \mu\text{g}/\text{cm}^2$)
Positive reaction	0.29 (± 0.16)	1.01 (± 0.38)		
Negative reaction	0.27 (± 0.08)	1.14 (± 0.43)	0.27 (± 0.09)	0.99 (± 0.31)

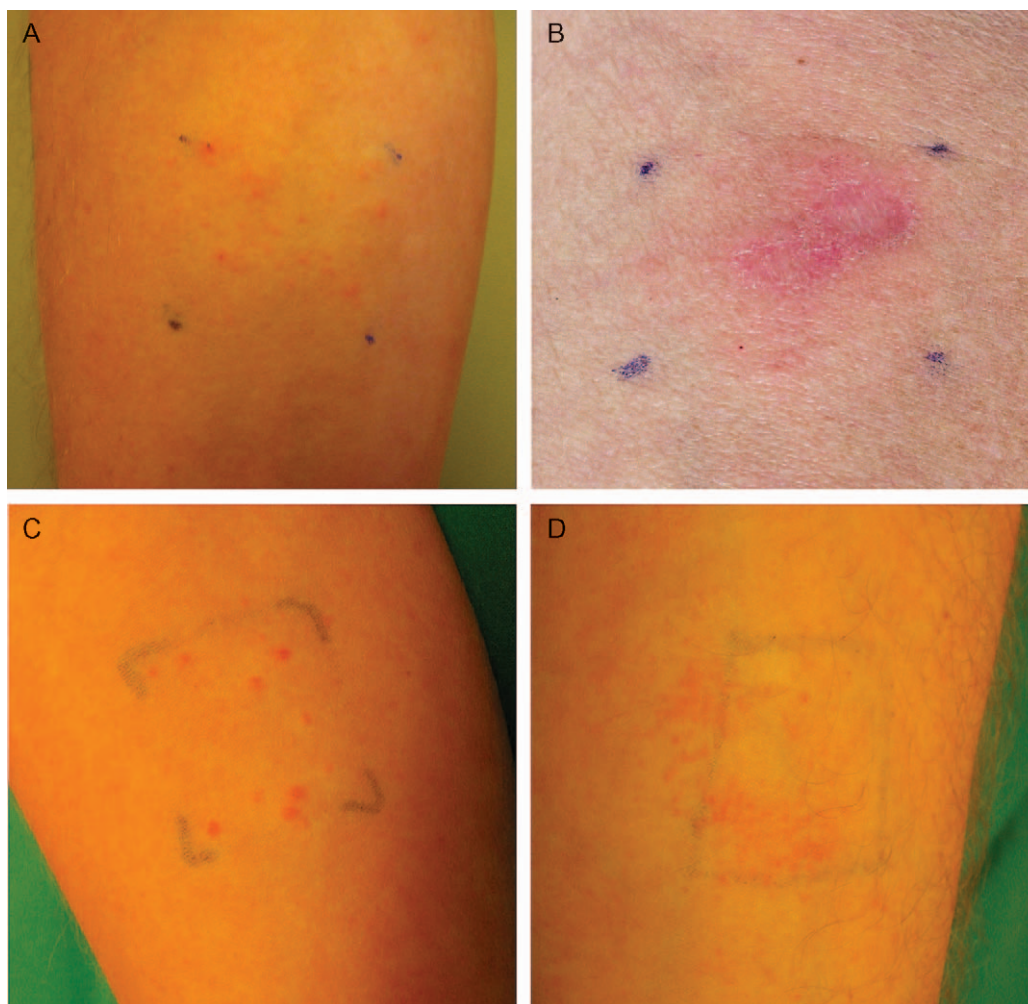


Fig. 3. Representative examples of positive reactions from repeated open application test 1 and 2. **a** A weak follicular reaction after repeated open application test (ROAT) application of $0.025 \mu\text{g}/\text{cm}^2$ methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) in ROAT 1. **b** A moderate eczematous reaction after ROAT application of $0.025 \mu\text{g}/\text{cm}^2$ MCI/MI in ROAT 1. **c** A weak follicular reaction after ROAT application of $0.094 \mu\text{g}/\text{cm}^2$ MCI/MI in ROAT 2. **d** A moderate eczematous reaction after ROAT application of $0.094 \mu\text{g}/\text{cm}^2$ MCI/MI in ROAT 2.

rate was zero, and only three patients experienced minor worsening in their hand eczema.

The overall results of ROAT 1 with $0.025 \mu\text{g}/\text{cm}^2$ MCI/MI and ROAT 2 with $0.094 \mu\text{g}/\text{cm}^2$ MCI/MI are given in Table 5. The ROAT results, expressed as days to a positive skin reaction dependent on MCI/MI concentration and time, are shown as a Kaplan–Meier plot in Fig. 4. None of the 10 nonallergic control subjects showed any positive reaction to $0.025 \mu\text{g}/\text{cm}^2$ MCI/MI in ROAT 1 or to $0.094 \mu\text{g}/\text{cm}^2$ MCI/MI in ROAT 2.

There was no statistically significant difference in the number of positive reactions, when comparing the number of positive reactions to $0.025 \mu\text{g}/\text{cm}^2$ MCI/MI in ROAT 1 with the vehicle control ($P = 0.0835$). A statistically significant result was observed for the number of positive reactions to $0.094 \mu\text{g}/\text{cm}^2$ MCI/MI in ROAT 2 with vehicle control ($P = 0.0022$). There was also a statistically

significant difference ($P < 0.0001$) observed when comparing the number of positive reactions in ROAT 1 (28%) and ROAT 2 (56%). All 7 subjects who reacted with a positive skin response in

Table 5. Repeated open application test (ROAT) outcome in methylchloroisothiazolinone/methylisothiazolinone (MCI/MI)-allergic individuals exposed to $0.025 \mu\text{g}/\text{cm}^2$ (ROAT 1) and $0.094 \mu\text{g}/\text{cm}^2$ MCI/MI (ROAT 2)

	ROAT 1	ROAT 2
Number of individuals included	25	25
Number of positive reactions	7	14
Average time to positive reaction	16.5 days	12.1 days
Number of weak reactions	5 (5–9)*	7 (4–7)*
Number of moderate reactions	2 (8–9)*	6 (8–12)*
Number of strong reactions	0	1 (11)*
Average skin grading for the ROAT	7.1	7.8

*Range of results from the ROAT readings.

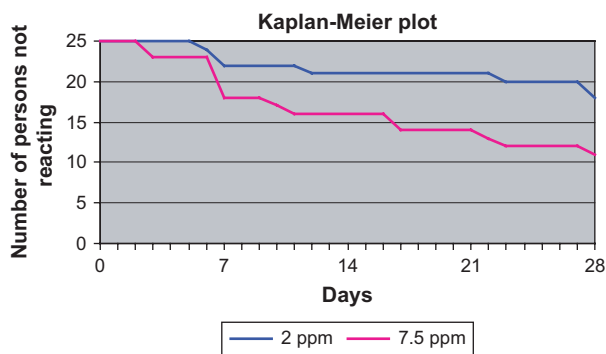


Fig. 4. Kaplan–Meier survival plot of time to positive skin response for methylchloroisothiazolinone/methylisothiazolinone-allergic individuals in repeated open application test 1 and 2.

ROAT 1 ($0.025 \mu\text{g}/\text{cm}^2$ MCI/MI) also reacted with a positive skin response in ROAT 2 ($0.094 \mu\text{g}/\text{cm}^2$ MCI/MI). 5 of these 7 patients reacted during the first week in ROAT 2 (Fig. 5).

The overall clinical impression of ROAT 1 and ROAT 2 is shown in Fig. 6. All subjects reacting to $0.025 \mu\text{g}/\text{cm}^2$ MCI/MI in ROAT 1 had either a similar strength of skin response (4/7) or worse (3/7) when exposed to the increased concentration of MCI/MI ($0.094 \mu\text{g}/\text{cm}^2$) in ROAT 2.

Discussion

The current study shows that elicitation of experimental ACD with MCI/MI depends on time and dose per unit area. This confirms the earlier studies conducted with the existing time response elicitation studies on isoeugenol (22) and suggests that allergens could be expected to behave similarly in these respects. While it was not possible to establish a definitive elicitation threshold for MCI/MI in this study, the data indicate that the elicitation threshold is in the proximity of $0.025 \mu\text{g}/\text{cm}^2$.

In addition, these ROAT data strongly support the conclusions of Fewings and Menné (5) that the

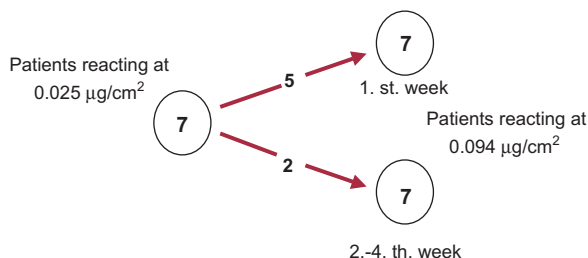


Fig. 5. Prediction of time to a positive skin response in repeated open application test (ROAT) 2 to $0.094 \mu\text{g}/\text{cm}^2$ methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) for patients already reacting in ROAT 1 to $0.025 \mu\text{g}/\text{cm}^2$ MCI/MI.

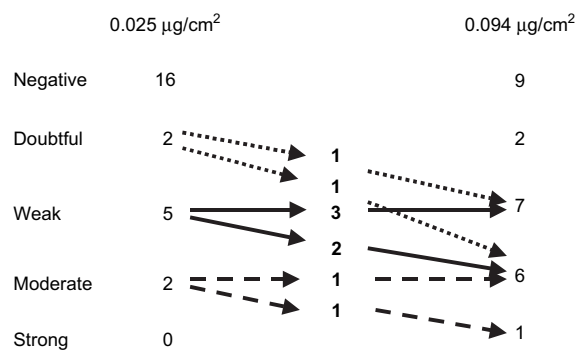


Fig. 6. The overall clinical picture of repeated open application test (ROAT 1) ($0.025 \mu\text{g}/\text{cm}^2$) and ROAT 2 ($0.094 \mu\text{g}/\text{cm}^2$ methylchloroisothiazolinone/methylisothiazolinone), with the number of positive reactions identified for different levels of skin response. Arrows connect the results of the individual test subjects.

use of MCI/MI in consumer rinse-off products involves negligible risk for the induction of contact allergy and that elicitation response to the use of such products is rare.

It is clear that in addition to potency, exposure is also a key determinant as to whether an allergen will elicit reactions in presensitized individuals. It is equally clear that other factors may also be expected to significantly influence the ability to manifest an elicitation response. As more studies become available, the relative importance of the factors such as potency, concentration (dose/unit area), time, influence of induction regimen, patch-test sensitivity and skin barrier integrity for the elicitation of ACD will be further understood (23, 24). As reported in the recent literature (25) that there is an increasing evidence that elicitation thresholds depend on factors that are additional to the inherent allergenic potency of the material, such studies will greatly add to our overall understanding of the elicitation of contact allergy.

However, it is already clear that the use of any ingredient, known to be a skin sensitizer in consumer products must be based on state-of-the-science risk assessment to ensure adequate consumer protection through the use of consumer products.

References

1. Cosmetic Ingredient Review Panel of the Cosmetic, Toiletry Fragrance Association. Final report on the safety assessment of methylisothiazolinone and methylchloroisothiazolinone. *J Am Coll Toxicol* 1992; 11: 75–128.
2. The Cosmetics Directive of the European Union. Dir. 76/768/EEC. Annex VI: List of preservatives which cosmetic products may contain.
3. Bruze M, Fregert S, Gruvberger B, Persson K. Contact allergy to the active ingredients of Kathon® CG in the guinea pig. *Acta Derm Venereol* 1987; 67: 315–320.

4. Warbrick E V, Dearman R J, Basketter D A, Kimber I. Influence of application vehicle on skin sensitisation to the isothiazolinone CMIT: an analysis using the local lymph node assay. *Contact Dermatitis* 1999; 41: 325–329.
5. Fewings J, Menné T. An update of the risk assessment for methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) with focus on rinse-off products. *Contact Dermatitis* 2000; 41: 1–13.
6. Tosti A. Prevalence and sources of Kathon CG[®] sensitization in Italy. *Contact Dermatitis* 1988; 18: 173–174.
7. Wilkinson J D, Shaw S, Andersen K E et al. Monitoring levels of preservative sensitivity in Europe. A 10 year overview (1991–2000). *Contact Dermatitis* 2002; 46: 207–210.
8. Gerberick G F, Robinson M K. A skin sensitization risk assessment approach for evaluation of new ingredients and products. *Am J Contact Dermat* 2000; 11: 65–73.
9. Robinson M K, Gerberick G F, Ryan C A, McNamee P M, White I, Basketter D A. The importance of exposure estimation in the assessment of skin sensitization risk. *Contact Dermatitis* 2000; 42: 251–259.
10. Gerberick G F, Robinson M K, Felter S P, White I R, Basketter D A. Understanding fragrance allergy using an exposure-based risk assessment approach. *Contact Dermatitis* 2001; 45: 333–340.
11. Kligman A M. The identification of contact allergens by human assay. II. Factors influencing the induction and measurement of allergic contact dermatitis. *J Invest Dermatol* 1966; 47: 375–392.
12. White S I, Friedmann P S, Moss C, Simpson J M. The effect of altering area of application and dose per area on sensitization by DNCB. *Br J Dermatol* 1986; 155: 663–668.
13. Upadhye M R, Maibach H I. Influence of area of application of allergens on sensitization in contact dermatitis. *Contact Dermatitis* 1992; 27: 281–286.
14. Björkner B, Bruze M, Dalquist I, Fregert S, Gruvberge B, Persson K. Contact allergy to the preservative Kathon[®] CG. *Contact Dermatitis* 1986; 14: 85–96.
15. Hannuksela M. Rapid increase in contact allergy to Kathon[®] CG in Finland. *Contact Dermatitis* 1986; 15: 211–214.
16. Frosch P J, Schulze-Dirks A. Contact allergy of Kathon[®] CG. *Hautarzt* 1987; 38: 422–425.
17. Hannuksela M, Salo H. The repeated open application test (ROAT). *Contact Dermatitis* 1986; 14: 221–227.
18. ICH Good Clinical Practices: Consolidated Guideline. CPMP/ICH/135/95 (available from <http://www.eudra.org/emea.html>).
19. Wilkinson D S, Fregert S, Magnussen B, Bandmann H J, Calnan C D, Cronin E. Terminology of contact dermatitis. *Acta Derm Venereol* 1970; 50: 287–292.
20. Johansen J D, Bruze M, Andersen K E, Frosch P J, Dreier B, White I R. The repeated open application test: suggestion for a scale of evaluation. *Contact Dermatitis* 1997; 39: 95–96.
21. Fisher R A, Yates F. *Statistical Tables for Biological, Agricultural and Medical Research*, 6th edition, Edinburgh, Oliver and Boyd, 1963.
22. 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.
23. Zachariae C, Hall B H, Cottin M, Cupferman S, Andersen K E, Menné T. Experimental elicitation of contact allergy from a diazolidinyl urea-preserved cream in relation to anatomical region, exposure time, and concentration. *Contact Dermatitis* 2005; 53: 268–277.
24. Villarama C D, Maibach H I. Correlations of patch test reactivity and the repeated open application test (ROAT)/provocative use test (PUT). *Food Chem Toxicol* 2004; 42: 1719–1725.
25. Hostynek J J, Maibach H I. Thresholds of elicitation depend on induction conditions. Could low level exposure induce sub-clinical allergic states that are only elicited under the severe conditions of clinical diagnosis. *Food Chem Toxicol* 2004; 42: 1859–1865.

Address:
Dr Claus Zachariae
Department of Dermatology
Gentofte Hospital
University of Copenhagen
Niels Andersensvej 65
2900 Hellerup
Denmark
Tel: +45 39773203
Fax: +45 39657137
e-mail: clza@gentoftehosk.kbhamt.dk