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Received Date : 04-Dec-2014

Revised Date : 04-Feb-2015

Accepted Date : 16-Feb-2015

Article type : Cutaneous allergy

Methylisothiazolinone in rinse-off products causes allergic contact dermatitis: a Repeated Open Application study

Running head: MI in rinse-off products

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Funding: Funding by the Swedish Research Council for Health, Working Life and Welfare (Forte); Hudfonden

Disclosures: All authors have no conflicts of interest to declare.

What's already known about this topic?

- The prevalence of contact allergy to methylisothiazolinone (MI) has increased dramatically during the recent years.

What does this study add?

- In a repeated open application test, we showed that nearly all MI-allergic subjects developed allergic contact dermatitis from using cosmetic rinse-off products containing allowed concentrations of MI (100 ppm or 50 ppm).
- The elicitation threshold is certainly far below 50 ppm
- The current levels of MI are not safe

Abstract/Summary

Background

In recent years, the prevalence of contact allergy to the preservative methylisothiazolinone (MI) has increased dramatically. Cosmetic products are one of the major sources of exposure.

Objectives

To examine whether allowed concentrations of MI in cosmetic rinse-off products have the potential to cause allergic contact dermatitis.

Materials/methods

19 MI-allergic subjects and 19 controls without MI allergy applied 2 liquid hand soaps five times per day on areas of 5*10 cm² on the ventral side of their forearms. One soap contained 100 ppm MI, the maximum allowed concentration in cosmetics, and was used by 10 allergic subjects and all controls. Another liquid soap with 50 ppm MI was used by 9 allergic subjects. As the negative control, all subjects used a similar soap that did not contain MI. The repeated open applications (ROAT) proceeded until a positive reaction occurred or up to 21 days. The study was conducted in a randomized and blinded fashion.

Results

10 out of 10 MI-allergic subjects developed positive reactions to the soap with 100 ppm and 7 out of 9 reacted to the 50 ppm soap, while none of the 19 controls had a positive reaction during 21 days of application. No reactivity was seen to the soap without MI. The difference in reactivity to MI between MI-allergic subjects and controls was statistically significant (Fisher's exact test, $p < 0.0001$).

Conclusions

Rinse-off products preserved with 50 ppm MI or more are not safe for consumers. No safe level has yet been identified.

Introduction

Since the late 1970s, methylisothiazolinone (MI) has been used in combination with methylchloroisothiazolinone (MCI) as a preservative in a wide range of chemicals and cosmetics (MCI/MI in the proportions 3:1, CAS number 55965-84-9, often referred to the trade name Kathon CGTM).¹ Since the early 2000s, MI has been used as an individual preservative in chemical products/mixtures, and in 2005 it was allowed for use in leave-on and rinse-off cosmetics (including products for personal hygiene) in a maximum concentration of 100 ppm.^{2,3} According to local lymph node assay (LLNA) data in mice, MI has an EC3 value of 0.4%⁴, and is thereby a strong skin sensitizer.^{5,6}

In recent years, the prevalence of contact allergy to MI has increased dramatically and the most recent reports from different European patch test centres indicate a sensitization frequency of around 5.5% (France) to 13% (Finland) in dermatitis patients.⁷⁻¹² Cosmetic products and paints are regarded to be important sources of exposure. Because of the increasing problems, MI was appointed as the American Contact Dermatitis Society Contact Allergen of the Year for 2013.¹³

Three different studies using repeated open application test (ROAT), intending to mimic exposure to MI by leave-on cosmetics, have been conducted in MI-allergic patients.¹⁴⁻¹⁶ They all show that leave-on cosmetics preserved with 100 ppm (0.01%) MI elicit allergic reactions in large proportions of MI-allergic subjects. There is presently no information regarding eliciting doses of MI in *rinse-off products* such as liquid hand soap and shampoo.

The aim of the present study was to examine whether cosmetic rinse-off products preserved with allowed concentrations of MI (100 ppm) and half that concentration (50 ppm) have the potential to elicit allergic contact dermatitis in previously sensitized individuals. This was investigated in a use test by repeated open applications (ROAT).

Materials and Methods

Subjects

The study adhered to the tenets of the Declaration of Helsinki and was approved by the regional ethics review boards in Stockholm, Sweden (ID: 2013/976-31/4) and Capital Region, Denmark (ID: H-4-2013-094). All subjects gave written informed consent before taking part in the study.

MI-allergic subjects

Dermatitis patients with contact allergy to MI were invited to participate in the study. They had been diagnosed by patch testing with MI (2000 ppm) at the out-patients clinics of the Centre for Occupational and Environmental Medicine, Stockholm County Council,

Stockholm, Sweden, or Gentofte Hospital, Copenhagen, Denmark between 2011 and 2013, or in 2012, respectively.

Inclusion criteria were confirmatory patch testing prior to inclusion showing at least one positive reaction to MI (minimum criteria: erythema and infiltration (+))¹⁷ and negative patch test to paraben mix. Exclusion criteria were age <18 years, eczema on the test areas, extensive exposure to ultraviolet light within the last 3 weeks (sunbathing, solarium), systemic immunosuppressive therapy, pregnancy and breast feeding.

In total, 19 MI-allergic subjects fulfilling the above criteria completed the ROAT (age 19-60, mean 40). In addition, two subjects were not included as they were test negative to MI in the confirmatory patch test; and one subject chose to discontinue the ROAT after 5 days of exposure, before any test reaction on the arms had developed.

Control subjects

Control subjects were recruited by advertisements on two websites with this purpose (www.studentkaninen.se and www.forsøgsperson.dk). Inclusion criteria were confirmatory patch testing prior to inclusion showing no patch test reactivity to MI or to paraben mix. Exclusion criteria were the same as for the MI-allergic subjects.

In total, 19 control subjects completed the ROAT (age 20-57, mean 37). In addition, two other control subjects were excluded; one due to problems following instructions, and one chose to discontinue after one day of participation.

The study procedure is described in Fig. 1.

Confirmatory patch test

Patch testing, prior to inclusion in the study, was performed in accordance with international recommendations.¹⁷ The MI-allergic subjects were patch tested with a serial dilution of MI in aqua to get an indication of their patch test reactivity. The following concentrations (%) of MI were tested: 0.2, 0.1, 0.05, 0.025, 0.01, and 0.0016, when described as $\mu\text{g MI}/\text{cm}^2$ this

corresponds to 60, 30, 15, 7.5, 3, and 0.48. In addition, they were tested with a vehicle control (aqua) and paraben mix (16% in petrolatum) (Chemotechnique, Vellinge, Sweden). The control subjects were patch tested with MI 0.2% in aqua and paraben mix only. Fifteen microliters of each patch test solution were applied by micropipette to Finn Chambers with filter paper on Scanpor tape (Epitest Ltd Oy, Tuusula, Finland; 8 mm internal diameter). The order of test substances was randomized. The tests were applied on the upper back for two days. Readings were performed at day 4 only, and the readings were blinded. In Sweden readings were performed by KY in combination with CL and/or AB, and in Denmark readings were performed by expert nurses, who do all readings in the clinic.

The reading scale used for the patch test, consisting of 8 steps, has previously been proposed and used by Fischer et al.¹⁸. The purpose of using this scale was to identify weaker skin reactions that are not considered to fulfil the criteria as positive allergic reactions in diagnostic patch testing. The threshold concentration for a positive response was defined as the lowest dose giving a visible reaction (minimum score 1) on day 4, if there was a continuous line of reactions from the highest dose and down.

MI (Microcare MT, 9.5% active ingredient in water, Thor, Kent, UK) from the same batch (no. rp-278529-1410, 2) was used for the patch test solutions and the liquid hand soaps which were all prepared by ACO Hud Nordic AB, Upplands Väsby, Sweden, in accordance with guidelines on good manufacturing practices (GMP) for cosmetics.

Repeated open application test (ROAT)

Materials

Three compositions of rinse-off test products were prepared specifically for this study. The composition reflected a simple liquid hand soap product with commonly used ingredients and concentrations. For details on the composition see Table 1. One liquid soap contained MI in a concentration of 100 ppm (0.48 $\mu\text{g}/\text{cm}^2$ per application), which is the maximum allowed concentration in cosmetics³, and one liquid soap contained 50 ppm MI (0.24 $\mu\text{g}/\text{cm}^2$ per application). The third product served as negative control and was identical except that it did not contain MI and was instead preserved with methyl- and ethylparaben. Pump soap packages dispensing approximately 0.24 ml per press (Rexam, Paris, France) were marked, filled and stored in a refrigerator until use. The packages were weighed before and after use in

order to assess the amount used by each subject, which the participants however were not informed about.

To confirm an accurate MI concentration, the liquid soap containing 100 ppm MI and the control soap were chemically analysed using HPLC with MS for quantification of the substance. This method has previously been described more in detail.²

Study design

Test areas of 5*10 cm² were marked on the ventral side of the participants' forearms. In a first step, 10 MI-allergic subjects and 19 control subjects applied the liquid soap with 100 ppm MI on one arm and the control soap without MI on the other arm. In a second step, 9 additional MI-allergic subjects applied the liquid soap with 50 ppm MI and the control soap without MI. The soaps were randomized to the test areas and the experiment was blinded, such that neither the subjects nor the assessor of the skin reactions knew which test area had been exposed to MI. The subjects got new soap packages every week and returned the used ones to the assessor.

The subjects were thoroughly instructed to apply the preparations 5 times per day, with a minimum of 2 h between each application, according to the following procedure:

- moisten the marked test area on one arm with water
- apply to the area the amount of liquid soap extruded by one press of the soap dispenser (approximately 0.24 ml)
- distribute the soap over the entire test area with the single-use applicator (plastic spoon, convex aspect)
- rinse off 20-25 seconds after the start of application and dry gently with paper tissue
- repeat the procedure on the other arm with the other product and a new applicator
- do not use any of your own products on the test areas.

The subjects were instructed orally and manually exactly how to apply the soaps. In addition, they were given written instructions with pictures illustrating in steps how to apply the soaps,

as well as a link to a YouTube clip showing the application procedure (<https://www.youtube.com/watch?v=zgHxJs8RrnM>). Text message reminders to be sent at time points decided by the subjects themselves were offered. The subjects were further instructed to apply a moisturizing cream (Decubal original clinic cream, Actavis Plc, Dublin, Ireland), supplied by the test leader, once daily on both arms when they had finished the day's soap applications. This was done to reduce possible irritation or dryness by the intense washing.

ROAT readings

Examination of the skin was performed once weekly or earlier if the participant reported a reaction. Reading was performed according to a previously proposed scale of evaluation.¹⁹ A positive reaction was defined as: *area of involvement >25% of the area of application, including erythema and signs of infiltration, i.e. at least 1 papule*. If a positive reaction occurred on any of the two arms, all further applications by the participant were stopped. If no reaction, the test proceeded for a maximum of 21 days. Readings were documented in a protocol and by photography. In Sweden readings were performed by KY in combination with CL and/or AB, and in Denmark readings were performed by ML in combination with JDJ.

Statistical analysis

Two-tailed Fisher's exact test was used for comparison of reactivity to the MI-preserved soaps between MI-allergic subjects and control subjects. McNemar's test was used to compare elicitation reactions to the same dose of MI per application in the ROAT and patch test ($0.48 \mu\text{g}/\text{cm}^2$). Kendall's tau-b was used to investigate a possible correlation between MI patch test threshold and accumulated dose MI until positive ROAT reaction. The two subjects that did not have any reaction in the ROAT were excluded from this analysis. Statistical significance was indicated by p values ≤ 0.05 . All statistical analyses were performed with SPSS software (version 22, IBM, Somers, NY).

Results

The ROAT results are summarized in Table 2. Individual data regarding patch test and ROAT reactions are found in Table 3. Examples of positive ROAT reactions are given in Fig. 2.

Patch test

All MI-allergic subjects included in the study reacted to doses down to $15 \mu\text{g}/\text{cm}^2$ (0.05%) (Table 3). Three subjects reacted to the lowest dose tested, $0.48 \mu\text{g}/\text{cm}^2$ (0.0016%).

ROAT

Ten out of 10 MI-allergic subjects developed positive reactions to the liquid soap with 100 ppm MI ($0.48 \mu\text{g}/\text{cm}^2/\text{application}$) within 4-11 days (median 7 days, average 7.3). Seven out of 9 of the MI-allergic subjects that used the product with 50 ppm MI ($0.24 \mu\text{g}/\text{cm}^2/\text{application}$) developed a positive reaction within 5-21 days (median 7 days, average 8). None of the MI-allergic subjects had a positive reaction to the control soap without MI.

None of the 19 control subjects without MI allergy developed positive reactions on any arm within 21 days of usage of the soap containing 100 ppm MI or the control soap.

The difference in response to the 100 ppm and 50 ppm MI-containing soap respectively in MI-allergic subjects compared with the control subjects was statistically significant (Fisher's exact test, $p=5.0 \times 10^{-8}$ and $p=0.00003$).

Product usage

The amount of liquid soap used per application differed only slightly when comparing the control group and MI-allergic group, and also when comparing control products and MI-containing products. The usage is summarised in Table 4.

Worth noting is that subject 12 had used considerably less soap (50 ppm MI) than expected, and thereby also in terms of applied dose of MI (table 3). This subject did not have a positive reaction in the ROAT.

Comparison of patch-test and ROAT reactivity to the same dose/application

10 MI-allergic subjects were exposed to $0.48 \mu\text{g}/\text{cm}^2$ per application in both the patch test and the ROAT. None of these subjects reacted to this dose in the patch test but all 10 had a positive reaction in the ROAT. The higher reactivity to the ROAT compared to the patch test was statistically significant in the McNemar's test ($p=0.00195$).

A comparison including all 19 MI-allergic subjects was also performed. In this case the dose per application of $0.48 \mu\text{g}/\text{cm}^2$ in the patch test was compared to a dose of application of $\leq 0.48 \mu\text{g}/\text{cm}^2$ in the ROAT ($0.48 \mu\text{g}$ $n=10$ or $0.24 \mu\text{g}$ $n=9$). Also in this comparison, the higher reactivity to the ROAT compared to the patch test was statistically significant ($p=0.000122$).

Correlation of patch test and ROAT reactivity

The investigation of a possible correlation between MI patch test threshold and accumulated dose MI until positive ROAT reaction with Kendall's tau-b gave a correlation coefficient of 0.381 (approximate $p=0.036$, exact $p=0.062$).

Discussion

Main findings

To the best of our knowledge this is the first study to examine elicitation responses to MI from rinse-off exposure. All MI-allergic subjects had positive allergic reactions to a rinse-off product preserved with 100 ppm MI, which is the maximum allowed concentration in cosmetics. When reducing the concentration to 50 ppm, 7 out of 9 additional MI-allergic subjects reacted. The difference in reactivity of the MI-allergic subjects compared to the control subjects was statistically significant. The lack of positive reactions in the control subjects and on the MI-allergic subjects control arm clearly demonstrates that the positive reactions were contact allergy reactions.

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This study demonstrates that the current levels of MI in rinse-off cosmetics are not safe for previously sensitized individuals.

Limitations and strengths of the study

It was decided to test only one dose per arm, which meant one dose MI per subject since the other arm was exposed to a control vehicle. This was done in order to minimize the obvious risk of contamination when rinsing off the product. This limits the amount of data generated by each subject in the study compared to a leave-on ROAT where multiple concentrations often are tested.

An obvious strength of a properly conducted ROAT is that it is closer to real life exposure than most other study designs. First, it is done with the target species, humans. Second, it mimics realistic vehicles as well as exposure patterns (frequency, application procedure etc.).

In the present study we increased the number of applications to 5 times per day. This is more than the usual ROAT procedure of 2 times per day, which have also been used in some recent rinse-off tests.^{20,21} Although 5 applications per day still may underestimate the total number of exposures for most people²² this was chosen for practical reasons, i.e. reasonable work load for subjects for good compliance with instructions.

Results in relation to other studies

Typically, the first signs of positive allergic reactions in our study were scattered erythema and papules/vesicles and at a later stage the reaction became more confluent. The same pattern of clinical signs has been described from other use tests with various haptens.²³⁻²⁵

At least three ROATs have been conducted studying leave-on exposure to MI.¹⁴⁻¹⁶ Although there are differences both in terms of study designs and results (with positive reactions to 100 ppm MI in 31% to 63% of subjects), the general picture is that leave-on products with 100 ppm MI elicits reactions in substantial proportions of the subjects. In the *leave-on* study performed by Lundov et al.¹⁴, which is probably most comparable to the present study, 63% of MI-allergic subjects reacted to preparations both with 100 ppm and 50 ppm MI. The higher response in our *rinse-off* study (although not statistically significant, Fisher's exact test

p=0.09) may, to some extent, be explained by the higher patch test reactivity to MI in our group of subjects.

Another possibility to take into consideration is that leave-on exposure has often been in focus, while the impact of frequent repeated rinse-off exposure may be underestimated. Under the conditions of our study, the relatively high, but realistic, frequency of exposure most likely had an impact on the response. Repeated low-dose exposure has been shown to be an important factor for elicitation as well as sensitization.²⁶⁻³⁰ In the study by Jensen et al, just 2 applications per day of a soap with 0.1% methyldibromo glutaronitrile (MDBGN) in MDBGN-allergic individuals elicited a reaction in 37% of subjects.²⁰ The authors also studied the effect of multiple applications giving the same total dose of MDBGN in one day as one application and found that these different types of exposures resulted in similar frequencies of elicitation reactions in pre-sensitized individuals.²⁷ This indicates that smaller repeated doses accumulate in the skin and can stimulate the immune system. In addition, combined exposures to allergens and irritants, in our case surfactants in the liquid soaps, may lead to reduced elicitation and sensitization thresholds.³¹⁻³³

Implications

ROAT data can contribute valuable information in the risk assessment process, especially when risk assessments based on sensitization testing have failed to predict the true risk of a hapten, as in the case of the preservative MDBGN³⁴ and now also MI. Previous risk assessments conducted in Europe and US, based on sensitization data, severely underestimated the allergy risk from preserving cosmetic products with MI.^{35,36} A recent opinion on MI by the Scientific Committee on Consumer Safety (SCCS) concluded, based largely on clinical data, that the current levels of MI in cosmetic products are not safe to the consumers. It was also concluded that elicitation data from rinse-off exposure was lacking. Under the conditions in our study, levels down to 50 ppm MI in rinse-off products were not safe, and the elicitation threshold is likely far below the tested doses. Risk managers and regulators urgently need to take proper actions to protect the consumers and workers.

Acknowledgements

We wish to thank Kerstin Skog in Stockholm and the staff at the allergy test clinic in Gentofte for their assistance in the patch test application. We thank Jan Hagberg and Tomas Lind for statistical support. In addition we thank Izabela Norin and Ulf Åkerström at ACO for valuable advice regarding suitable composition and packaging of the liquid hand soaps.

Tables

Table 1. Composition of the rinse-off products used in the repeated open application test (ROAT).

Substance (INCI-name)	CAS	Content in control soap (w/w %)	Content in soap with MI 100 ppm (w/w %)	Content in soap with MI 50 ppm (w/w %)
Aqua	7732-18-5	83	83	83
Sodium laureth sulfate	68585-34-2	9.0	9.0	9.0
Cocamidopropyl betaine	61789-40-0	5.0	5.0	5.0
Peg-7 glyceryl cocoate	66105-29-1, 68201-46-7	1.0	1.0	1.0
Sodium chloride	7647-14-5	0.87	1.4	1.4
Methylparaben	99-76-3	0.40	-	-
Ethylparaben	120-47-8	0.40	-	-
Citric acid	77-92-9 / 5949-29-1	0.12	0.13	0.13
Methylisothiazolinone	2682-20-4	-	0.010	0.0050

MI, methylisothiazolinone; INCI, International Nomenclature of Cosmetic Ingredients

Table 2. Number of subjects who developed positive reactions to liquid hand soaps by rinse-off repeated open application test (ROAT). The soaps contained different concentrations of methylisothiazolinone (MI). A positive reaction was defined as: *area of involvement >25 % of the area of application, including erythema and signs of infiltration, i.e. at least 1 papule.*

Liquid soap MI concentration	MI-allergic (pos/tested)	Controls (pos/tested)
100 ppm	10/10	0/19
50 ppm	7/9	Not tested
0 ppm	0/19	0/19

Table 3. Threshold dose for positive reaction in patch test and accumulated dose until positive reaction in a rinse-off repeated open application test among methylisothiazolinone-allergic subjects. Subjects 1-10 used the test product with 100 ppm MI and subjects 11-19 used the test product with 50 ppm MI.

Subject	Patch test threshold in $\mu\text{g MI/cm}^2$ (%)	Accumulated applied dose of MI ($\mu\text{g/cm}^2$) until assessed as positive reaction in ROAT	Calculated dose of MI ($\mu\text{g/cm}^2$) per application in ROAT ^a	Number of days until assessed as positive reaction in ROAT ^b
1 (SE)	3 (0.01)	25.43	0.53	9
2 (SE)	3 (0.01)	15.43	0.44	7
3 (SE)	3 (0.01)	16.63	0.48	7
4 (SE)	3 (0.01)	16.61	0.47	7
5 (SE)	3 (0.01)	17.35	0.50	7
6 (DK)	15 (0.05)	21.60	0.57	11

7 (DK)	3 (0.01)	8.00	0.36	4
8 (DK)	3 (0.01)	14.60	0.42	7
9 (DK)	3 (0.01)	14.00	0.42	7
10 (DK)	15 (0.05)	10.80	0.45	7
11 (DK)	3 (0.01)	12.40	0.19	13
12 (DK)	3 (0.01)	No reaction	0.15	-
13 (DK)	3 (0.01)	8.10	0.23	7
14 (SE)	0.48 (0.0016)	6.81	0.22	7
15 (SE)	0.48 (0.0016)	8.35	0.24	7
16 (SE)	3 (0.01)	6.60	0.22	5
17 (SE)	0.48 (0.0016)	11.81	0.22	12
18 (SE)	3 (0.01)	No reaction	0.22	-
19 (SE)	7.5 (0.025)	21.19	0.19	21

DK, Denmark; SE, Sweden;

^a Calculation based on amount used (by weighing containers before and after) and dividing by the total number of exposures.

^b There were variations in the number of times the subjects applied the soaps, although they were assessed at the same day. This was mainly because some subjects stopped the applications earlier than the reading day due to skin reactions.

Table 4. Amount of liquid hand soap used, averaged over the methylisothiazolinone-allergic subjects (n=19) and control subjects (n=19), in a rinse-off repeated open application test. The lower *total amount* of soap used by the MI-allergic subjects is because the experiment was stopped after a positive reaction.

Subjects	Type of soap	Amount (g) liquid hand soap/application (range)	Total amount (g) liquid hand soap used
MI-allergic	control soap	0.22 (0.16-0.26)	10.5
MI-allergic	MI-containing soap	0.22 (0.15-0.27)	10.3
control	control soap	0.22 (0.19-0.25)	22.9
control	MI-containing soap	0.21 (0.18-0.25)	22.6

MI, methylisothiazolinone.

Legends to figures

Figure 1. Flowchart illustrating the study procedure in a rinse-off repeated open application test, including methylisothiazolinone-allergic subjects (MI-pos) and methylisothiazolinone-negative control subjects (MI-neg).

Figure 2. Images concerning the rinse-off repeated open application test. (a, b, c) Examples of positive reactions (subject 15, 1, and 17 in Table 3). (d) Negative result in a control subject after 21 days of application. (e) The pump soap packages (red for left and blue for right arm). (f) Application of the soap.

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