

Patch testing with serial dilutions of various isothiazolinones in patients hypersensitive to methylchloroisothiazolinone/methylisothiazolinone

Marléne Isaksson, Birgitta Gruvberger and Magnus Bruze

Department of Occupational and Environmental Dermatology, Skåne University Hospital, Lund University, SE-205 02 Malmö, Sweden

doi:10.1111/cod.12184

Summary

Background. In 2005, methylisothiazolinone (MI) on its own came into use as a preservative. Prior to that, MI was always present together with methylchloroisothiazolinone (MCI). Can the pattern of reactivity to the separate active ingredients in allergic patients tell us something about the primary sensitizer?

Objectives. To investigate the potential pattern of cross-reactivity between the isothiazolinones tested, and to find the minimal elicitation concentration for each chemical, in order to determine whether the primary sensitizer is MCI or MI.

Methods. Patients reacting to MCI/MI and/or MI were additionally patch tested with MCI/MI, MCI, MI, 2-n-octyl-4-isothiazolin-3-one (OIT) and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (dichloro-OIT) in serial dilutions.

Results. Three different groups of reactors were seen. One group did not react to MI; another group reacted to both MCI and MI, but had higher patch test reactivity to MCI; and a third group reacted to both MCI and MI with very similar patch test reactivity, but reacted more often to OIT and dichloro-OIT.

Conclusions. Patch testing with the active ingredients of MCI/MI in serial dilutions could give information on the primary sensitizer.

Key words: contact allergy, patch testing, methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) 3:1 CAS no. 55965-84-9; cross-reactivity; methylchloroisothiazolinone MCI CAS no. 26172-55-4; methylisothiazolinone (MI) CAS no. 2682-20-4; 2-n-octyl-4-isothiazolin-3-one (OIT) CAS no. 26530-20-1; 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (dichloro-OIT) CAS no. 64359-81-5.

Preservatives containing methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) have been used extensively since the 1980s. Since 2005, MI on its own has been available as a preservative in cosmetics and

toiletries. To investigate whether dermatitis patients react to MI, a test preparation containing MI only was added to our baseline series in 2003. Patients reacting to MCI/MI and/or MI were additionally patch tested with various isothiazolinones in serial dilutions in an attempt to determine whether the primary sensitizer is MCI or MI. This study was aimed at describing the patterns seen.

Correspondence: Marléne Isaksson, Department of Occupational and Environmental Dermatology, Skåne University Hospital, SE-205 02 Malmö, Sweden. Tel: +46 (0) 40 331760; Fax: +46 (0) 40 33 11 92. E-mail: marlene.isaksson@med.lu.se

Conflicts of interest: The authors declare that they have no conflicts of interest.

Accepted for publication 26 November 2013

Materials and Methods

The study period lasted for almost 3 years, from 17 March 2003 to 31 December 2005.

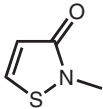
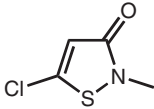
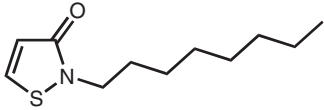
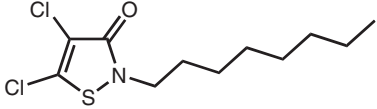
 MI	2-Methyl-4-isothiazolin-3-one CAS no. 2682-20-4 MW = 115.2 logP _{o/w} = - 0.11
 MCI	5-Chloro-2-methyl-4-isothiazolin-3-one CAS no. 26172-55-4 MW = 169.6 logP _{o/w} = 0.60
 OIT	2-n-Octyl-4-isothiazolin-3-one CAS no. 26530-20-1 MW = 213.3 logP _{o/w} = 3.6
 Dichloro-OIT	4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one CAS no. 64359-81-5 MW = 282.2 logP _{o/w} = 4.9

Fig. 1. Molecular structures for methylisothiazolinone (MI), methylchloroisothiazolinone (MCI), 2-n-octyl-4-isothiazolin-3-one (OIT), and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (dichloro-OIT).

The Swedish baseline series was purchased from Chemotechnique Diagnostics (Vellinge, Sweden). The following biocides were used: (i) Kathon® CG (at the time, from Rohm and Haas, Philadelphia, PA, USA), which consists of the active ingredients MCI (1.125%) and MI (0.375%); (ii) Neolone™ 950 (Rohm and Haas), which contains only water and MI at 9.5%, according to its material safety data sheet (in our baseline series, it was first tested at a concentration of 475 ppm, then at 950 ppm, and finally at 1000 ppm); (iii) 2-n-octyl-4-isothiazolin-3-one (OIT) isolated from Skane™ M-8 (Rohm and Haas) at our laboratory; (iv) MCI and MI isolated from Kathon® CG at our laboratory; and (v) 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (dichloro-OIT), isolated at our laboratory from Rocima™ 342 (Acima Chemical Industries, Im Ochsensand, Buchs, Switzerland) (Fig. 1).

Chemicals

Acetic acid was obtained from Fisher Scientific UK (Loughborough, UK). Acetonitrile, ethyl acetate and methanol were obtained from J. T. Baker (Mallinckrodt Baker B.V., Deventer, The Netherlands). The water was Millipore water.

Preparative high-performance liquid chromatography (HPLC)

For isolation of MCI and MI, a gradient HPLC system was used [fully described in (1)]. Isolation and purification of OIT and dichloro-OIT were performed with an automatic Nova Prep 200 system (R&S Technology, Wakefield, RI, USA) equipped with an L-7400 UV detector (Hitachi, Tokyo, Japan). The process was computer-controlled with TurboPrep Applications Software (R&S Technology). The column (250 × 50 mm) was packed with Kromasil C₁₈, 7 μm, 100 Å (Eka Nobel, Bohus, Sweden). Elution was performed with solvent A (methanol/water 60:40 vol/vol) and solvent B (methanol/ethyl acetate 80:20 vol/vol). The ultraviolet detector was operated at a wavelength of 280 nm. Flow rates of 100 ml/min and injection volumes of 30–100 ml were used. For dichloro-OIT, isocratic elution with 75% solvent B was used. The dichloro-OIT eluted at 6 min, and from 7 to 10 min the column was washed with 100% solvent B. Linear gradient elution was used for the isolation of OIT, starting at 20% solvent B, reaching 100% solvent B at 12 min, and being maintained there for 5 min. OIT eluted at 9.5 min.

Table 1. Description of the 1734 dermatitis patients tested with the baseline series during the study, and the number and sex of those reacting positively to methylchloroisothiazolinone/methylisothiazolinone (MCI/MI)

Patients tested with the baseline series		Contact allergy to MCI/MI				
Total number	Females (%)	MCI/MI 200 ppm (%)	Males (%)	Mean age (years)	Females (%)	Mean age (years)
1734	1040 (60.0)	46 (2.7)	19 (2.7)	51.9	27 (2.6)	41.1

Table 2. Data on methylisothiazolinone (MI) patch test preparations used and rates of contact allergy to MI in consecutively tested dermatitis patients in Malmö

Concentration (ppm)	Vehicle	Dose (µg/cm ²)	Total number of tested individuals; males (%); females (%)	Contact allergy rate (%)	Rate (%) of MI-positive/MCI/MI-negative	Years	Positive patients (no.)
475	Aqua	14.3	100; 40 (40.0); 60 (60.0)	1.0	0	2003	16
950	Aqua	28.5	1457; 631 (43.3); 826 (56.7)	0.3	0	2003–2005	12, 13, 15, 17, 18
1000	Aqua	30.0	181; 68 (37.6); 113 (62.4)	0.6	0	2005	14

MCI, methylchloroisothiazolinone.

Patch testing

Patch testing was performed according to International Contact Dermatitis Research Group (ICDRG) recommendations with the Finn Chambers® (Ø 8 mm) test system (Epitest Ltd Oy, Tuusula, Finland) secured with Scanpor® tape (Norgesplaster A/S, Vennesla, Norway). Fifteen microlitres of each test solution was micropipetted on to the filter paper discs (2). Tests were left on the upper back for 48 hr, and readings were performed on D3 and D7 according to ICDRG criteria.

In March 2003, Neolone™ 950 0.5% aq. (corresponding to 475 ppm MI) was inserted into the baseline series; MCI/MI 0.02% aq. has been present as a part of this for decades. The concentration of Neolone™ 950 was increased to 1.0% aq. (950 ppm MI) a month later, as we had not seen any adverse reactions, including patch test sensitization. In October 2005, the patch test concentration of MI was raised to 1000 ppm. To investigate the reactivity to potentially cross-reacting substances, a 'Neolone series' was made up, consisting of serial dilutions of MCI/MI at concentrations ranging from 200 down to 1.6 ppm, MCI at concentrations ranging from 150 down to 1.25 ppm, MI at concentrations ranging from 1000 down to 2 ppm, OIT at concentrations ranging from 1000 down to 7.8 ppm, and Neolone™ 950 at concentrations ranging from 950 down to 2 ppm. Initially, this series was patch tested in selected cases depending on the reactivity to MCI/MI and/or MI or the exposure pattern, but in late November 2004 this series (no. I) was introduced for testing of all patients allergic to MCI/MI and/or MI in the baseline series. From December 2004 onwards, this series was changed to Neolone series II, which also contains serial dilutions of dichloro-OIT at concentrations ranging from 100 down to 0.8 ppm. When a patient was tested with the

Neolone series, all dilutions of the respective sensitizer or various dilutions of the respective ingredients were tested.

Statistics

Fisher's exact two-tailed test was used to determine whether or not supposedly primary sensitization to MI was linked to contact allergy to OIT. The difference was considered to be significant at $p < 0.05$.

Results

Test results are shown in Tables 1–3. Test results from D3 to day D7 are compiled into one column, with only the highest test reactivity for the respective concentration noted. From this point in the article, we refer to the active ingredients instead of the various biocides when discussing the test results and their implications.

Baseline series

Table 1 shows the details of the 1734 dermatitis patients patch tested with the baseline series. Table 2 shows the number of patients tested with the various concentrations of MI and which patients reacted positively. Of the 7 patients positive to the respective MI preparation in the baseline series, all were tested with Neolone series I or II or with various dilutions of the respective ingredients.

Neolone series

Nineteen dermatitis patients were tested with various dilutions of the Neolone series. Table 3 shows patch test reactions to various isothiazolinones in 9 patients

Table 3. Patch test reactions to aqueous solutions of methylchloroisothiazolinone (MCI)/methylisothiazolinone (MI), MI and MCI, and ethanol solutions of 2-n-octyl-4-isothiazolin-3-one (OIT) and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (dichloro-OIT), in 9 patients with positive reactions to MCI/MI and to MCI and negative reactions to MI, in 5 patients with positive reactions to both MCI/MI, MCI and MI (patients 10–14) and with high patch test reactivity to MCI, and in 5 patients with positive reactions to both MCI/MI, MCI and MI (patients 15–19) and with high patch test reactivity to MI. The table is a summary of results from testing with the baseline series and additional serial dilutions

		Cases																		
	Concentration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Test substance	(ppm)	F	M	M	F	M	M	F	F	F	M	M	F	F	F	F	M	M	M	M
Kathon CG ^a (MCI/MI) ^b	200	++	+++	++	+++	+ (D10) ^c	+	++	+	+	++	+++	+++	+++	++	++	+++	+++	++	++
Neolone 950 ^d (MI) ^b	1000	NT	NT	NT	NT	NT	–	–	–	–	NT	NT	NT	NT	++	NT	NT	NT	NT	NT
Neolone 950 ^d (MI) ^b	950	–	NT	–	–	–	NT	NT	NT	NT	–	–	++	+	NT	+	NT	++	++	–
Neolone 950 ^d (MI) ^b	475	NT	–	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	+++	NT	NT	NT
Kathon CG ^a (MCI/MI) ^e	200 to 1.6	+++	NT	+	+	+++	+	–	++	+	+	+++	+	+	++	+	++	+++	++	++
	50			200	100	100	200	200	200	200	50	50	25	100	100	3.15	50	50	50	12.5
Neolone 950 ^d (MI) ^e	950 to 2	–	NT	–	–	–	–	–	–	–	+	+	+	+	+	+	++	++	++	+
	950			950	950	950	950	950	950	950	475	950	119	238	950	238	30	30	30	2
MI ^g ^f	1000 to 2	–	NT	–	–	–	–	–	–	–	+	+	+	–	–	+	++	++	++	+
	1000			1000	1000	1000	1000	1000	1000	1000	500	125	500	1000	62	31	31	31	31	2
MCI ^g ^g	150 to 1.25	+++	+	+	++	++	–	++	–	–	+	+++	+++	+	++	+	+++	++	++	+
	37.5	75	150	150	75	150	75	150	150	150	37.5	37.5	150	150	37.5	75	37.5	75	1.25	+
Skane M-8 ^h (OIT) ^e	1000 to 7.8	–	NT	–	–	–	–	++	–	–	+	–	–	–	–	+	++	–	–	+
	1000			1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	62.5	7.8	1000	1000	7.8
Dichloro-OIT ^e ⁱ	100 to 0.8	NT	NT	–	–	NT	–	–	–	–	–	NT	–	NT	–	+	NT	NT	–	+
				100	100		100	100	100	100	100		100		100	0.8			100	1.5

^aKathon CG containing active ingredients MCI/MI at the stated concentration.

^bPresent in the baseline series and tested on test occasion I.

^cDoubtful reaction on D7; retested at 200 and 300 ppm MCI/MI, with positive reactions to both on D4.

^dNeolone 950 containing active ingredients MI at the stated concentration.

^eTested on test occasion II.

^fMI isolated at our laboratory.

^gMCI isolated at our laboratory.

^hSkane M-8 containing active ingredients OIT at the stated concentration.

ⁱDichlorinated OIT isolated at our laboratory.

NT, not tested.

The lowest concentration giving a positive reaction when dilution series were tested is marked. When a negative reaction is noted, the concentration tested is marked.

with positive reactions to MCI/MI and MCI and negative reactions to MI (patients 1–9). Table 3 shows 5 patients with positive reactions to both MCI/MI, MCI and MI (patients 10–14) and with high patch test reactivity to MCI, that is, at least two dilution steps higher than for MI. Table 3 also shows patch test reactions to various isothiazolinones in the 5 patients (patients 15–19) with positive reactions to MCI/MI, MCI and MI and with very similar reactivity to MI and MCI, that is, at most one step difference in dilution step between MI and MCI, or with high patch test reactivity to MI. Three of the 5 patients also reacted to OIT, and 2 of the 3 also reacted to dichloro-OIT.

MCI/MI

Of the 46 patients reacting to MCI/MI in the baseline series, 41 were also tested with one of three NeoloneTM 950 test preparations in the baseline series. Of these 41,

7 were positive to the tested MI preparation and 34 were negative. Three of the 34 were later tested with a Neolone series, and then reacted positively to MI (patients 10, 11, and 19; Table 3).

Discussion

Since the early 2000's, biocides containing exclusively MI without MCI have been on the market, implying various patterns of exposure to MI and MCI, with, consequently, various possible modes of sensitization.

The 41 patients (41 of 46 MCI/MI-allergic patients also tested with MI) with contact allergy to MCI/MI can be divided into two groups: (A) without allergic patch test reactions to MI (the 31 patients negative to NeoloneTM 950 in the baseline series, some of whom are patients 1–9 tested with Neolone series I or II); and (B) with allergic patch test reactions to MI (the 10 allergic to MI among

the 41 MCI/MI-allergic patients). This second group can be further divided into: (a) high patch test reactivity to MCI/MI and MCI as compared with MI (at least a two-step difference in the dilution series) (patients 10–14); and (b) high patch test reactivity to MI as compared with MCI, or the same reactivity, or, at most, one dilution step difference in reactivity to MI as compared with MCI (patients 15–19).

In our previous article on primary MI sensitization, we concluded that primary sensitization to MI differs from primary sensitization to MCI/MI, in which the sensitization is directed to the active ingredient MCI, and that the cross-reaction pattern in these two circumstances differs (3). Both human studies and animal studies support this (1, 4–6).

Primary sensitization to MI differs from primary sensitization to MCI/MI. A human study has shown that patients sensitized to MCI/MI only react to MCI when tested with equimolar concentrations of MCI and MI (5). A guinea-pig maximization test (GPMT) has also shown MCI to be a stronger sensitizer than MI (4). The same GPMT failed to show cross-reactions to MI and OIT in animals primarily sensitized to MCI (4). This type of pattern is supported by cases 1–9, in which the contact allergy is directed towards MCI/MI. When these patients were patch tested with the separate components of MCI/MI, they only reacted to MCI (if they reacted at all), although they were tested with a 10–20-fold higher concentration of MI than is present in our MCI/MI baseline test preparation, namely 200 ppm, corresponding to MCI 150 ppm/MI 50 ppm. Nor was there any cross-reactivity to OIT or dichloro-OIT in those tested with these two substances, except in patient 8, who reacted to 1000 ppm OIT. The reason for this is unclear, and nothing in the history suggests exposure to this preservative. The 31 patients who reacted to MCI/MI but not to Neolone™ 950 in the baseline series can also be allocated to this group. The pattern of reactivity is a pattern that we have seen since the 1980s in patients sensitized to MCI/MI, and still see (6). Interestingly, 3 of the MCI/MI-allergic patients did not react to MCI when tested with this active ingredient. Several explanations are possible. Those 3 patients had very weak contact allergy to MCI/MI, and we know that sometimes a patient reacts to a mix but not to the individual constituents when they are tested separately. Another explanation could be that substances other than MCI and MI could be responsible. It has been shown that 4,5-dichloro-2-methyl-4-isothiazolin-3-one is present in the MCI/MI mixture as an impurity, and this substance is a sensitizer (5, 7).

Patients 10–14 can be attributed to group Ba, as they reacted to both MCI and MI but they had higher patch

test reactivity to MCI than to MI, which is in favour of primary MCI/MI sensitization, and thus subsequent MCI sensitization with cross-reactivity to MI. This is a pattern seen in the two aforementioned human studies, in which patients sensitized to MCI/MI were patch tested with both the active ingredients separately, and where all patients tested positively to MCI (1, 6) and the majority reacted negatively to MI (1, 6). In one of the studies in which workers had been occupationally sensitized to MCI/MI, one tested positively to MI, and this patient reacted positively to a concentration of MCI seven times lower than that of MI. In that study, we concluded that the most likely explanation was a cross-reaction between MCI and MI (6).

Furthermore, patients 15–19 can be placed in group Bb, in which a different pattern is seen, with reactivity to both MCI and MI, and with either high reactivity to MI as compared with MCI, resulting in positive patch test reactions to lower concentrations of MI than to MCI, or the same reactivity to MCI as to MI, or, at most, one dilution step difference in reactivity for MI as compared with MCI and with cross-reactions to OIT and dichloro-OIT.

This is an opposite pattern to that seen in the aforementioned human study, in which patients sensitized to MCI/MI were patch tested with equimolar concentrations of both the active ingredients separately, and where all patients reacted positively to MCI (5) and no patients reacted positively to MI (5). This pattern (Bb) suggests primary MI sensitization. Indeed, 2 of the 5 patients in Table 3 were considered to be primarily sensitized to MI, namely patients 16 and 19. Patient 16 has been reported on separately by us, with probable primary sensitization to MI after a chemical burn (3). He was found to already react 10 days after the introduction of MI into our baseline series. He also had strong contact allergy to OIT without known exposure to this preservative. Animal studies lack sufficient data to determine whether the positive test reactions to MCI and OIT in patients 16 represent cross-reactions. In a GPMT with MI as the primary sensitizer, 31.3% were sensitized to MI, and cross-reactions to MCI were seen in 12.5% and cross-reactions to OIT in 0%. Even though the figures for MI and MCI were non-significant, the authors concluded that possible cross-reactivity to MCI was indicated (4). In patients 17 and 18, no reactions were noted for OIT, but, in patients 15 and 19, positive reactions to several concentrations of OIT were seen, as well as to dichloro-OIT. In patient 15, according to the hospital file, the patch tests had become partly loosened from the back, which may explain the negative reactions to the highest test concentrations of OIT. She was later retested with 1000, 500 and 250 ppm OIT, and showed positive reactions to all three concentrations.

From a chemical point of view, cross-reactivity to OIT can be expected with MI as the primary sensitizer, as OIT is chemically more closely related to MI than to MCI (6) (Fig. 1). Moreover, the positive reactions to dichloro-OIT in only 2 patients with contact allergy to OIT indicates that there might be cross-reactivity between these two substances as well. Furthermore, a higher tendency for there to be simultaneous reactions between OIT and MI was seen in those presumably primarily sensitized to MI, that is, reacting to similar or lower concentrations of MI as compared with MCI [3 of 5 (patients 15, 16, and 19) versus 2 of 13 (patients 7 and 10) in Table 3; $p = 0.10$; Fisher's exact test, two-sided], partly supporting cross-reactivity between MI and OIT. In contrast to patients 16 and 19, in which we suspect primary sensitization to MI, we lack information on patients 15, 17 and 18 with respect to probable primary sensitization to MI. Patient 15 was a teacher without known exposure to MI, and patient 17 had a nummular dermatitis without known exposure to MI. Patient 18 worked in a paint factory with known exposure to MCI/MI and MI, and had to stop working there, owing to work-related dermatitis. When he obtained another job, the dermatitis cleared completely.

In conclusion, in patients reacting to both MCI/MI and MI, high patch test reactivity to MCI is in support of MCI

being the primary sensitizer, with cross-reactivity to MI. No cross-reactivity to OIT is expected. In patients reacting to both MCI/MI and MI, high patch test reactivity to MI is in support of MI being the primary sensitizer, with cross-reactivity to MCI. Cross-reactivity to OIT is also expected.

All 46 patients reacting to MCI/MI should, of course, avoid contact with preservatives containing this combination. Those reacting only to MCI/MI 200 ppm and not to MI may tolerate cosmetics and toiletries preserved with Neolone™ 950 at 100 ppm. To elucidate this further, we conducted a double-blind repeated open application test on patients reacting positively to MCI/MI 100 ppm and/or MI at any of the three concentrations (1000, 1500 or 2000 ppm MI), in which a cream containing MI at 100 ppm and a control cream was used (8). In that study, we showed that a patient who reacts positively to MCI/MI 100 ppm will get dermatitis if exposed to a cream containing MI at 100 ppm ($p = 0.016$). The explanation is probably cross-reactivity between MCI/MI and MI. This means that we should tell our patients sensitized to MCI/MI to avoid contact with MI-preserved products, or at least with those preserved with high concentrations, such as 100 ppm. From our study, it is also obvious that an individual who is allergic to MI should avoid products containing this preservative.

References

- 1 Bruze M, Dahlquist I, Fregert S et al. Contact allergy to the active ingredients of Kathon® CG. *Contact Dermatitis* 1987; **16**: 183–188.
- 2 Frick-Engfeldt M, Gruvberger B, Isaksson M, Hauksson I, Pontén A, Bruze M. Comparison of three different techniques for application of water solutions to Finn Chambers®. *Contact Dermatitis* 2010; **63**: 284–288.
- 3 Isaksson M, Gruvberger B, Bruze M. Occupational contact allergy and dermatitis from methylisothiazolinone after contact with wallcovering glue and after a chemical burn from a biocide. *Dermatitis* 2004; **15**: 201–205.
- 4 Bruze M, Dahlquist I, Fregert S et al. Contact allergy to the active ingredients of Kathon® CG in the guinea pig. *Acta Derm Venereol (Stockh)* 1987; **67**: 315–320.
- 5 Bruze M, Dahlquist I, Gruvberger B. Contact allergy to dichlorinated methylisothiazolinone. *Contact Dermatitis* 1989; **20**: 219–220.
- 6 Isaksson M, Bruze M, Gruvberger B. Cross-reactivity between methylchloroisothiazolinone/methylisothiazolinone, and other isothiazolinones in workers at a plant producing binders for paints and glues. *Contact Dermatitis* 2008; **58**: 60–62.
- 7 Bruze M, Gruvberger B, Persson K. Contact allergy to a contaminant in Kathon CG in the guinea pig. *Dermatosen* 1987; **35**: 165–168.
- 8 Isaksson M, Gruvberger B, Gonçalo M et al. Repeated open application test with methylisothiazolinone in individuals sensitive to methylchloroisothiazolinone/methylisothiazolinone. In manuscript.