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<b>DATA EVALUATION RECORD</b>
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**STUDY TYPE:** Skin Sensitization non-guideline (Repeat Open Application Test)-Human**PC CODE:** 107104**DP BARCODE:****TXR#:****TEST MATERIAL (PURITY):** [patch test]: methylisothiazolone (9.7% in 10% ethanol/90% water), or 9.7% methylisothiazolone with 9.2 ug phenoxyethanol/cm<sup>2</sup>. [repeat open application test]: methylisothiazolone (various concentrations) in phenoxyethanol.**SYNONYMS:** MI, MIT, methylisothiazolinone**CITATIONS:** Michael D. Lundov, Claus Zachariae and Jeanne D. Johansen (2011):  
Methylisothiazolinone contact allergy and dose-response Relationships. *Contact Dermatitis* 64: 330-336. MRID 50030303.**LABORATORY:** Conducted at the Department of Dermato-Allergology, National Allergy Research Centre, Copenhagen University Hospital Gentofte, DK-2820 Gentofte, Denmark, and Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, DK-2820 Gentofte, Denmark.**EXECUTIVE SUMMARY:**

Methylisothiazolone (MI) was examined for concentrations that elicited dermal sensitization in human volunteers using both a patch test protocol and a Repeat Open Application Test (ROAT). The study was performed in 11 individuals determined to be previously allergic to MI and 14 control subjects without an allergy to MI. In the first experiment, patch testing was performed using 12 decreasing doses of MI (60, 30, 15, 8.82, 4.41, 2.94, 1.47, 0.441, 0.21, 0.147, 0.105 and 0.0105 µg MI/cm<sup>2</sup>). The purpose of the patch test study was two-fold: (1) to examine the influence of including phenoxyethanol in the MI patch test on reactivity to MI; and (2) to use a previously developed model equation to determine if patch test results could be used to predict responses in the ROAT. Patch tests were applied on the back and occluded for 2 days. Readings from day 3 and day 4 post-exposure were used in statistical calculations, using the scale of Fisher et al.

The ROAT study used the same participants as for the patch test. The conduct of the patch testing and the ROAT portion occurred concurrently. For the ROAT, study participants applied 3 different concentrations of MI and a control solution in a 20 µl volume to a 3 x 3 cm area on the volar aspect of the forearm twice a day for up to 21 days. The intent was to mimic the use of a cosmetic cream applied daily. Concentrations used in the ROAT test were 0, 0.21, 0.105 and 0.0105 µg MI/cm<sup>2</sup> per application.

Results of patch testing with MI showed that all participants reacted to the 60, 30, 15, and 8.82 µg MI/cm<sup>2</sup> concentrations. The lowest eliciting concentration in the patch test was 1.47 µg MI/cm<sup>2</sup>, where 6 participants (55%) showed reactions.

In the ROAT study, 9 of the 11 MI-allergic test subjects completed the 21 day study duration. One test subject completed only 19 days of the protocol due to travel; one subject lost the test materials and missed 4 days of test material application (which days the applications were missed was not stated). Seven test subjects (64%) reacted to the highest dose of MI (0.21 µg/cm<sup>2</sup>). The same 7 test subjects also reacted to the middle dose of MI (0.105 µg/cm<sup>2</sup>). Reactions at the high and mid dose were statistically significant from control. Two (18%) reacted to the lowest dose (0.0105 µg/cm<sup>2</sup>); this was not statistically significantly different from control. None of the participants reacted to the control solution, and none of the control subjects developed any reactions in the ROAT.

In comparing the frequency of reactions to the dose per application in the ROAT and the same dose used in the patch test, none of the participants developed a reaction to the patch test MI doses of 0.21, 0.105 and 0.0105 µg/cm<sup>2</sup>, but in the ROAT, as noted, reactions were noted from the repeated dermal administration of the same concentrations.

The Lowest Adverse Effect Level in this study from the ROAT is 0.0105 MI µg/cm<sup>2</sup>.

This study is classified as **acceptable/non-guideline**. It was not submitted by the registrant for fulfillment of a guideline, but provides quantitative information on elicitation thresholds to MI in humans and can be used to derive a point of departure.

**COMPLIANCE:** This is a published study and as such, did not contain statements of compliance or confidentiality.

## I. MATERIALS AND METHODS

### A. MATERIALS:

<b>1. <u>Test Materials:</u></b>	Methylisothiazolone
<b>Description:</b>	Antimicrobial
<b>Lot/Batch #:</b>	Not provided
<b>Purity:</b>	9.7% active ingredient (Rohm & Hass Europe, Morges, Switzerland) in 10% ethanol and 90%

**CAS # of TGAI:** 2682-20-4

**2. Vehicle and/or positive control:** Neolone 950™; 10% ethanol and 90% water

### B. STUDY DESIGN and METHODS:

The objective of the present study was to experimentally determine eliciting doses of MI dermal sensitization in a patch test and in a repeated open application test (ROAT). The influence of phenoxyethanol on reactivity to MI in the patch test was also examined. The ROAT study is designed to represent more realistic dermal exposures that might occur to potential dermal sensitizers and potential allergic contact dermatitis reactions in people (i.e. repeated, non-occluded exposures).

#### **Study Participants**

According to the paper, 52 people were originally identified from records at Gentofte Hospital from 2005 as having had a positive sensitization reaction to MI at concentrations of 30, 31.5, 45 or 60 µg/cm<sup>2</sup>. Additional people were also recruited who had a positive reaction to methylchloroisothiazolinone (MCI)/MI (100 ppm) from 2000 to 2005, as approximately 40% of MCI/MI-allergic patients have a concomitant reaction to MI. As noted in the paper, “Fifty MCI/MI-allergic patients were invited, and 5 agreed to participate. Before inclusion into the general study, the MCI/MI-allergic patients were patch tested with MI (60 µg/cm<sup>2</sup>).”

Ultimately, as noted, “11 test subjects with MI contact allergy were recruited into the study, 2 women and 9 men aged 37–68 years (mean 49.7 years). No statistically significant difference was found in patch test reactivity between participants and non-participants.”

For this study, *inclusion criteria* for test subjects included age over 18 years, and having a positive sensitization reaction to MI or MCI/MI as noted above. *Inclusion criteria* for control subjects included age of 18 years and not having an allergy to MI or MCI/MI.

*Exclusion criteria* for all subjects included age < 18 years, eczema on the tested area, exposure to ultraviolet light within the last 3 weeks and during the study (e.g. sunbathing or solarium), systemic immunosuppressive therapy, pregnancy, breast feeding, and not being able to cooperate.

### **IRB Approval and Informed Consent**

According to the paper, “All control and test subjects received written and oral information and signed a written consent prior to enrolment in the study. The study was performed according to the Helsinki II declaration, and was approved by the local ethics committee (Capital Region of Denmark, H-2-2010-015).”

### **Patch Testing**

The following methodology on patch testing is reproduced from the study report:

“The patch test series consisted of 12 decreasing doses of MI (CAS 2682-20-4) in Neolone 950™ 9.7% active ingredient (Rohm & Hass Europe, Morges, Switzerland) in 10% ethanol and 90% aqua, and the same 12 doses of MI combined with 9.26 µg phenoxyethanol/cm<sup>2</sup> (CAS 122-99-6; Sigma Aldrich, Schnelldorf, Germany) in the same vehicle. The dose of phenoxyethanol corresponded to a concentration of 0.4%; the combination of this and MI was shown to be an effective preservative in a previous study (8). The blank was phenoxyethanol (9.26 µg/cm<sup>2</sup>) in 10% ethanol and 90% aqua. The doses of MI in the patch test were: 60, 30, 15, 8.82, 4.41, 2.94, 1.47, 0.441, 0.21, 0.147, 0.105 and 0.0105 µgMI/cm<sup>2</sup>. Control subjects were patch tested with only 60 µgMI/cm<sup>2</sup> and the blank.”

“Fifteen microlitres of each dilution was applied on a filter disc in a Finn Chamber® (Epitest, Oy, Finland) on Scanpor® tape (Norgesplaster A/S, Alfarma, Vennessla, Norway). The patch tests were applied on the upper back and occluded for 2 days. Readings were performed on D2, D3 or D4, and on D7, but only reactions from D3 or D4 were used for statistical calculations.”

Reactions to patch testing were scored according to the published paper of Fischer et al. (2011), and shown below:

- 0 - no reaction
- 1- few papules with no erythema and no infiltration
- 2- faint erythema with no infiltration or papules
- 3 - faint erythema with few papules and no homogeneous infiltration
- 4 – erythema and homogeneous infiltration
- 5 - erythema, infiltration, and a few papules
- 6 - erythema, infiltration, and papules
- 7 - erythema, infiltration, papules, and a few vesicles
- 8 - intensive erythema, infiltration, and vesicles

## ROAT Study

The intent of this portion of the study was to mimic the use of a cream preserved with three different concentrations of MI. Assuming the application of 4.2 mg cream/cm<sup>2</sup>/day, the concentrations of MI tested in the ROAT were 0.21, 0.105 and 0.0105 µg MI/cm<sup>2</sup> per application.

The following is reproduced from the study report regarding the ROAT methodology:

“For the ROAT portion of the study, the participants were instructed to apply 20 µl from four different bottles twice a day on four areas on the volar aspect of the forearm. Each area was 3 × 3 cm. Solutions were applied with a fixed-volume micropipette (Acura 815, 20 µl; Buch & Holm, Herlev, Denmark). Each bottle was numbered 1–4, the numbers corresponding to areas on the forearm, also numbered 1–4. The solutions were spread out on the entire area with the tip of the pipette, and allowed to dry by evaporation.”

“Reactions were routinely read on D2, D3 or D4, D7, D14, and D21, and additionally if a reaction occurred between visits.”

Scoring in the ROAT was based on a system developed by Johansen et al. (1997) and illustrated in Johansen et al. (2015) as shown below:

**Table 3.** Modified scale for reading repeated open application test results

Score points per criterion	0	1	2	3	4
1. Involved area of application	0	1–24%	<b>25–49%</b>	50–89%	90–100%
2a. Erythema (involvement)	None	<b>Spotty</b>	Homogeneous	–	–
2b. Erythema (strength)	None	<b>Weak</b>	Medium	Strong	–
3. Papules	None	<5	5–10	>10	Homogeneous infiltration
4. Vesicles	None	<5	5–10	>10	Confluent

The scale applies to a 3 × 3-cm<sup>2</sup> application area. The minimum requirement for a positive test is marked in bold, equivalent to that of 5 points (74, 75).

Each variable (1–4) must be given a score from 0 to 2–4. A positive reaction is characterized by erythema and infiltration as represented by papules, as a minimum, and the reaction should cover altogether at least 25% of the test area. The single scores are added, and a positive reaction will range from 5 points to a maximum of 17 points.

As noted in the report, “If an area scored 5 or above...exposure to this area was terminated. If no reactions occurred or an area scored under 5, all exposures were terminated after 21 days. The threshold dose was the lowest dose with a score of 5 or above, or the lowest dose giving a visible reaction that remained at D21 if the exposure had not been terminated. Readings in both the patch test and the ROAT were blinded and performed by a trained nurse from the allergy laboratory.”

## RESULTS

### Patch Test Results

A total of 11 test subjects and 14 control subjects participated in the study. Nine of the 11 were patients with a previous positive patch test reaction to MI. Five MCI/MI-allergic patients were patch tested with MI. Two developed a positive reaction and were included in the study.

The summary of the patch test results is shown in the following table, reproduced from the paper:

**Table 2.** Number and percentage of test subjects reacting to methylisothiazoline in the patch test with and without phenoxyethanol (9.24  $\mu\text{g}/\text{cm}^2$ )

Patch test concentration ( $\mu\text{g}$ methylisothiazoline/ $\text{cm}^2$ )	Reactions, n (%)	
	Without phenoxyethanol	With phenoxyethanol
60	10 (91)	10 (91)
30	10 (91)	10 (91)
15	10 (91)	10 (91)
8.82	10 (91)	10 (91)
4.41	8 (73)	10 (91)
2.94	7 (64)	6 (55)
1.47	6 (55)	6 (55)
0.441	0	0
0.21	0	0
0.147	0	0
0.105	0	0
0.0105	0	0

As illustrated above, all participants in the patch test study showed a positive reaction to MI at 60, 30, 15, and 8.82  $\mu\text{g}/\text{cm}^2$  MI. No reactions were observed at 0.441  $\mu\text{g}/\text{cm}^2$  and below. There was no significant effect of phenoxyethanol on the dose-response to patch testing of MI. The dose-response for positive reactions, as shown in the figure on the right above, shows no significant difference in the presence or absence of phenoxyethanol.

### ROAT Test Results

The results of the ROAT test are shown in the table below, reproduced from the paper.

Results of ROAT Testing with MI	
Dose (ug MI/cm <sup>2</sup> )	Response n (%)
0.21	7(64)
0.105	7(64)
0.0105	2(18)

#### **E. REVIEWER'S CONCLUSIONS:**

The current study was conducted to examine doses of MI causing contact allergy in a patch test and a repeated open application test (ROAT). While the author made an attempt to employ a model equation to determine whether patch test results could be used to convert to ROAT doses, the review only focuses on the ROAT portion of the study.

The present ROAT study utilized a study population of 11 human test subjects who were demonstrated to be sensitized to MI from previous patch test results, and were again confirmed to be sensitized in a patch test conducted in this study. It also included 14 human control subjects who did not have an allergy or sensitivity to MI. The ROAT study was designed to mimic repeated dermal exposure to a leave-on product containing MI at concentrations used currently in cosmetics. The study authors note that "results cannot be directly compared with exposures from cosmetics or other products, because the vehicle may influence the reactivity in already sensitized individuals." However, there is no direct comparison made to other products in this study. It is noted that other ROAT studies using MI or MCI/MI as the test substance have shown low elicitation threshold concentrations. In Yazar et al 2015 (*British Journal of Dermatology* 173: 115-122; MRID 50035301), a positive reaction was observed in a ROAT study in 7/9 subjects (77%) to MI at 0.24  $\mu\text{g}/\text{cm}^2$ . In Zachariae et al 2006 (*Contact Dermatitis* 55: 160-166; MRID 50035302), positive reactions were observed with MCI/MI at 0.025 and 0.094  $\mu\text{g}/\text{cm}^2$  (7 of 25 subjects (28%) responding at 0.025  $\mu\text{g}/\text{cm}^2$  and 14/25 subjects (56%) responding at 0.094  $\mu\text{g}/\text{cm}^2$ . Compared to Lundov et al., these studies did not conduct as extensive a dose-response. These studies nonetheless provide a weight of evidence to support the derivation of a point of departure for MI from the results of Lundov et al.

The Lowest Adverse Effect Level in this study from the ROAT is 0.0105  $\mu\text{g}/\text{cm}^2$ .

As this study was obtained from the peer reviewed open scientific literature, the OPP guidance document “Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment (USEPA, 2012),” is also applicable when considering the use of open literature studies for risk assessment purposes. This guidance document presents criteria for screening of studies, and criteria for whether the study is of sufficient quality to be used quantitatively. Screening criteria include the following:

1. The toxic effects are related to defined chemical exposure;
2. The toxic effects are on an appropriate test animal species;
3. The presence or absence of toxicological effects is observed;
4. A chemical concentration/dose or application rate is reported;
5. An explicit duration of exposure is included;
6. Toxicology information is reported for the chemical of interest or its structural analog;
7. The article is available in the English language;
8. The study results are presented as a full article (i.e., not an abstract);
9. The paper is a publically available document;
10. The paper is the primary source of the data;
11. Treatment(s) are compared to acceptable controls;
12. The location of the study (e.g., laboratory vs. field) is reported;
13. Adequate data are provided on the chemical tested (i.e., test article characterization);
14. Adequate data are provided on the species tested;
15. The study results (findings) are adequately reported; and
16. The study findings are relevant to assessing human health risks

The current study meets all of the screening criteria. From review of this study, it is concluded that the study is appropriate for quantitative use, i.e. establishing a point of departure for risk assessment. This is concluded based on the study meeting the criteria as established in the guidance as follows:

- The dose from the open literature study is lower (*i.e.*, more sensitive) than the lowest dose from a comparable registrant-submitted study – this criterion is met; there was no registrant submitted study, but the proposed point of departure is lower than data from other published studies.
- The open literature data are reported in (or have the ability to be converted to) units that can be compared to other study results- results are reported in  $\mu\text{g}/\text{cm}^2$ , which can be compared to other studies- this criterion is met.
- Sufficient information is provided in the open literature to substantiate whether the study conclusions/endpoints/doses are accurate, reliable, and reasonable and a judgement can be made that the study findings could potentially be replicated – it is the judgement of the reviewer that this criterion has been met.

Given the above, there are still some weaknesses of this study:



- Low number of participants in this study
- Males and females are not represented equally
- The history of exposure to MI is not known for each individual
- The individual ROAT data results were not available from the study authors for statistical analysis (personal communication with study authors)
- It was not possible to reproduce several of the reported statistical analyses in the paper
- Logistic regression of the ROAT test results has high uncertainty because the logistic regression was only fitted to three dose levels and so numerous curves with different shapes could be fit to the same data