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DATA EVALUATION RECORD

STUDY TYPE: Skin Sensitization non-guideline (Repeat Open Application Test)-Human

PC CODE: 107104 **DP BARCODE:**
TXR#:

TEST MATERIAL (PURITY): methylisothiazolone (9.5% in water)

SYNONYMS: MI, MIT, methylisothiazolinone

CITATIONS: Yazar, K.; Lundov, M.D.; Faurschou, A.; Matura, M.; Boman, A.; Johansen, J.D.; Liden, C. (2015): Methylisothiazolinone in rinse-off products causes allergic contact dermatitis: a repeated open application study. British Journal of Dermatology 173: 115-122. MRID 50035301.

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Center for Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden.

EXECUTIVE SUMMARY:

Methylisothiazolone (MI) was examined for the potential to cause allergic contact dermatitis at or below the allowed concentrations of MI (100 ppm) found in cosmetic rinse-off products. Half of the allowed concentration (50 ppm) was also tested. The study was performed in 19 individuals determined to be previously allergic to MI and 19 controls with no allergy to MI. Subjects allergic to MI were first confirmed to be sensitive through patch testing using a series of MI dilutions (0.2, 0.1, 0.05, 0.025, 0.01 and 0.0016 % solution or 60, 30, 15, 7.5, 3 and 0.48 ug/cm^2). Patch tests were applied on the upper back and occluded for 2 days. Readings were performed on day 4 only and were scored according to the scale of Fisher et al (Br J Dermatol 2007; 157:723–729).

For the repeat open application test (ROAT), three liquid rinse-off products prepared specifically for this study were tested. One liquid soap contained MI in a concentration of 100 ppm (0.48 ug /cm^2 per application), which is the maximum allowed concentration in cosmetics, and one liquid soap contained 50 ppm MI (0.24 ug /cm^2 per application).The third product served as negative control and was identical except that it did not contain MI and instead used the preservatives methyl- and ethylparaben. The same participants in the patch test study participated in the ROAT. The ROAT was carried out as follows: 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, nine 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. Area of application was a 5 x 10 cm^2 area volar aspect of the forearm twice a day for up to 21 days. Exposure duration was 20-25 seconds per application, with rinse-off and gentle drying with paper towels after each application.

Results of patch testing with MI showed that all test participants reacted to the 15 µg MI/cm^2 concentration, and three participants reacted to 0.48 µg MI/cm^2. In the ROAT study, 10/10 subjects were positive in the ROAT at 100ppm (0.48 µg/cm^2 per day). Seven of nine subjects were positive in the ROAT at 50 ppm (0.24 µg/cm^2 per day). Both responses were statistically significant vs the control group, where no positive reaction was observed.

The results of this study, where positive reactions were observed in 7/9 subjects (77%) to MI at 0.24 µg/cm^2, are similar to results of other ROAT studies where low elicitation threshold concentrations were reported for MI and MCI/MI. In Zachariae et al. (*Contact Dermatitis* 55: 160-166 (2006); MRID 50035302), positive reactions were observed with MCI/MI at 0.025 and 0.094 µg/cm^2 (7 of 25 subjects (28%) responding at 0.025 µg/cm^2 and 14/25 subjects (56%) responding at 0.094 µg/cm^2). In Lundov et al., an 18% response was observed at a concentration of approximately 0.0105 µg/cm^2 in the ROAT portion of the study. These studies provide a weight of evidence to the results of Lundov et al for deriving a point of departure for an elicitation threshold to MI.

The Lowest Adverse Effect Level (LOAEL) for this study is 0.24 µg/cm^2; there was no NOAEL established in this study.

This study is classified as **acceptable/non-guideline.** It was not submitted by the registrant for fulfillment of a guideline, but provides information on elicitation thresholds to MI in humans. This study is considered adequate for qualitative use in risk assessment in a weight-of-evidence determination for dermal sensitization elicitation threshold to MI.

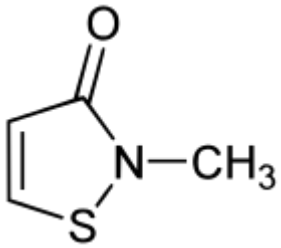
COMPLIANCE: This is a published study and as such, did not contain statements of compliance or confidentiality.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Materials: Methylisothiazolinone
Description: Liquid Soap Preparation (commercial)
Lot/Batch #: no. rp-278529-1410, 2
Purity: 9.5% active ingredient in water

CAS # of TGAI: 2682-20-4



2. Vehicle and/or positive control: liquid hand soap product "containing commonly used ingredients and formulations" according to the paper. These include water, sodium laureth sulfate, cocamidopropyl betaine, peg-7 glyceryl cocoate, sodium chloride, and citric acid. Negative control soap contained methyl- and ethylparaben as preservative in place of MI.

B. STUDY DESIGN and METHODS:

The objective of the present study was to examine whether allowed concentrations of MI in cosmetic rinse-off products have the potential to cause allergic contact dermatitis. To this end, human subjects were recruited for patch testing of MI at various concentrations to determine the presence of contact allergy, and for testing in the ROAT protocol to determine if the allowed concentration of MI (100ppm) and half that concentration (50ppm) had the potential to elicit contact dermatitis in these already sensitized individuals when the product is a rinse-off product.

Study Participants

According to the paper, "The study adhered to the tenets of the Declaration of Helsinki and was approved by the regional ethics review boards in Stockholm, Sweden (ID no. 2013/976-31/4) and Capital Region, Denmark (ID no. H-4-2013-094). All subjects gave written informed consent before taking part in the study."

Patients with dermatitis and contact allergy to MI were invited to participate in the study. They had been diagnosed by patch testing with MI (2000 ppm) at the outpatients clinics of the Centre for Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden, or Gentofte Hospital, Copenhagen, Denmark. In total, 19 test subjects and 19 control subjects participated in the study.

For this study, *inclusion criteria* for test subjects were confirmatory patch testing prior to inclusion in the study showing at least one positive reaction to MI [minimum criteria: positive erythema and infiltration, and negative patch test to paraben mix]. *Inclusion criteria* for control subjects were no allergy or sensitivity to 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, and breast feeding.

IRB Approval and Informed Consent

According to the paper, "The study adhered to the tenets of the Declaration of Helsinki and was approved by the regional ethics review boards in Stockholm, Sweden (ID no. 2013/976-31/4) and Capital Region, Denmark (ID no. H-4-2013-094). All subjects gave written informed consent before taking part in the study."

Patch Testing

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

"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 µg MI 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, and 15 mL of each patch-test solution was 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 2 days. Readings were performed at day 4 only, and the readings were blinded. In Sweden, the readings were performed by K.Y. in combination with C.L. and/or A.B., and in Denmark, the readings were performed by expert nurses, who do all readings in the clinic."

The scale of Fischer et al. (Br J Dermatol 2007; 157:723–729) was used for scoring of dermal responses. This scale was used to identify weaker patch test 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.

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 determine the potential for contact allergy after use of a rinse-off product that contains MI. Concentrations of 100 ppm (0.48 µg/cm^2) and 50 ppm (0.24 µg/cm^2) were used in this study. The 100ppm concentration represents the allowed concentration of MI in cosmetic products (at the time of publication of this paper; there is debate ongoing about the level of MI that should be allowed in cosmetics).

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

"Test areas of 5 x 10 cm^2 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, nine 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 instructed thoroughly to apply the preparations five times per day, with a minimum of 2 h between each application."

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. This is the same scale use by Lundov (2011).

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%	**25–49%**	50–89%	90–100%
2a. Erythema (involvement)	None	**Spotty**	Homogeneous	–	–
2b. Erythema (strength)	None	**Weak**	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^2 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, "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 one papule'. If a positive reaction occurred on either of the two arms, all further applications by the participant were stopped. If no reaction, the test proceeded for a maximum of 21 days."

RESULTS

Patch Test Results

The patch testing results in the paper stated that all of the test subjects reacted to the 15 µg/cm^2 concentration. The data also showed that 13 of 19 test subjects reacted to the 3 µg/cm^2 concentration, and three test subjects reacted to the lowest dose tested in the patch test (0.48 µg/cm^2).

ROAT Test Results

For those test subjects using the 100 ppm (0.48 µg/cm^2) concentration in the ROAT, the applied doses as shown in Table 3 of the paper ranged from 0.36-0.53 µg/cm^2. According to the paper, all test subjects (10/10) developed positive reactions in the ROAT, with reactions occurring within 4-11 days (7.3 days average). For those test subjects using the 50 ppm (0.24 µg/cm^2) concentration, 7 of the 9 developed positive reactions within 5-21 days (8 days on average). None of the test subjects had a positive reaction to the soap without MI. No control subject had a reaction to any soap application.

According to the paper, "The difference in response to the 100 ppm and 50 ppm MI-containing soap, respectively, in MI-allergic subjects compared with control subjects was statistically significant (Fisher's exact test, P = 5x 10^{-8} and P = 0.00003."

Comparison of Patch test and ROAT results at the same Concentration

According to the paper, the results from the 10 test subjects exposed to the 0.48 µg/cm^2 concentration in the Patch test were compared to the results of the participants' reaction to the ROAT test at this same concentration. The paper stated that "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 with the patch test was statistically significant in the McNemar's test (P = 0.00195)." This statement does not agree with the claim earlier in the paper that three participants did react to the 0.48 µg/cm^2 concentration from the patch test.

E. REVIEWER'S CONCLUSIONS:

The current study was conducted to examine doses of MI causing contact allergy in a patch test and a ROAT using a rinse-off product.

The present ROAT study utilized a study population of 19 test subjects who were demonstrated to be sensitized to MI following a patch test conducted in this study, and 10 control subjects who did not have an allergy or sensitivity to MI. The ROAT study was designed to mimic repeated dermal exposure to a rinse-off product containing a level of MI that is currently used in cosmetic products of this type. The soap used, according to the paper, contained "commonly used ingredients and concentrations." Thus, it was not to be expected that this soap differed in any significant way from what is currently marketed and what people are exposed to, although the exact composition of the soap is not known.

The results of the ROAT study showed that 7 of 9 test subjects exposed to the lowest concentration tested in the ROAT (0.24 µg/cm^2) showed a positive response to MI. As this concentration is half of the currently allowed concentration of MI, the study authors suggest that levels of MI currently used in rinse-off cosmetics are not safe for previously sensitized individuals.

The results of this study, where positive reactions were observed in 7 of 9 test subjects (77%) to MI at 0.24 µg/cm^2, are similar to results of other ROAT studies where low elicitation threshold concentrations were reported for MI and MCI/MI. In Zachariae et al. (*Contact Dermatitis* 55: 160-166 (2006); MRID 50035304), positive reactions were observed with MCI/MI at 0.025 and 0.094 µg/cm^2 (7 of 25 subjects (28%) responding at 0.025 µg/cm^2 and 14/25 subjects (56%) responding at 0.094 µg/cm^2. In Lundov et al., (*Contact Dermatitis* 64: 330-336,2011), an 18% response was observed at a concentration of approximately 0.0105 µg/cm^2 in the ROAT portion of the study. These studies together provide a weight of evidence in support of derivation of a point of departure from Lundov et al. for an elicitation threshold for MI.

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. It is concluded that the study is appropriate for quantitative use as part of a weight of evidence determination in conjunction with other ROAT studies that have been reviewed (MRIDs 50035302, 50035303, 50035304) in addition to the present study. This is concluded based on 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 not met as this study did not show the lowest 'dose' in comparison to the Lundov et al. study.
• 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 µg/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.

Some weaknesses of this study include: the number and sex of the study participants was not provided; it is not clear whether the study design was blinded.