EPA Reviewer: Tim McMahon, Ph.D **Signature**: _____
RASSB, Antimicrobials Division (7510P) **Date**: 11/29/2016
EPA Secondary Reviewer: LCDR Jonathan Leshin, Ph.D. **Signature**: _____
RASSB, Antimicrobials Division (7510P) **Date:** 11/29/2016

DATA EVALUATION RECORD

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

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

TEST MATERIAL (PURITY): Methylchloroisothiazolinone/Methylisothiazolone (MCI/MI aqueous solution). Patch testing: 100ppm MCI/MI. ROAT study: 7.5 ppm and 2 ppm in two separate studies.

SYNONYMS: Kathon CG®; MCIT/MIT

CITATIONS: Zachariae, C.; Lerbaek, A.; McNamee, P.; Gray, J.; Wooder, M.; Menne, T. (2006): 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. *Contact Dermatitis* 55: 160-166. MRID 50035302.

LABORATORIES: Department of Dermatology, Gentofte Hospital, University of Coenhagen, 2900 Hellerup, Denmark; Proctor and Gamble Technical Centres Limited, Rusham Park Technical Centre, Whitehall Lane, Egham, Surrey TW20 9NW, UK; Rohm and Haas, Herald Way Coventry CV# 2RQ, UK. MRID 50035302.

EXECUTIVE SUMMARY:

This study examined the influence of time and dose per unit area on elicitation threshold for MCI/MI using a repeated open application test (ROAT) protocol. A double blind, placebo controlled dose-response ROAT was conducted in 25 subjects with confirmed allergy to MCI/MI by patch test results and 10 non-MCI/MI allergic control subjects. The first ROAT study exposed all test and control subjects to 0.025 µg/cm^2 (2 ppm) MCI/MI for 4 weeks. Subjects were then allowed a 4 week washout period. A second ROAT was then conducted on these same subjects using 0.094 µg/cm^2 (7.5 ppm) MCI/MI for 4 weeks.

In the first ROAT (0.025 µg/cm^2), 7 of the 25 test subjects showed a positive reaction with an average time to reaction of 16.5 days. Five weak, 2 moderate, and 0 strong reactions were

observed. In the second ROAT (0.094 µg/cm^2), 14 of 25 test subjects showed a positive result with and average time to reaction of 12.1 days. Seven weak, 6 moderate, and 1 strong reaction were observed. The difference in the number of positive reactions to the 0.025 µg/cm^2 application of MCI/MI was stated by the investigators as being not significantly different than vehicle control, while the number of positive reactions at 0.094 µg/cm^2 was significantly different from vehicle control. The number of positive reactions in the ROAT at 0.094 µg/cm^2 was statistically different than the number of positive reactions at 0.025 µg/cm^2. All subjects reacting to the 0.025 µg/cm^2 concentration had either a similar or worse strength skin reaction at 0.094 µg/cm^2 MCI/MI. The control subjects had no reaction to either concentration during the ROATs. While it was not possible to establish an elicitation threshold for MCI/MI in this study, the data suggest that the LOAEL is in the area of 0.025 µg/cm^2. It would likely be somewhat lower, as this concentration showed a 28% response rate based on the reactions of the 25 test subjects.

The results of this study, where positive reactions were observed with MCI/MI at 0.025 and 0.094 µg/cm^2 (7 of 25 test subjects (28%) responding at 0.025 µg/cm^2 and 14/25 subjects (56%) responding at 0.094 µg/cm^2), are supported by the results of other studies on MI and MCI/MI showing low elicitation threshold concentrations. In Yazar et al. (British Journal of Dermatology 173: 115-122 (2015); MRID 50035301), a positive reaction was observed in a ROAT study in 7/9 subjects (77%) to MI at 0.24 µg/cm^2. In Lundov et al., an 18% response to MI was reported at 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 supporting derivation of a point of departure for an elicitation threshold to MI.

This study is classified as **acceptable/non-guideline.** It was not submitted by the registrant for fulfillment of a guideline, and cannot be used quantitatively, but provides qualitative information in a weight-of-evidence for determination of the 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: Chloromethylisothiazolinone/Methylisothiazolinone (aqueous solution 3:1 ratio of MCI/MI)

Description: antimicrobial
Lot/Batch #: Not provided
Purity: not provided

2. Vehicle and/or positive control: The MCI/MI test material did not have a specific description in this study. It was used as an aqueous solution in this study for patch testing and for the ROAT studies. The vehicle control was deionized water with 10% ethanol.

B. STUDY DESIGN and METHODS:

The objective of the present study was to determine the effect of time and dose per unit area on the elicitation threshold for MCI/MI. Two ROAT studies were designed to investigate these questions.

Study Participants

Twenty-nine eczema patients were enrolled in this study. These patients were all over 18 years of age and had previously demonstrated contact allergy to MCI/MI at 100ppm, a standard patch test concentration. The subjects were re-tested in a patch test to confirm contact allergy for this study. Ten additional volunteers were recruited as controls and tested negative to MCI/MI at 100ppm.

Patches in the patch test were applied for two days to the upper back using Finn chambers and Scanpor tape. Readings were conducted on day 2, 3-4, and 7.

For this study, *inclusion criteria* other than being 18 years of age or older were not stated.

Exclusion criteria were stated as "pregnant or lactating women and subjects who had a current acute or chronic widespread eczema at any site were excluded from the study."

IRB Approval and Informed Consent

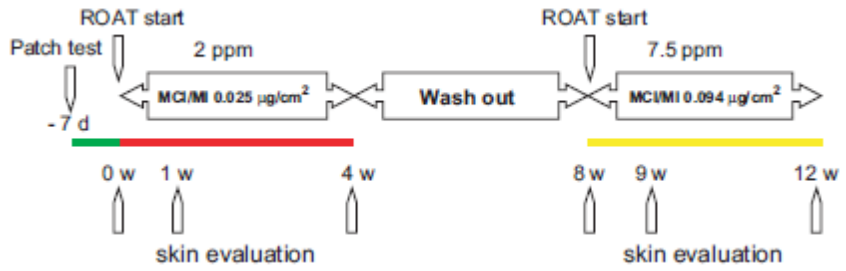
According to the paper, "The local ethics committees approved the study, and all participants were included after informed consent was obtained from them. The study was performed in accordance with Good Clinical Practice Guidelines."

Patch Testing

Patch testing was conducted with 100 ppm MCI/MI using 15 microliters of solution on a 0.5 cm^2 patch. Patch testing was also conducted with vehicle control (deionized water in 10% ethanol). Twenty-nine MI-allergic subjects were tested. Four of these potential test subjects had reactions that were negative or were doubtful and these subjects did not participate further in the study.

ROAT Study

The ROAT studies were designed as double-blind, placebo-controlled studies. The study design is depicted in the following figure, reproduced from the paper:



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

"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 x 3 cm on the volar part of either the left or right forearm. 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. In the first ROAT period (ROAT 1), test material containing 0.025 mg/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 mg/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."

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.
Scoring in the ROAT was made according to a grading scale as presented, reproduced below from the paper:

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					

This scoring method is the same as that used by Lundov et al. (*Contact Dermatitis* 64: 330-336. MRID 50030303), as published by Johansen et al. (1997) and illustrated in Johansen et al. (2015).

RESULTS

Patch Test Results

The patch testing results in the paper were presented as part of the results shown in Table 3 of the paper, shown below.

Table 3. Results of the 29 patients diagnostically patch tested to methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) [3 μg/cm^2 aq. (100 p.p.m.)]

Patch test results	MCI/MI (3 μg/cm^2) (aq.) (100 p.p.m)
+++	1
++	8
+	16
Negative or doubtful	4

As noted previously, four potential test subjects had negative/doubtful reactions to the patch test and did not participate in the ROAT portion of the study.

ROAT Test Results

The results of the ROAT study are also shown below (Table 5, reproduced from the report):

Table 5. Repeated open application test (ROAT) outcome in methylchloroisothiazolinone/methylisothiazolinone (MCI/MI)-allergic individuals exposed to 0.025 μg/cm^2 (ROAT 1) and 0.094 μg/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.

In the first ROAT (0.025 μg/cm^2), 7 of the 25 test subjects showed a positive reaction with an average time to reaction of 16.5 days. Five weak, 2 moderate, and 0 strong reactions were observed. In the second ROAT (0.094 μg/cm^2), 14 of 25 test subjects showed a positive result with an average time to reaction of 12.1 days. Seven weak, 6 moderate, and 1 strong reaction were observed. The difference in the number of positive reactions to the 0.025 μg/cm^2 application of MCI/MI was stated by investigators as not significantly different than vehicle control, while the number of positive reactions at 0.094 μg/cm^2 was significantly different from vehicle control. The number of positive reactions in the ROAT at 0.094 μg/cm^2 was statistically different than the number of positive reactions at 0.025 μg/cm^2. All subjects reacting to the 0.025 μg/cm^2 concentration had either a similar or worse strength skin reaction at 0.094 μg/cm^2 MCI/MI. The vehicle control data were not presented in this study.

E. REVIEWER'S CONCLUSIONS:

The present ROAT study utilized a study population of 25 test subjects who were demonstrated to be sensitized to MCI/MI from patch testing conducted previously, and 10 control subjects without an allergy or sensitivity to MCI/MI. The ROAT study was designed to investigate the effect of time and concentration (dose per unit area) on elicitation threshold of MCI/MI.

The study was conducted to examine doses of MCI/MI causing contact allergy in a patch test and two repeated open application tests with two concentrations of MCI/MI using an aqueous solution that was applied and left on. The stated purpose of the paper – to evaluate the importance of time and dose per unit area on elicitation threshold- was addressed in the study design. It is clear that the 0.094 μg/cm^2 concentration resulted in a higher percentage of positive responses than the 0.025 μg/cm^2 concentration. The data suggest that the average time to develop a positive reaction was also shorter at the higher concentration of MCI/MI, and there was a slight shift in the severity of the reaction at the higher concentration. The influence of time on elicitation was illustrated in a Kaplan-Meier plot of days of exposure vs. the number of persons not reacting to MCI/MI in the ROAT tests. This plot appears to show that the number of persons not reacting to MCI/MI at the 0.094 μg/cm^2 concentration is lower than that of those not reacting

at the 0.025 µg/cm^2 concentration. Since the duration of exposure to MCI/MI was 4 weeks at each concentration, it seems that the only variable investigated was concentration, and not time. It should be expected that a higher concentration of a contact allergen such as MCI/MI would result in a higher response rate. The initial decrease in those not responding between the two concentrations was not discussed in the paper, and it is not possible to tell whether the differences between the two concentrations are significant.

The results of the ROAT study showed positive reactivity to the ROAT test at 0.025 µg/cm^2 concentration and can be considered the LOAEL; no NOAEL was established.

The present study tested two concentrations of MCI/MI and did not test MI. However, the lowest concentration of MCI/MI eliciting a positive response in this study (0.025 µg/cm^2) can be used in conjunction with results Yazar et al. 2015 (*British Journal of Dermatology* 173: 115-122), who reported positive responses in a human ROAT study with MI at 0.24 µg/cm^2, and Lundov et al. (*Contact Dermatitis* 64: 330-336) who observed a positive response to a concentration of 0.0105 µg MI/cm^2 in a ROAT study. The data in this study lends a weight of evidence to supporting derivation of a point of departure from the Lundov et al. study for an elicitation threshold to MI.

This study is classified as **acceptable/non-guideline.** It was not submitted by the registrant for fulfillment of a guideline, but provides qualitative information in a weight-of-evidence for determination of the dermal sensitization elicitation threshold to 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 does not meet all of the screening criteria; criterion #13 was not met (there was little information on the test article characterization). While not all characteristics are provided for the test article, there was sufficient information to draw some conclusions about the article as a whole. It is known that MCI/MI is manufactured as a 3:1 ratio mixture, and the results are consistent with MCI/MI as a potent dermal sensitizer. The study is appropriate for qualitative use as part of a weight of evidence determination in conjunction with other ROAT studies that have been reviewed (MRIDs 50035301, 50035303, 50035304) in addition to the present study. This is concluded based on the interpretation of 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 criteria is not met as the 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.

Weaknesses of this study were: the statistical results of Fisher exact tests could be reproduced, but the logistic regression analysis comparing the two doses could not be reproduced.