

# Can glutathione-containing emollients inactivate methylchloroisothiazolinone/methylisothiazolinone?

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Many persons are exposed to preservatives/biocides based on methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), since toiletries and cosmetics, as well as products/water-based systems used occupationally, may all contain MCI/MI. In toiletries and cosmetics, the MCI/MI concentration is often below 15 ppm (0.0015%). In some industries, workers handle high concentrations of MCI/MI, which can cause chemical burns and induce sensitization if skin is exposed. Contact allergy to MCI/MI is common and reports on chemical burns have been published. Thus, there is a need for prevention of skin diseases caused by MCI/MI. The inactivation of MCI/MI by glutathione (GSH) in emollients, containing different amounts of lipids, was studied by HPLC. Various amounts of GSH were added to Fenuril®, Essex®, and Locobase®, giving 3 preparations of each emollient containing 0.10%, 0.50% and 2.0% GSH, respectively. The inactivation of 15 ppm MCI/MI and the total inactivating capacity of GSH in these preparations, kept at room temperature and refrigerated, was studied over a period of 6 months. The inactivating capacity of GSH in the emollients was almost equivalent, regardless of the lipid contents of the emollients, type of storage and age. On the other hand, the GSH concentration in the emollients had a crucial importance on the inactivation of MCI/MI. Emollients containing 2% GSH were capable of inactivating up to 2400 ppm MCI/MI.

**Key words:** emollients; glutathione; inactivation; methylchloroisothiazolinone; methylisothiazolinone; preservatives; skin care creams; prevention; allergic contact dermatitis; chemical burns.  
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*Accepted for publication 17 December 1997*

Methylchloroisothiazolinone / methylisothiazolinone (MCI/MI) are active ingredients in several preservatives. Many persons are exposed to MCI/MI, since toiletries and cosmetics, as well as products/water-based systems used occupationally, may all contain MCI/MI. The MCI/MI concentration, especially in consumer products, is often low, in most products below 15 ppm (0.0015%) (1). Nevertheless, many consumers have been sensitized when using such products (2). Furthermore, in some industries workers handle concentrated solutions of MCI/MI. A single exposure to high concentrations of MCI/MI can cause a severe chemical burn as well as induce sensitization, since MCI/MI are both strong irritants and sensitizers (3–9).

Sodium bisulfite has been shown to have the capacity to inactivate MCI/MI, with subsequent reduction of the irritating and sensitizing capacity

(10). However, sodium bisulfite has some potential hazardous effects (11–13). On theoretical grounds, glutathione (GSH) should be an alternative as a more appropriate inactivator of MCI/MI, since it is an endogenous non-toxic substance. The aim of this study was to investigate chemically the inactivation of MCI/MI by GSH in 3 common emollients on the Swedish market containing various amounts of lipids.

## Materials and Methods

### *Substances*

Kathon® CG, containing 1.125% 5-chloro-2-methyl-4-isothiazolin-3-one (MCI) and 0.375% 2-methyl-4-isothiazolin-3-one (MI), was obtained from Rohm and Haas Company, Philadelphia,

USA. Glutathione of high quality was purchased from ICN, Biomedicals Inc., Costa Mesa, USA. The structures of MCI/MI and GSH are shown in Fig. 1.

### Emollients

From the hospital pharmacy 3 common emollients on the Swedish market, Fenuril® (F), Essex® (E), and Locobase® (L), manufactured by Pharmacia Upjohn AB, Stockholm, Schering-Plough, Stockholm, and Yamanouchi, Malmö, respectively, were purchased. The content of lipids in the 3 emollients is 18.5% w/w (F), 28% w/w (E), and 70% w/w (L), respectively.

### Preparation of GSH-containing emollient

To 3 jars, containing 599.4, 597.0, and 588.0 g of one and the same emollient, 0.60 g, 3.00 g, and 12.00 g GSH, respectively, was added and the mixtures were stirred for 20 min. After the additions the preparations contained 0.10%, 0.50%, and 2.0% w/w GSH, respectively. The same procedure was performed with all 3 emollients. Henceforth these GSH-containing preparations of F, E and L are called F 0.1, F 0.5, F 2.0, E 0.1, E 0.5, E 2.0 and L 0.1, L 0.5, L 2.0, respectively.

### High-performance liquid chromatography (HPLC)

An isocratic HPLC system, consisting of an SP Spectra Series P200 pump, an SP Spectra System UV1000 detector and an SP ChromJet integrator, was used. The system was equipped with a Rheodyne 7125 injector and with a 20  $\mu$ l loop. The column (4 mm i.d.  $\times$  250 mm) was packed with 5  $\mu$  Nucleosil C<sub>18</sub>. The UV detector was operated at 280 nm and the flow rate was 1 ml/min. The mobile phase was methanol/acetonitrile/water 20/10/70 v/v. The detec-

tion limit has been shown to be 0.05 ppm of MCI/MI in a water solution (1).

### Inactivation of MCI/MI with aqueous GSH

To 2.0 ml freshly prepared aqueous GSH 0.10% w/v, 20  $\mu$ l of a solution containing 1500 ppm MCI/MI was added and mixed for 1 min. Samples from the reaction mixture were injected into the chromatograph at the reaction times 2, 5, 10, 15, 20, 30 and 60 min and the peaks in the chromatograms were compared to the peaks corresponding to MCI and MI in a chromatogram of Kathon® CG. Corresponding investigations were performed with aqueous GSH 0.50% and 2.0%, respectively.

### Inactivation of MCI/MI by GSH in emollients

**Inactivation of 15 ppm MCI/MI.** All 9 GSH-containing preparations were investigated concerning inactivation of MCI/MI. 20  $\mu$ l of a dilution of Kathon® CG containing 1500 ppm (0.15%) MCI/MI was added to 2.00 g of a GSH-containing preparation and mixed vigorously for 1 min. 1.00 g of the MCI/MI-containing preparation was weighed and 9.0 ml of an extraction solution and some boiling chips were added. The mixture was shaken vigorously for about 1 min and then the extracts were injected into the HPLC. About 3 min passed from the addition of MCI/MI to the emollient until injection into the HPLC. The same procedure was performed with the emollients without GSH. The peaks in the chromatograms were compared to the peaks corresponding to MCI and MI in a chromatogram of Kathon® CG. Duplicate samples were prepared and analyzed.

3 different extraction solutions were used: (i) the mobile phase (methanol/acetonitrile/water 20/10/70) (F preparations), (ii) methanol/water/acetonitrile 10/35/55 (E preparations), and (iii) ethanol/water 50/50 (L preparations).

**Inactivating capacity of GSH in emollients.** In order to determine the total inactivating capacity of GSH in emollients, various amounts of MCI/MI were added to the GSH-containing preparations until there was no more inactivating capacity according to the HPLC analyses. To give a total amount of 2.00 g sample to analyze, 20 up to 640  $\mu$ l of Kathon® CG (15000 ppm MCI/MI) were added to 1.98 to 1.36 g GSH-containing emollient, respectively. The sample preparations and extraction procedures were performed in the same way as in A. The extracts were injected into the HPLC.

### Stability investigation

In order to investigate the stability of GSH in the emollients, each GSH-containing preparation was

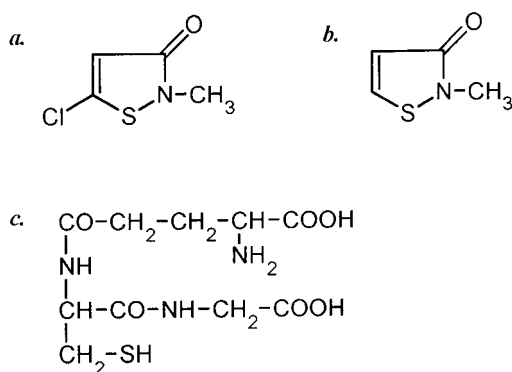


Fig. 1. The structures of (a) 5-chloro-2-methyl-4-isothiazolin-3-one (MCI), (b) 2-methyl-4-isothiazolin-3-one (MI), and (c) glutathione.

divided in 2 equal parts and kept in 2 separate dark glass jars. One jar was kept at room temperature (20–22°C) and the other one refrigerated (6–7°C). The inactivation of MCI/MI was investigated as described in A and B. The inactivation of 15 ppm MCI/MI was studied every 2 to 4 weeks over a period of 6 months, and the capacity studies were performed every 1 to 2 months over the same period.

### Results

The concentration of MCI/MI decreased slowly after addition to a 0.10% aqueous solution of GSH (Fig. 2). At 20 min's reaction time, 0.9 of the added 15 ppm MCI/MI was detected. 1.0 ppm MCI/MI was demonstrated after 5 min's reaction with 0.50% GSH (Fig. 2). Aqueous GSH at 2.0% inactivated MCI/MI more rapidly and after 2 min only 0.3 ppm MCI/MI could be detected (Fig. 2).

After addition of 15 ppm MCI/MI to F, E, and L without GSH, 14.7, 15.0 and 14.7 ppm MCI/MI, respectively, was detected by HPLC. The results of inactivation of 15 ppm MCI/MI by GSH in emollients are shown in Figs. 3a–c. After addition of 15 ppm to freshly prepared GSH-containing emollients at 2.0%, 0.1 ppm–0.4 ppm MCI/MI was detected and the highest demonstrated MCI/MI concentrations were 1.6 ppm after about 18 weeks (Fig. 3c). Equivalent results were obtained for refrigerated preparations and preparations kept at room temperature (Fig. 3c).

After addition of 15 ppm MCI/MI to freshly prepared GSH-containing emollients at 0.50%, 0.1–0.6 ppm MCI/MI was demonstrated. During the study period, the MCI/MI concentration tended to increase (Fig. 3b). The highest detected MCI/MI concentration was 8.9 ppm. The results were similar regardless of storage (Fig. 3b).

1.6–2.9 ppm MCI/MI was detected after addition of 15 ppm MCI/MI to freshly prepared GSH-con-

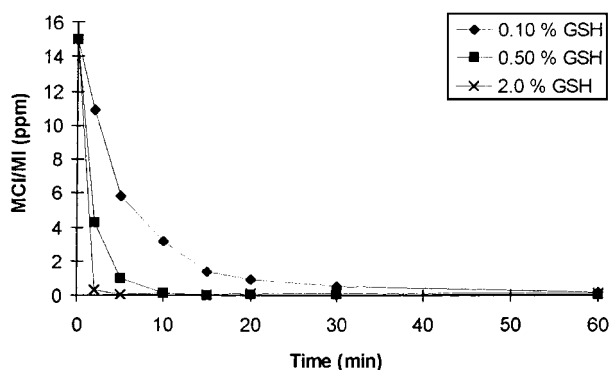


Fig. 2. The inactivation of 15 ppm methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) with aqueous glutathione (GSH) solutions at 0.10%, 0.50% and 2.0%, respectively.

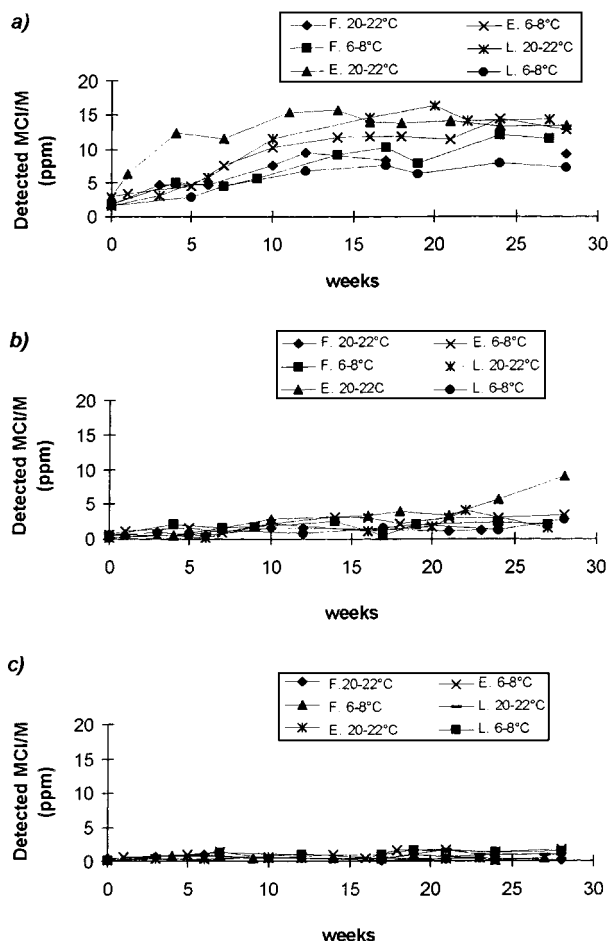


Fig. 3. The inactivation of 15 ppm methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) added to samples of glutathione-containing emollients, Fenuril® (F), Essex® (E), and Locobase® (L) over a period of 6 months. (a) Inactivation of 15 ppm MCI/MI by 0.10% glutathione (GSH) in F, E, and L kept at room temperature (20–22°C) and refrigerated (6–7°C), respectively. (b) Inactivation of 15 ppm MCI/MI by 0.50% GSH in F, E, and L kept at 20–22°C and 6–7°C, respectively. (c) Inactivation of 15 ppm MCI/MI by 2.0% GSH in F, E, and L kept at 20–22°C and 6–7°C, respectively.

taining emollients at 0.10% (Fig. 3a). The inactivating capacity decreased continuously during the period, with no apparent relation to storage (Fig. 3a).

The total inactivating capacities of 0.10%, 0.50% and 2.0% GSH in F, E, and L, respectively, were very similar within the respective concentration group, regardless of storage and age. Therefore only 1 emollient from each concentration group is shown in Fig. 4. GSH in L 2.0 was shown to inactivate up to 2400 ppm MCI/MI independent of age (Fig. 4c), and GSH in E 0.5 was capable of inactivating a few hundred ppm MCI/MI (Fig. 4b). Fig. 4a shows an almost linear graph, demonstrating GSH in F 0.1 to have a limited inactivation capacity.

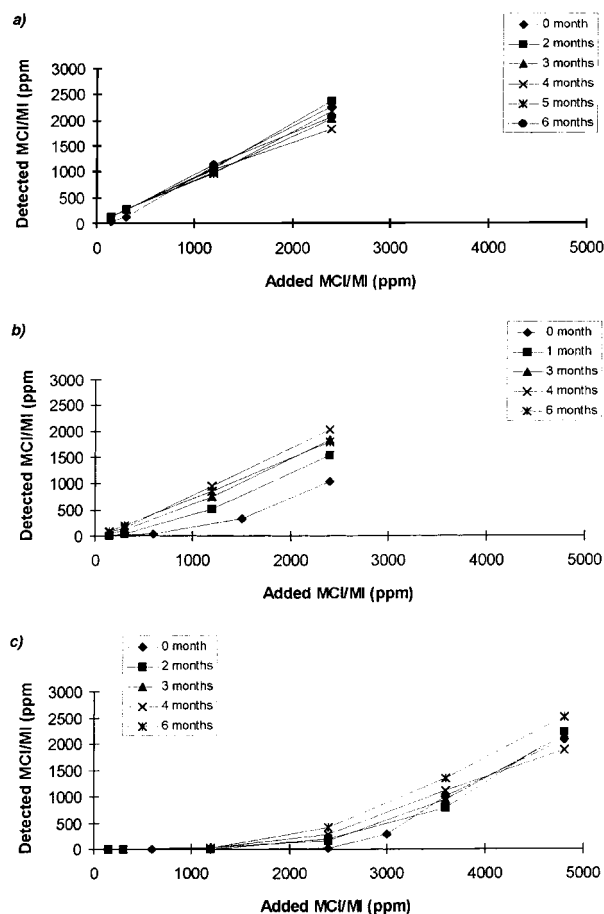


Fig. 4. The inactivating capacity of glutathione (GSH) in Fenuril® (F), Essex® (E), and Locobase® (L) over a period of 6 months. (a) Inactivating capacity of 0.10% GSH in F kept at 20–22°C. (b) Inactivating capacity 0.50% GSH in E kept at 20–22°C. (c) Inactivating capacity of 2.0% GSH in L kept at 20–22°C.

### Discussion

By HPLC technique it was shown that MCI/MI was inactivated by GSH (Fig. 2). The concentration of MCI/MI was reduced from 15 to 5.8 ppm during the first 5 min when added to aqueous GSH 0.10%, and after 20 min 0.9 ppm MCI/MI could be detected, which indicates a long reaction time. When the inactivation of MCI/MI by GSH in emollients was studied, samples from the reaction mixture were analyzed as soon as 3 min after the addition of MCI/MI, and thus not awaiting completion of the reaction. However, to prevent MCI/MI causing chemical burns, as well as sensitization and elicitation of allergic contact dermatitis on exposed skin, it is likely that the inactivation of MCI/MI must occur as soon as possible after exposure. Therefore, in this study a short reaction time before analysis was chosen. Especially for the low-concentration GSH-containing emollients, small variations in the reaction times before analysis may give different results, which in part

may account for the fluctuations noted in some figures (Fig. 2).

The selected emollients contain different amounts of lipids. However, independent of the lipid content of the emollients, GSH was shown to inactivate MCI/MI to about the same extent. Neither storage (at room temperature or refrigerated), nor age of the GSH-containing emollients seemed to be significant for the inactivation capacity of GSH, although there were olfactorial signs of degradation of GSH to volatile mercapto-containing substances. However, it is known that only minute amounts of such substances are needed to give malodor. The mercapto compounds seemed to be generated when the emollient did not fill up the whole jar. There were no signs of such degradation in tubes where there was no space of free air between the GSH-containing emollient and the lid.

As expected, it was shown that the concentration of GSH in the emollients is crucial for inactivation of 15 ppm MCI/MI, as well as higher concentrations of MCI/MI.

The results of the *in vitro* study based on chemical methodology encourage further studies on the possibility of using emollients containing 2% GSH (i) in industries where MCI/MI are handled, to prevent sensitization and elicitation of allergic contact dermatitis, and (ii) in individuals hypersensitive to MCI/MI, to prevent elicitation of allergic contact dermatitis after unintentional exposure to MCI/MI. The results also encourage further studies on the use of aqueous solutions of higher concentrations of GSH to prevent chemical burns. Experimental studies in humans hypersensitive and non-hypersensitive to MCI/MI, as well as animal studies, are required to investigate these issues.

### Acknowledgements

The authors are grateful to Lena Persson for valuable technical assistance. This investigation was supported by a grant from the Swedish Council for Work Life Research.

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