# An Italian multicentre study on methylchloroisothiazolinone/methylisothiazolinone contact sensitivity: understanding the structure–activity relationship

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The mixture methylchloroisothiazolinone (MCI)/methylisothiazolinone (MI) is a well-known contact sensitizer (1). However, studies to determine the primary sensitizer in this blend are rare, and have been performed only in small groups of patients (2–4). Herein, we present a larger study on humans in which we used the structure–activity relationship of the molecules to explain (i) why MCI is the stronger allergen, and (ii) why cross-reactivity between MCI and MI can be expected.

## Materials and Methods

From January 2016 to December 2016, eight patch-testing Italian clinics conducted a study on 141 patients (34 men and 107 women; mean age 44.1 years) recently found to be sensitized to MCI/MI 0.02% aq. We recalled patients with positive patch test reactions to MCI/MI during the period 2014–2016, mainly resulting from non-occupational exposure (cosmetics). The patients were patch tested with MCI and MI at the same concentrations as in the blend: MCI 0.015% aq. (provided by the Laboratory of the Department of Occupational and Environmental Dermatology, Lund University, Malmö, Sweden) and MI 0.005% aq. (FIRMA, Florence, Italy). We also tested MI 0.2% aq. (FIRMA) (1, 5). In this study, micropipettes were used for dosing, and haptens (0.2 ml) were simultaneously applied. Occlusion was performed for 2 days with Haye's Test Chambers® (Haye's Service, Alphen aan den Rijn, The Netherlands) on Soffix® tape (Artsana, Grandate, Italy), and evaluation was performed on day (D) 2, D4, and D7, according to ESCD guidelines (6).

## Results

The patch test results are shown in Fig. 1. One hundred and ten subjects (78.1%) reacted to MCI: 60 (42.6%) reacted only to MCI, and 50 (35.5%) reacted to both MCI and MI. Only 16 (11.3%) subjects reacted only to MI. Fifteen subjects (10.6%) reacted neither to MI nor to MCI.

## Discussion

As previously reported (2, 4, 7, 8), MCI was found to be the stronger sensitizer, which shows that primary sensitization to MCI still occurs, although the amount of exposure to MCI through MCI/MI via cosmetics and in occupational settings has become rather low. This cannot be explained by simple structural analysis, as this shows similar polarization of negative charges (electron cloud accumulation) around the oxygen atom (O) in MCI and MI, although positive charges (electron-deficient areas)

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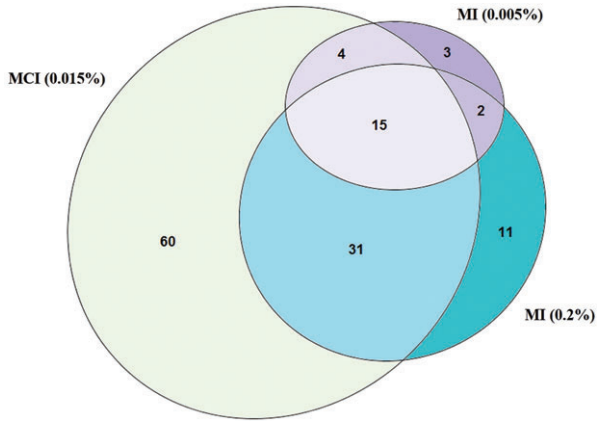



**Fig. 1.** Distribution of isolated and concurrent reactions to methylisothiazolinone (MI) 0.2%, MI 0.005% and methylchloroisothiazolinone (MCI) 0.015% in 141 patients who reacted positively to MCI/MI 0.02%, presented in an area-proportional Venn diagram. Fifteen patients did not react to MI nor MCI.

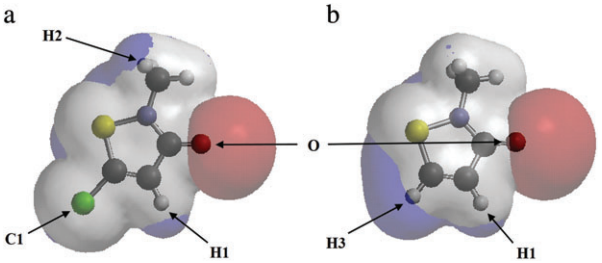



**Fig. 2.** Structure–activity relationship of methylchloroisothiazolinone (MCI) **(a)** and methylisothiazolinone (MI) **(b)** for negative charges (electron cloud accumulation, in red) and positive charges (electron deficient areas, in blue). O, oxygen atom; H1, hydrogen atom of the thiazolidine ring; H2, hydrogen atom of the methyl group; H3, hydrogen atom of the thiazolidine ring *ortho* to the ketone group; Cl, chlorine atom.

are arranged in slightly different ways in the two structures. In fact, the positive charges in MCI are distributed around the hydrogen atom of the thiazolidine ring (H1) and on two of the three hydrogen atoms of the methyl group (H2). In contrast, MI has positive charges distributed mainly around the two hydrogen atoms of the thiazolidine ring (H1 and H3), particularly near the hydrogen atom *ortho* to the ketone group (H3) (Fig. 2). The strong charge polarization of MCI increases its ability to undergo dipole–dipole interactions with other polarized molecules such as proteins, and to be involved in nucleophilic attacks with consequent addition reactions (9). Moreover, the reactivity of MCI with other organic molecules is enhanced by the bulky dimensions of the chlorine atom (10), which make it a good leaving group in all substitution reactions.

Fifty patients reacted to both MCI and MI, suggesting cross-reaction between the two molecules (11). In order to explain this, the moiety composed of a carbonyl group (C=O) with an unsaturated bond (i.e. a double bond) in the $\alpha-\beta$ position, which is common to many allergens (i.e. acrylic derivatives) (12), characterizes the two molecules. Substituents attached to this basic structure can modify the polarization, reactivity, shape and size of the two molecules, but the reaction mechanisms are superimposable (13). However, a study conducted on three-dimensional reconstructed human epidermis showed that MI reacts exclusively with cysteine thiol residues, whereas MCI reacts with histidine and lysine, making cross-reactivity between MI and MCI difficult to explain (14). Indeed, co-sensitization is still a potential explanation, as humans are usually exposed to both molecules in the environment. On the other hand, the 50 recently sensitized patients with positive patch test reactions to MCI/MI are probably sensitized primarily to MI (because of the more widespread exposure to MI than to MCI), with cross-reactivity to MCI.

An interesting finding of our study was that solitary sensitization to MI (without concomitant reactions or cross-reaction to MCI) occurred in only 16 of 66 (24.2%) MI-sensitized patients (MCI: solitary sensitization in 54.5%), pointing to a rather asymmetric cross-reactivity profile (15). Further studies are needed to verify this. Seven subjects (5.0%) reacted only to the lower concentration of MI (0.005%). This is surprising, as we used the same methodology in the participating clinics, and reactions only to lower MI or MCI/MI concentrations are rarely reported (4, 16). Finally, in the patients who did not react either to MI or to MCI, a compound allergy could be hypothesized. Moreover, the presence of a sensitizing impurity, such as 4,5-dichloro-2-methyl-4-isothiazolin-3-one (17), or other decomposition by-products of the two molecules in the commercial raw materials should not be excluded.

In conclusion, this large cohort study on patients with positive patch test reactions to MCI/MI suggests an original chemical approach for understanding the stronger sensitizing capacity of MCI and the possible but not frequent cross-reactivity between MCI and MI.

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