

# Thimerosal positivities: patch testing to methylmercury chloride in subjects sensitive to ethylmercury chloride

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The aim of this paper was to evaluate whether methylmercury chloride (MeHgCl) aq., when patch tested in a group of thimerosal-positive subjects reacting to ethylmercury chloride (EtHgCl), might be a reliable model for the better understanding of interactions between alkylmercury compounds and the skin. 19 out of 21 consecutive patients who previously had given positive patch-test reactions to both ethylmercury chloride 0.0165% eth.(EtHgCl, 0.615 mM) and MeHgCl 0.031% aq.(1.23 mM), and negative reactions to thiosalicylic acid 0.05% (3.24 mM) aq./eth. 50/50, were repatch tested to 8  $\mu$ l of MeHgCl 0.031% aq. and to 8  $\mu$ l of aq. solutions containing MeHgCl mixed with cysteine, glutathione, ZnSO<sub>4</sub>, Mg SO<sub>4</sub>, Mn SO<sub>4</sub>, ZnCl<sub>2</sub>, MgCl<sub>2</sub> and Mn Cl<sub>2</sub>, respectively. The results showed that cysteine, glutathione and Zn(II) salts were able to abolish the positive reactions, demonstrating the rôle played by both thiol groups and Zn(II) itself. Patch tests concomitantly carried out in 16 out of 19 patients to 8  $\mu$ l of aqueous MeHgCl and to 8  $\mu$ l of aqueous solutions containing MeHgCl and MeHgCl mixed to fragment 56–61 of metallothionein I (MT I), MT I and MT II-Zn, respectively, revealed that all the MTs tested were able to reduce or to inhibit the reactions, demonstrating the effect of the thiol groups. Due to the close chemical similarities to EtHgCl and to its water solubility, MeHgCl seems to be a suitable model for evaluating the reactivity of alkylmercury compounds in the skin. We speculate that both EtHg- and MeHg-derivatives are xenobiotics with similar reactivity. However, the lack of clinical relevance of the reactions to both alkyl compounds lead us to conclude that, since environmental exposure does not seem to play a pivotal rôle, they probably have mostly to do with compounds included in the standard series, and are elicited by reduced function of physiological SH chelators.

**Key words:** alkyl mercury compounds; allergic contact dermatitis; divalent ions; SH groups; metallothioneins; methylmercury chloride; ethylmercury chloride; thimerosal; patch test standard series.  
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In recent years, dermatologists have repeatedly debated the reason for positive patch test reactions to thimerosal (TH), a preservative containing an alkylmercury moiety (ethylmercury (EtHg)) which is considered to be responsible for the majority of the reactions (1–3).

In June 1997, the International Conference on Human Health Effects of Mercury Exposure held in Tørshavn (4) sounded the alarm over the risks of the increasing environmental mercury pollution. Only 1 paper on the harmful cutaneous effects of mercury exposure was presented there (5). This was quite surprising considering that some mercury compounds, notably alkyl compounds, are more dangerous than others (6, 7) and easily penetrate the skin (8). Furthermore, the skin is one of

the largest organs of the body, continuously exposed to many hazardous environmental agents, and one of the most prominent sites of xenobiotic metabolism (9).

In a previous paper (10), we reported that a group of subjects reacting to both TH and to ethylmercury chloride (EtHgCl), when repatch-tested to some organomercury compounds such as mersalyl acid, p-amino-phenylmercuric acid, mercury acetate and methyl mercury chloride, were able to give constantly positive reactions only to methylmercury chloride (MeHgCl), an organomercury alkyl compound differing from EtHgCl only by the length of the side chain. Because of these results, we supposed that organomercury alkyl compounds played a pivotal role in TH reactions.

Subsequently, we showed that, as found in biological systems, both thiol groups and zinc (Zn) were in some way involved in cutaneous reactions to EtHgCl (11). Ethanolic solutions containing EtHgCl mixed with L-cysteine, glutathione and ZnCl<sub>2</sub>, respectively, when patch tested in EtHgCl-positive subjects, exerted an inhibiting or reducing effect on the positive reactions. Taken together, these results and the lack of the clinical relevance in TH-positive reactions, led us to speculate that positive subjects might belong to a group with reduced activity of physiological SH-chelating groups.

In the light of these considerations, and in accordance with the opinion expressed by Aberer & Kränke (3), there is a need not only to carry out further epidemiological studies of the effects exerted by environmental mercury pollutants, but also to try to better understand the mechanistic details of their cutaneous interactions.

In previous patch testing (11), the solubility of EtHgCl in ethanol did not permit a complete appreciation of the details of the Zn-inhibiting effect. Since other Zn salts, such as sulfate, were not soluble in ethanol, it was impossible to ascertain, for example, whether the Zn inhibiting action was due to Zn itself or to the counterion. Counterions are reported to exert an influence on the actions of Ni inside the skin (12–14). In addition, the solubility of EtHgCl in ethanol did not allow us to evaluate whether the compound could interact with some Zn-containing biomolecules, for example metallothioneins (MT). MTs are a group of low-molecular-weight metal-binding proteins with high cysteine content, which may be induced by relatively low concentrations of some metals and have the ability to bind selectively Zn, Cd, Hg and Cu. They are involved in the detoxification of these metals and free radicals (15). MTs are constitutively expressed in normal and pathologic mice (16) and human skin (17). It has recently been reported that in mice, MTs may be involved in the epidermal hyperplasia maintaining essential zinc concentration (18).

On the basis of the observed findings, we speculated that the inhibiting Zn action was due to at least 3 mechanisms: the anti-inflammatory properties of the element; the induced production of metallothioneins; the substitution of Zn contained in some biomolecules by organomercury alkyl compounds (11).

The close structural similarities between EtHgCl and MeHgCl and the constant presence of concomitant positive reactions to both, directed us to evaluate whether MeHgCl might be a useful model for the better understanding of interactions between organomercury alkyl compounds and the

skin. The water solubility of MeHgCl allows the performance of patch tests with mixed aqueous solutions containing more Zn salts, SH-containing substances and Zn-containing biomolecules such as Zn-MTs, to be monitored.

### Materials and Methods

21 consecutive subjects (16 female, 5 male, mean age 24.2 years), who had previously given positive patch-test reactions without any clinical relevance only to TH 0.1% pet. (Hermal) included in our standard series, gave their consent to this study. They were simultaneously repatch-tested to 8  $\mu$ l of the following solutions: EtHgCl 0.0165% (0.615 mM) eth., MeHgCl 0.031% (1.23 mM) aq., thiosalicylic acid 0.05% (3.24 mM) aq./eth.(50/50). 15 days later, 19 of them were simultaneously repatch-tested to 8  $\mu$ l of the following solutions in accordance with the following plan: MeHgCl 0.031% aq., MeHgCl 0.031% aq.+cysteine (0.058%, 3.69 mM, molar ratio 1:3) MeHgCl 0.031%+glutathione (0.114%, 3.69 mM, molar ratio 1:3). A month later, the 19 subjects were divided into 2 groups of 10 and 9 subjects, respectively, and simultaneously repatch-tested to 8  $\mu$ l of the following solutions in accordance with the following plan:

Group A (10 subjects): MeHgCl 0.031% aq., MeHgCl 0.031% aq.+ZnSO<sub>4</sub> (1%, 55.7 mM, 1:3 w/w), MeHgCl 0.031% aq.+MgSO<sub>4</sub> (1%, 40.6 mM, 1:3 w/w), MeHgCl 0.031% aq.+MnSO<sub>4</sub> (1%, 59.1 mM, 1:3 w/w). Group B (9 subjects): MeHgCl 0.031% aq., MeHgCl 0.031% aq.+ZnCl<sub>2</sub> (1%, 73.4 mM, 1:3 w/w), MeHgCl 0.031% aq.+MgCl<sub>2</sub> (1%, 49.2 mM, 1:3 w/w), MeHgCl 0.031% aq.+MnCl<sub>2</sub> (1%, 50.5 mM, 1:3 w/w).

2 months later, 6 subjects belonging to group A and 6 subjects belonging to group B were simultaneously repatch-tested to 8  $\mu$ l of the following solutions: MeHgCl 0.031% aq., MeHgCl 0.031% aq.+MT I fragment 56–61 (1.23 mM, 1:1 molar ratio), MeHgCl 0.031% aq.+metallothionein I (1 mg/ml), MeHgCl 0.031% aq.+metallothionein II-Zn (1 mg/ml). Patch tests were carried out using Al-Test on Leucotest for all the solutions. The panels containing Al-Tests were arranged in a vertical row and applied to the back. The distance between the patches was 3 cm. Patch tests were read in all subjects at 48 h. and scored in accordance with ICDRG guidelines. Visual score was used in comparing the differences between the positive responses found to MeHgCl and those to the mixed solutions.

The substances used were: sodium ethylmercurythiosalicylate (Sigma, purity: 98%, MW 404.8), MeHgCl (Erba, purity: 98%, MW 251.10), EtHgCl (Alfa, purity: 97.7%, MW 265.13), thiosal-

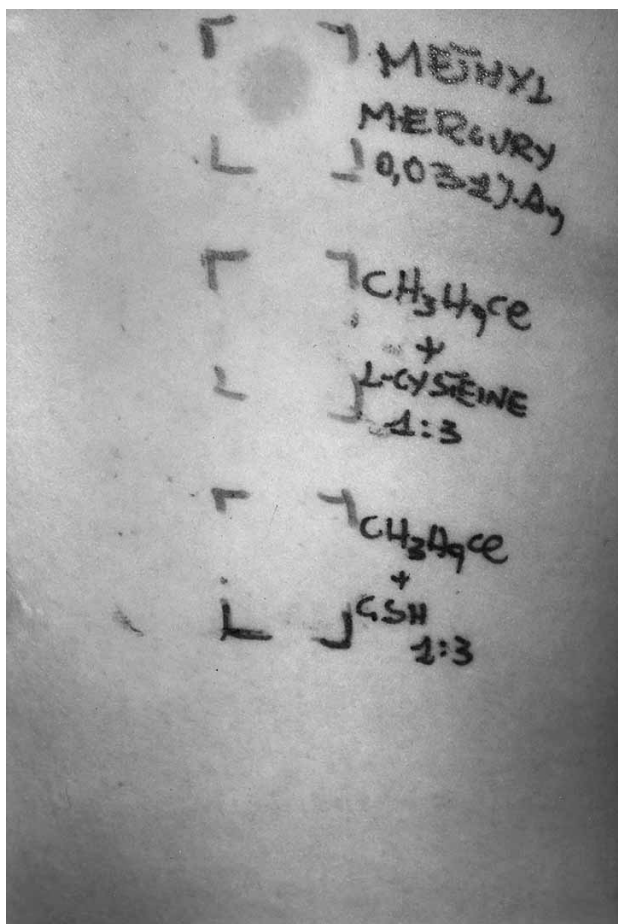


Fig 1. Positive patch test reactions to MeHgCl inhibited by mixed solutions containing cysteine and glutathione, respectively.

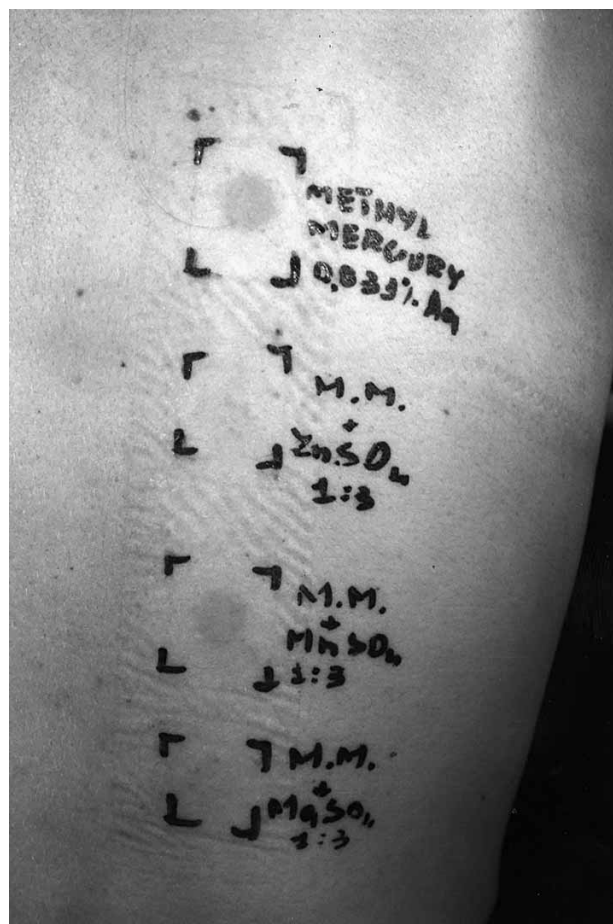


Fig 2. Mixed solution containing MeHgCl and ZnSO<sub>4</sub> inhibiting the positive reaction to MeHgCl.

icylic acid (Aldrich, purity: 97%, MW 154.19), glutathione, reduced form (Sigma, purity 98–100%, MW 307.3), cysteine hydrochloride hydrate (Sigma, purity 98%, MW 157.6) ZnSO<sub>4</sub>·7 H<sub>2</sub>O (Erba, purity 98%, MW 179.45), MgSO<sub>4</sub>·7 H<sub>2</sub>O (Erba, purity 99%, MW 246.48), MnSO<sub>4</sub> (Erba, purity 99%, MW 169.1), ZnCl<sub>2</sub> (Fluka, purity 98%, MW 136.29), MgCl<sub>2</sub>·6 H<sub>2</sub>O (Merck, purity 99%, MW 203.30), MnCl<sub>2</sub> (Fluka, purity: 99%, MW 197.91), metallothionein I fragment 56–61 (Sigma, acetate salt, from mouse liver, M.W.627.8), metallothionein I (Sigma, essentially salt free, from rabbit liver, Cd content: 6.0%, Zn content: 0.6%), metallothionein II, Zn (Sigma, from rabbit liver, Zn content: 7.0%, Cd content: 0.5%) .

The different compounds in mixed samples are present in a final concentration.

### Results

Whilst 19 of the 21 subjects gave concomitant patch-test positive responses to both EtHgCl and

MeHgCl, the remaining 2 gave a positive response to thiosalicylic acid alone. The positive reactions to MeHgCl were always abolished or reduced either by the solutions containing L-cysteine or by those containing glutathione (Fig. 1).

*Group A.* The mixed solutions containing ZnSO<sub>4</sub> abolished the positive reactions to MeHgCl in all subjects. MgSO<sub>4</sub> reduced the reactions in 2 subjects and had no effect in the remaining 8. MnSO<sub>4</sub> had no effect in any of the tested patients (Fig. 2).

*Group B.* The mixed solutions containing ZnCl<sub>2</sub> abolished the positive reaction to MeHgCl in 8 subjects and reduced it in the remaining 1. The mixed solutions containing MgCl<sub>2</sub> abolished the positive reaction to MeHgCl in 1 subject, reduced it in 2 subjects and had no effect on it in the remaining 6. The mixed solutions containing MnCl<sub>2</sub> abolished the reaction in 1 subject and had no effect on it in the remaining 8 (Fig. 3).

All the MTs tested exerted an inhibiting or reducing effect on the positive reactions (Fig. 4). However, the inhibiting effect exerted by the mixed

Table 1. Patch test results to MTs in 12 MeHgCl-positive patients

| Subject no.         | Degree of reaction |   |   |   |   |   |   |   |   |    |    |    |
|---------------------|--------------------|---|---|---|---|---|---|---|---|----|----|----|
|                     | 1                  | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| MeHgCl 0.031% aq.   | 3                  | 3 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 3  | 2  | 2  |
| fragment (m.r. 1:1) | 1                  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 0  |
| MT II Zn (1 mg/ml)  | 1                  | 2 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1  | 1  | 0  |
| MT I (1 mg/ml)      | 1                  | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  |

solutions containing the fragment 56-61 was stronger than that exerted by either MT I or MT II (Table 1).

### Discussion

Patch tests to organomercury alkyl compounds and to thiosalicylic acid, confirming previous results (10), revealed that the large majority (>90%) of reactions in TH-positive subjects, were concomitantly caused by EtHgCl and MeHgCl. The aqueous solutions containing MeHgCl mixed with L-cysteine and glutathione showed behaviour mim-

icking those of previously tested ethanolic solutions of EtHgCl (11). They were always able to abolish or reduce all positive reactions to MeHgCl, demonstrating the involvement of SH groups even in positive MeHg reactions.

On testing to aqueous solutions containing MeHgCl mixed with sulfates and chlorides of divalent ions, the results stressed that Zn, amongst the divalent ions tested, was the only one able to reduce or abolish all positive reactions. On the whole, when evaluating the interactions of MeHg with SH-containing substances and Zn, MeHg seemed to represent a reliable model in demon-

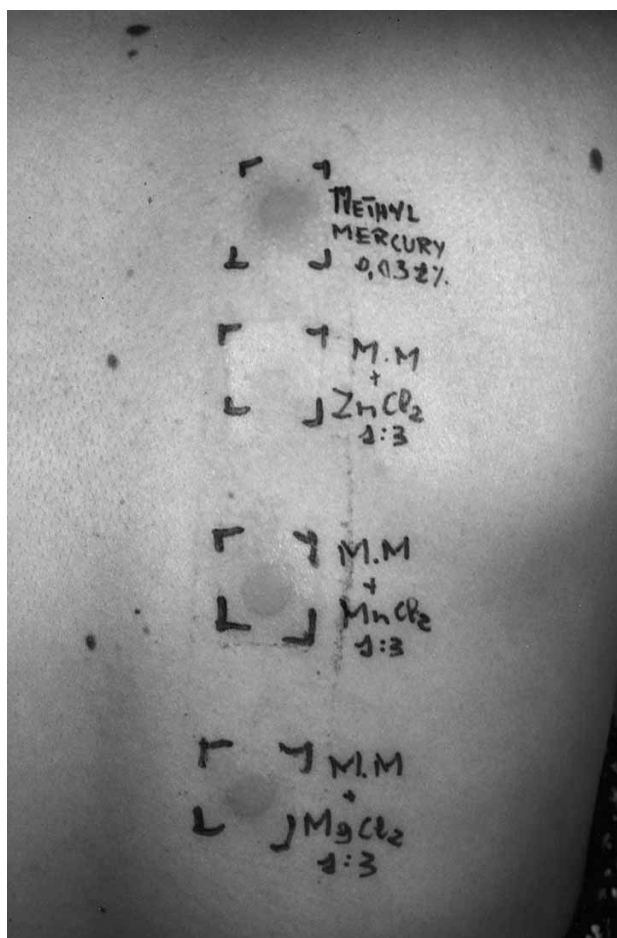


Fig 3. Mixed solution containing MeHgCl and  $ZnCl_2$  inhibiting the positive reaction to MeHgCl.

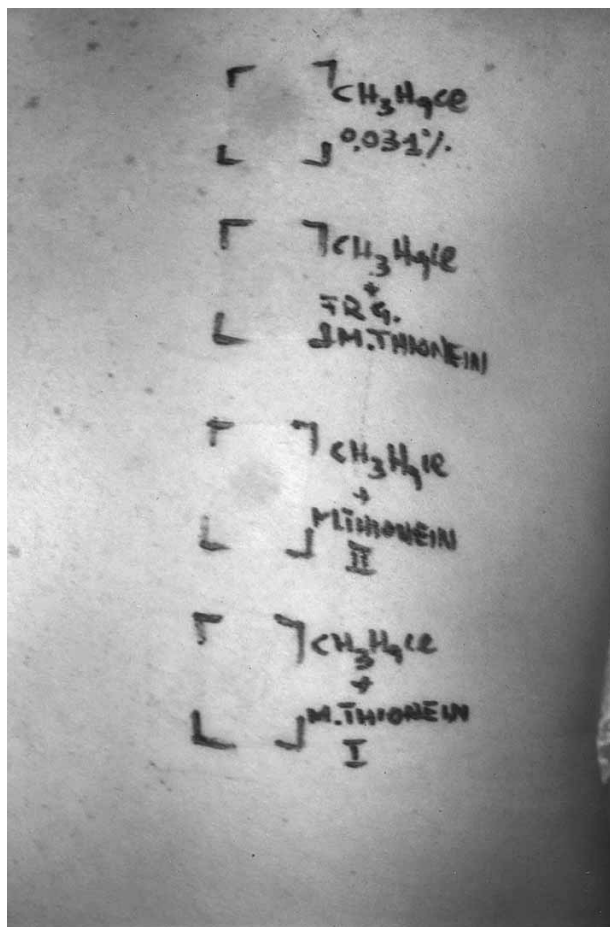


Fig 4. Positive reactions to MeHgCl inhibited by mixed solutions containing MT fragment and MTI, respectively, and reduced by that containing MT II Zn.

strating the reactivity of alkylmercury compounds inside the skin. In addition, the patch tests to MeHg permitted us to establish that the inhibiting action depended on Zn itself, and was not influenced by the nature of the counterions utilised.

Further confirmation of the rôle played by thiols in the cutaneous reactions to organomercury alkyl compounds came from the tests performed with mixed solutions containing commercially available MTs. There are at least 4 structurally distinct subgroups of MT, which differ in their aminoacid sequences and net charge (15). The activity of MTs seems to be mainly dependent upon the thiol groups that are known to participate, with different co-ordination properties, in metal binding. The sequences of the cysteines, notably Cys-Cys, Cys-X-Cys and Cys-X-Y-Cys are considered to be the most important factors in conditioning their affinity for metals and their binding (15).

In our tests, we used the MT I fragment 56–61 from mouse liver containing Lys-Cys-Thr-Cys-Cys-Ala, MT I from rabbit liver containing Cd 6.0% and Zn 0.6%, and MT II-Zn from rabbit liver containing Zn 7.0% and Cd 0.5%. All the MTs tested, at the utilised concentration, interacted with MeHg to cause inhibition or reduction of the positive reactions. Whilst the MT I fragment 56–61, containing only 3 cysteines, abolished nearly all the MeHg reactions, MT I and MT II Zn, containing about 30% of cysteines, seemed to give a less evident abolishing effect. Probably, the small number of patients tested and the different concentrations do not permit us to draw valid conclusions as to the possible role exerted by Zn and Cd contained in MTs. However, these preliminary results may indicate that cutaneous MTs are involved, mostly with their cysteines, in the cutaneous detoxification of organomercury alkyl compounds.

The question may arise as to whether in TH-sensitive subjects there is cutaneous reactivity to either of the alkylmercury compounds or to both. Even if the patch tests did not allow determination of the metabolic pathway of the organomercury alkyl compounds inside the skin, the results obtained permit us to hypothesise that EtHgCl and MeHgCl are 2 reactive xenobiotics with chemical similarities so close that, once applied to the skin, the skin reacts against each as if they were identical.

The last question concerns environmental exposure to alkylmercury compounds. As previously stated for EtHg reactions (1,10), even the positive reactions to MeHg seemed to occur without previous skin contacts. Despite the richness in sulfur of the stratum corneum, its continuous shedding makes the dermal uptake from atmospheric mercury vapour unimportant (19). By contrast, even if

the gastrointestinal absorption of MeHg is normally high, this exposure does not seem to cause harmful cutaneous effects. It has been recently stressed that mercury released by amalgam fillings may be methylated by bacteria commonly found in dental plaque and in the oral cavity. After a latent period of weeks, these absorbed compounds may cause various symptoms according to the organs affected (20). However, the relationship between absorption of alkyl mercury and appearance of cutaneous symptoms needs to be further clarified. In conclusion, to date, there is no certain proof that increasing mercury environmental pollution represents a high risk to the skin.

From all these considerations, the suspicion arises that positive reactions to organomercury alkyl compounds have mostly to do with their inclusion in patch test series, and that a still unknown defect of the function of low-molecular-weight thiol compounds, which promote transport both into and out of the cells, underlies their harmful effect.

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