

## Preservatives in Moisturizers on the Swedish Market

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The presence of 9 common preservatives was investigated in 100 moisturizers by high-performance liquid chromatography.

According to the manufacturers/suppliers 88 of the 100 moisturizers contained at least one of the 9 preservatives. This information was erroneous in 9 cases (10.2%). When the moisturizers were investigated for the presence of the 9 preservatives which should not be present in the moisturizers according to the manufacturers/suppliers, at least one of the preservatives was detected in 17 moisturizers (17.0%).

Parabens were the most common preservatives. The concentrations of the 9 preservatives did not exceed the maximum concentrations allowed in cosmetics.

When a patient with suspected allergic contact dermatitis tests positively to a preservative, the assessment of present clinical relevance requires demonstration of exposure to the sensitizer. This demonstration cannot rely solely on information on the packages or from the manufacturers/suppliers but has often to be supplemented by chemical analysis. **Key words:** allergic contact dermatitis; formaldehyde releasers; high-performance liquid chromatography; methylchloroisothiazolinone/methylisothiazolinone; methylchloroglutaronitrile; parabens.

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Moisturizers are used daily by many people to keep their skin smooth. Since most moisturizers contain water, preservatives are required to prevent growth of micro-organisms. Most preservatives are sensitizers and contact allergy to them is common. To demonstrate contact allergy to most preservatives, higher concentrations of the preservatives than those used in moisturizers are required. Therefore, a preservative-hypersensitive person may test negatively to a moisturizer containing the preservative, although the exposure to the moisturizer is clinically relevant.

In the present study the presence of 9 common preservatives in 100 moisturizers was investigated and compared with the information given by the manufacturers, and, when present, with preservatives labelled on the packages. These selected preservatives, which are also commonly used in epicutaneous patch-testing, were parabens, imidazolidinyl urea (Imid), diazolidinyl urea (Diazol), methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), methylchloroglutaronitrile (MDBGN), quaternium 15 (Quat), formaldehyde, DMDM hydantoin (DMDMH) and 2-bromo-2-nitropropane-1,3-diol (BNPD).

(The names of the preservatives are according to the International Nomenclature of Cosmetic Ingredients (INCI)).

### MATERIALS AND METHODS

#### Commercial products

One hundred moisturizers, together with information about what preservatives were used in every product, were obtained from 32 manufacturers/suppliers. The moisturizers were not necessarily freshly prepared, but still far from the expired date. The products were kept at room temperature during the time of investigation. These moisturizers are well-known and common on the Swedish market and were selected to represent manufacturers from different geographical areas and moisturizers to be used in the face, on the body and the hands.

During the investigation period there were no signs of deterioration of any moisturizer. However, no chemical investigations of the stability of the preservatives were carried out.

#### Reference substances

The preservatives were obtained from various suppliers (Table I). Usually a mixture of parabens is used, as a preservative and the separate parabens are not always specified. In this study methyl, ethyl, propyl and butyl paraben were used as paraben reference substances. Kathon CG, consisting of 1.125% MCI and 0.375% MI, was used when standard solutions of MCI/MI were prepared.

#### The investigative process

Out of 100 moisturizers 88 contained one or more of the 9 selected preservatives according to the manufacturers/suppliers. Five moisturizers contained no preservatives and 7 moisturizers contained other preservatives (Fig. 1).

The investigation that followed consisted of two different parts.

I. Identification and quantitative determination of the 9 preservatives in those 88 moisturizers which contained the selected preservatives according to the manufacturers/suppliers (Fig. 1).

II. Screening of the 9 preservatives in the 100 moisturizers. Only those preservatives which had not been declared by the manufacturers/

Table I. The manufacturers of the reference preservatives

| Preservative  | Manufacturer                             |
|---|--|
| Methyl, ethyl, propyl and butyl paraben                       | Fluka Chemica, Buchs, Switzerland        |
| Imidazolidinyl urea   | ICN Biomedicals Inc., Costa Mesa, USA    |
| Diazolidinyl urea   | ICN Biomedicals Inc., Costa Mesa, USA    |
| Kathon CG (methylchloroisothiazolinone/methylisothiazolinone) | Rohm and Haas Company, Philadelphia, USA |
| Methylchloroglutaronitrile                                    | Schülke & Mayr, Hamburg, Germany         |
| Quaternium 15   | Sigma Chemicals Co, St. Louis, USA       |
| Formaldehyde  | Acros Organics, Geel, Belgium            |
| DMDM hydantoin  | McIntyre Group LTD, Illinois, USA        |
| 2-Bromo-2-nitropropane-1,3-diol                               | Boots Company, Nottingham, England       |

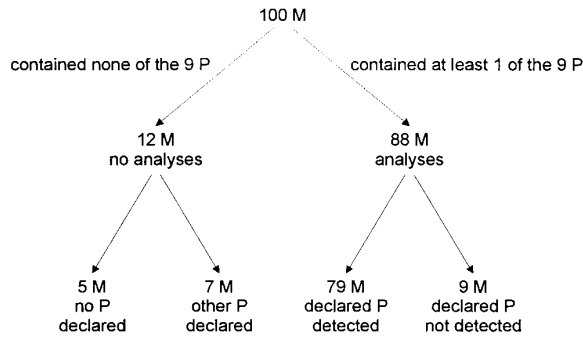


Fig. 1. Study I. Investigative process of 100 moisturizers with regard to 9 preservatives declared by the manufacturers/suppliers and the results of chemical analyses of those moisturizers containing at least one of the 9 preservatives. M = moisturizer, P = preservative.

suppliers were investigated. The detection limit was set at 1/10 of the legal maximum level in cosmetics.

#### High-performance liquid chromatography (HPLC)

Two separate isocratic HPLC systems were used. One system consisted of an SP Spectra Series P200 pump, an SP Spectra System UV1000 detector and an SP ChromJet integrator. The other system consisted of an LDC ConstrMetric III pump, an LDC SpectraMonitor III detector and an LKB 2210 printer. Both systems were equipped with Rheodyne 7125 injectors and with 20- $\mu$ l loops. Two columns (4 mm i.d.  $\times$  250 mm and 4 mm i.d.  $\times$  150 mm) packed with Nucleosil C18 5  $\mu$ m and 3  $\mu$ m, respectively, were used. The flow rate was 1 ml/min.

To identify the respective preservative and determine the concentration of parabens, MCI/MI and formaldehyde in the moisturizers, HPLC methods published in the literature were used (1–4).

For the corresponding investigations of DMDMH, MDBGN and BNPD in the moisturizers, available HPLC methods (5–7) were modified. Concerning the analysis of DMDMH, diethylether was used in the extraction procedure instead of dichloromethane. Mixtures of methanol and water were used as mobile phases instead of those recommended when MDBGN and BNPD were analysed.

To identify and quantify Quat, an HPLC method from Pharmacia & Upjohn, Denmark, was used (8).

There were no chemical methods available for analysis of Imid and Diazol, so HPLC methods were developed (to be published). In brief, a moisturizer was extracted with a mixture of ethanol and water, and the extract was then injected into the HPLC. In the chromatogram of a standard solution of Imid a specific pattern of peaks was demonstrated. This specific finger print was used to detect and determine the concentration of Imid in the extract of the moisturizers. Diazol could be detected in a similar way, with another combination of peaks in the chromatogram giving another specific finger print.

The mobile phases and the wavelength settings concerning the various methods are listed in Table II, as well as detection limits and recoveries.

#### Recovery

To estimate the recovery of a preservative, a known amount of the preservative was added to 3 different moisturizers not containing the actual preservative in at least duplicate samples. The preservative was added to the moisturizer to give a concentration corresponding to the maximum concentration allowed. After the addition the samples were analysed according to the specific methods. In Table II the recoveries from the investigated preservatives are listed.

#### Sample preparation

About 1 g of the moisturizer was accurately weighed and 10–20 ml of the respective mobile phase was added. Some boiling chips were added

before the mixture was shaken vigorously for about 1 min. The mixture was then filtered through a Millipore filter 1.2  $\mu$ m or Munktells paper filter nr 3 before the filtrate was injected into the HPLC. This sample preparation was used in most cases. Occasionally, an extraction procedure with diethylether was necessary to eliminate interferences from fat ingredients in the moisturizers.

## RESULTS

According to the manufacturers/suppliers 5 moisturizers did not contain any preservative and 7 moisturizers contained other preservatives than those under discussion. Therefore, in study I 88 moisturizers were investigated. In 9 out of the 88 moisturizers (10.2%) the preservatives declared by the manufacturers/suppliers could not be detected (Fig. 1).

In study II the screening of the preservatives not declared by the manufacturers/suppliers demonstrated that 16 out of the 100 analysed moisturizers contained one of the 9 preservatives and one of the 100 moisturizers contained 2 of the 9 preservatives. Thus, in 17 out of 100 moisturizers (17.0%) preservatives not declared by the manufacturers/suppliers were detected. Among these 17 products the detected preservatives were listed on the packages of 3 products (Fig. 2).

Fifty-four out of the 100 moisturizers had some or all ingredients listed on the packages. In 9 cases (16.7%) the declaration on the packages regarding the preservative/preservatives was not in accordance with the information given by the manufacturers/suppliers. Chemical analyses demonstrated that the information given on the packages was correct in 4 and erroneous in 3 cases, respectively. Furthermore, in 2 cases 2 different preservatives not included in this study were not among the ingredients labelled on the packages, although declared by the manufacturer/supplier.

Parabens were the most common preservatives. Seventy-five moisturizers contained a mixture of parabens and 5 moisturizers contained only one paraben, which means that 80.0% of the moisturizers contained parabens (Table III). The highest concentration of one paraben allowed in a cosmetic product is 0.4% w/w. The paraben concentrations in the 5 moisturizers containing one paraben were 0.016%–0.209% w/w. For mixtures of parabens the highest total paraben concentration allowed is 0.8% w/w. The total concentration range of parabens in the 75 moisturizers was 0.088%–0.837% w/w. Among the parabens methyl and propyl paraben were the most commonly used (Table IV).

The formaldehyde releasers Imid, Diazol, DMDMH, BNPD and Quat were demonstrated in 35 moisturizers. Formaldehyde, which was neither declared by any manufacturer/supplier nor labelled on any product, was demonstrated in 10 moisturizers (10.0%), with the concentration range 0.02%–0.09% w/w. In 9 of the formaldehyde-containing moisturizers formaldehyde releasers were demonstrated.

MCI/MI was demonstrated in 6 moisturizers and the concentration range was 0.00036–0.00147% w/w (3.6–14.7 ppm).

The concentrations of the 9 preservatives in the 100 investigated moisturizers did not exceed the maximum concentration allowed in the Swedish legislation, which is in accordance with the EEC Cosmetic Directive (9) (Table III).

## DISCUSSION

This study confirms that parabens, and in particular methyl and propyl paraben, are widely used as preservatives in

Table II. Chromatographic conditions for HPLC analyses of 9 preservatives in 100 moisturizers, including detection limits and recoveries as well as recommended use concentrations (based on the literature and information from manufacturers as there are no official recommendations) of the preservatives in creams and lotions

MCI/MI = methylchloroisothiazolinone/methylisothiazolinone, Form = formaldehyde, DMDMH = DMDM hydantoin, MDBGN = methylidibromoglutaronitrile, BNPD = 2-bromo-2-nitropropane-1,3-diol, Quat = quaternium 15, Imid = imidazolidinyl urea, and Diazol = diazolidinyl urea

| Preservative | Chemical method Ref. | Mobile phase   | UV detection nm | Detection limit in % w/w |   | Recovery in % w/w (range)  | Recommended use concentration |
|--------------|----------------------|--|-----------------|--------------------------|---|----------------------------|-------------------------------|
|              |                      |  |                 | Study I                  | Study II  |                            |                               |
| Parabens     | 1                    | Tetrahydrofuran/water/<br>methanol/acetonitrile<br>5/60/10/25 v/v  | 280             | <0.004 <sup>a</sup>      | 0.04 <sup>a</sup>   | 100 (100–100) <sup>a</sup> | 0.18                          |
|              |                      |  |                 | <0.006 <sup>b</sup>      | 0.04 <sup>b</sup>   | 100 (100–100) <sup>b</sup> | 0.02                          |
|              |                      |  |                 | <0.004 <sup>c</sup>      | 0.04 <sup>c</sup>   | 100 (100–100) <sup>c</sup> |                               |
|              |                      |  |                 | <0.007 <sup>d</sup>      | 0.04 <sup>d</sup>   | 100 (100–100) <sup>d</sup> |                               |
| MCI/MI       | 2                    | Methanol/acetic acid (0.07 mole/l) 20/80 v/v   | 280             | <0.00003                 | 0.00015   | 101 (99–102)               | 0.00075                       |
|              |                      |  |                 | 3                        | Methanol/acetonitrile/<br>0.45% acetic acid<br>10/10/80 v/v |                            |                               |
| Form         | 4                    | Acetonitrile/water<br>50/50 v/v  | 345             | <0.0002                  | 0.02  | 102 (100–104)              |                               |
| DMDMH        | 5                    | Phosphate buffer<br>pH 2.1   | 220             | <0.005                   | 0.06  | 99 (98–100)                | 0.15–0.4                      |
| MDBGN        | 6                    | Methanol/water<br>40/60 v/v  | 210             | <0.003                   | 0.01  | 96 (95–98)                 | 0.01–0.04                     |
| BNPD         | 7                    | Methanol/water<br>25/75 v/v  | 240             | <0.002                   | 0.01  | 100 (98–102)               | 0.01–0.1                      |
| Quat         | 8                    | 1000 ml water + 25 ml<br>acetonitrile + 700 µl<br>triethylamine + 0.96g<br>sodium pentane<br>sulfonate +<br>phosphoric acid to<br>pH 3.0 | 200             | <0.0007                  | 0.02  | 99 (98–100)                | 0.02–0.3                      |
| Imid         |                      | Water  |                 |                          |   |                            |                               |
| Diazol       |                      | Water  | 210             | <0.05                    | 0.05  | 96 (95–97)                 | 0.1–0.5                       |

<sup>a</sup> methyl, <sup>b</sup> ethyl, <sup>c</sup> propyl, <sup>d</sup> butyl paraben.

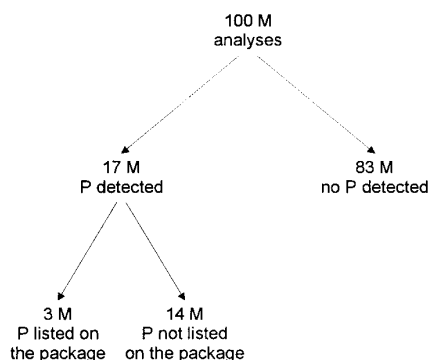


Fig. 2. Study II. Analyses of moisturizers with regard to those preservatives which had not been declared by the manufacturers/suppliers. M = moisturizer, P = preservative.

moisturizers. In a recent Danish study the presence of parabens in 215 cosmetic products was investigated. Of all products, 93% and 99% of the leave on products were demonstrated to contain parabens (10). Methyl paraben was present in 98% of the paraben-containing products. Recently, a Swedish multicentre patch test study showed that 19 of 3,690 patients with

dermatitis (0.5%) tested positively to parabens (11). Though parabens are common preservatives in Sweden, the frequency of contact allergy to parabens was lowest among the preservatives in the standard test series (11).

When cosmetics and toiletries belonging to patients hypersensitive to Kathon CG (which contains MCI/MI) were analysed in a previous study, 38 of 123 analysed products (30.9%) contained MCI/MI (2). In 16 of the 56 leave on products (28.6%) MCI/MI was demonstrated. A similar result was obtained by Rastogi, who in the late eighties found MCI/MI in 31% of the 49 leave on products among 156 of the most commonly used cosmetic products in Denmark (3). In the present study MCI/MI was detected in only 6 of the 100 moisturizers, which may reflect an intentional change to less sensitizing preservatives by the manufacturers. MCI/MI was neither declared by the manufacturer/supplier nor listed on the package of the moisturizer containing the highest concentration of MCI/MI.

Formaldehyde was detected in 10 of the moisturizers. In one product the concentration exceeded the level (0.05% w/w) for which declaration of formaldehyde on the product is required. The detection limit for formaldehyde when screening for this preservative in study II was 0.02% w/w, so it cannot be excluded, it is actually likely, that additional moisturizers, and

Table III. The number of moisturizers out of 100 in which 9 preservatives, together with the concentration range, were detected. In study II the preservatives were analysed with a detection limit of 1/10 of the legal maximum level in cosmetics

| Detected preservative                                 | No. of products |         |          | Concentration range % w/w |           | Max. allowed concentration in cosmetics % w/w (ref. 9) |
|---|-----------------|---------|----------|---------------------------|-----------|--|
|   | Total           | Study I | Study II | Study I                   | Study II  |  |
| Parabens  | 80              | 78      | 2        |                           |           |  |
| one paraben   | 5               | 4       | 1        | 0.016–0.209               | 0.04      | 0.4  |
| more than one   | 75              | 74      | 1        | 0.088–0.837               | 0.51      | 0.8  |
| Imidazolidinyl urea                                   | 23              | 21      | 2        | 0.04–0.59                 | 0.39–0.56 | 0.6  |
| Formaldehyde  | 10              | 0       | 10       |                           | 0.02–0.09 | 0.2  |
| Methylchloroisothiazolinone/<br>methylisothiazolinone | 6               | 5       | 1        | 0.00036–<br>0.00101       | 0.00147   | 0.0015   |
| Diazolidinyl urea                                     | 6               | 6       | 0        | 0.21–0.40                 |           | 0.5  |
| Methyldibromoglutaronitrile                           | 4               | 3       | 1        | 0.020–0.029               | 0.01      | 0.1  |
| DMDM hydantoin  | 3               | 3       | 0        | 0.052–0.236               |           | 0.6  |
| 2-Bromo-2-nitropropane-<br>1,3-diol                   | 2               | 0       | 2        |                           | 0.01–0.02 | 0.1  |
| Quaternium 15   | 1               | 1       | 0        | 0.0058                    |           | 0.2  |

Table IV. The number out of 80 paraben-containing moisturizers in which methyl, ethyl, propyl and butyl paraben were detected, and the concentration range

| Detected paraben | No. of products |          | Concentration range % w/w |           |
|------------------|-----------------|----------|---------------------------|-----------|
|                  | Study I         | Study II | Study I                   | Study II  |
| Methyl paraben   | 77              | 2        | 0.032–0.431               | 0.24–0.37 |
| Ethyl paraben    | 35              | 0        | 0.011–0.257               |           |
| Propyl paraben   | 73              | 1        | 0.010–0.406               | 0.14      |
| Butyl paraben    | 36              | 0        | 0.010–0.170               |           |

particularly those preserved with formaldehyde releasers, contained formaldehyde at lower concentrations than 0.02%. The presence of formaldehyde in the moisturizers may depend on (i) intentional addition of formaldehyde as a preservative in the moisturizer or in the raw material, (ii) release from formaldehyde releasers or (iii) degradation of polyethylene glycol ethers in the moisturizers (12). In 9 out of the 10 formaldehyde-containing moisturizers the presence of formaldehyde (0.02%–0.09% w/w) most likely depended on release from formaldehyde releasers, since 4 products each contained either Diazol or Imid, and one product contained Quat. In the remaining formaldehyde-containing moisturizer none of the 5 investigated formaldehyde releasers was detected. In this moisturizer the presence of formaldehyde may derive from anyone of the formaldehyde sources listed above, including formaldehyde releasers not investigated.

Formaldehyde is not recommended for use in moisturizers. Usually the recommended concentrations of preservatives in cosmetics include the highest concentration and the range down to concentrations 10 times lower. Therefore, in study II the detection limits of the preservatives were set at 1/10 of the highest concentration allowed.

Among the 9 preservatives MCI/MI and formaldehyde are the most investigated concerning clinical relevance with regard to allergic contact dermatitis. It is known that the test reactivity decreases rapidly when MCI/MI-hypersensitive patients are patch tested with serial dilutions of MCI/MI from concentra-

tions giving a moderate patch test reaction (13). When use tests with creams or lotions, containing around 15 ppm (0.0015%) MCI/MI, were carried out in MCI/MI-allergic patients twice daily for up to 2 weeks a positive use test was noted in approximately 30–50% (13, 14).

Jordan et al. (15) demonstrated that patch tests with low concentrations of formaldehyde applied continuously in formaldehyde-sensitive subjects for 1 week elicited positive test reactions. In some subjects positive reactions to 30 ppm (0.003%) were noted after testing for 120 h. In another experiment in the referred study subjects from the previous tests reacted to creams containing 0.1% Quat when patch tested in the same way as with formaldehyde (15). Recently Flyvholm et al. (16) studied the threshold concentration for occluded patch tests to formaldehyde in 20 formaldehyde-sensitive patients. The authors concluded that 250 ppm (0.025%) formaldehyde was the threshold concentration.

To diagnose and prevent allergic contact dermatitis from a preservative in moisturizers the presence and absence, respectively, of the preservative in the moisturizers has to be known or shown. With chemical methodology the presence or absence can be ascertained, but the laboratory facilities required for the analysis are not accessible to most dermatologists. Therefore, the dermatologist and the patient have to rely on the ingredient labeling on moisturizers or the information given directly by manufacturers/suppliers. However, as this study shows, this information is for too many moisturizers erroneous and misleading. Consequently, today and in future we will fail to diagnose and prevent allergic contact dermatitis from preservatives in some patients unless it is possible to encourage the manufacturers/suppliers to be more careful and conscious of their responsibilities concerning the information given on preservatives in moisturizers.

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