

Comparison of objective and sensory skin irritations of several cosmetic preservatives

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There are many cosmetic ingredients, such as preservatives and fragrances, known to elicit adverse effects. The aim of this study was to investigate the side-effects of cosmetic preservatives, by evaluating objective and subjective skin irritation. The method comprised of 2 parts. In part 1, we tried to compare 24-hr patch test results with the sensory irritation potential of several preservatives. In part 2, skin cumulative irritation test for 21 days and sensory irritation test were performed to compare various combinations of preservatives in 4 types of formulations. Our data showed that methylparaben, ethylparaben, propylparaben, butylparaben, phenoxyethanol (PE) and chlorphenesin (CPN) have similar objective skin irritation potential at the minimal inhibitory concentration of each preservative, but CPN has higher potential than other preservatives in subjective irritation. Sensory irritation of preservatives changed according to formulation type, and PE combined with CPN highly increased irritation. There was correlation between antimicrobial activity and skin objective irritation but not sensory irritation. Influence on skin sensory irritation varies with the combination of preservatives. Therefore, for the development of new preservatives and cosmetics, it is important to evaluate skin sensory irritation of preservatives used in cosmetic products according to the type of formulations.

Key words: formulation type; objective irritation; preservatives; sensory irritation. © Blackwell Munksgaard 2007.

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A significant number of consumers believe that they have sensitive skin. However, their concepts of sensitive skin are not always consistent. It is becoming more important to evaluate objective skin irritation in addition to subjective sensory irritation potential in developing cosmetic products.

Sensory skin irritation represents the array of bothersome symptoms such as stinging, burning and itching that can occur even without visual signs of skin inflammation (1) and is sometimes called 'subjective' irritation to differentiate it from objective irritation with signs of erythema and scaling (2). This often leads to consumer dissatisfaction, and it has been regarded as one of the important side-effects of using cosmetics. It is necessary to develop new products with minimum sensory irritation that are more tolerable to consumers. However, the mechanism of sensory irritation is poorly understood.

There are many cosmetic ingredients known to elicit adverse effects, such as preservatives and fragrances (3, 4). *P*-Hydroxybenzoic acid esters (parabens) are the most frequently used preservatives causing sensory skin irritation in cosmetics

(5). This is because parabens are widely used as preservatives in foods and cosmetics as well as in drugs. They are not only rapidly absorbed, metabolized and excreted but also have proved to be very effective antimicrobial agents. As the chain length of the ester group of paraben increases, antimicrobial activity increases, while water solubility decreases (6). Parabens meet several of the criteria as an ideal preservative possessing broad-spectrum antimicrobial activities with great safety (i.e. relatively non-irritating, non-sensitizing and low toxicity) (7). Phenoxyethanol (PE), a preservative with aromatic ether, is used in cosmetics. According to the classification scheme of the Cosmetic Ingredient Review report, PE is practically non-toxic to humans when administered orally or percutaneously. Chlorphenesin (CPN) is a potent antibacterial and antifungal agent for common dermatophytes, with low toxicity (8, 9). PE and CPN are used with other preservatives such as parabens because of relatively low antimicrobial activities.

In spite of mixed preservatives being more frequently used than single preservatives in cosmetic

formulation, there have been few studies of skin side-effects related to combined use. Further, there have been few subjective irritation reports of side-effects from preservatives, especially in regards to combined use or change of formulations.

The aim of this study was to investigate the side-effects of cosmetic preservatives as single or combined ingredient by evaluating objective and subjective skin irritations.

This study is comprised of 2 parts:

- In part 1, we tried to find the relationship between skin irritancy potential and preservative effects. To compare adverse skin reaction potential according to antimicrobial activity, we used the minimal inhibitory concentration (MIC) for *Pseudomonas aeruginosa*.
- In part 2, irritation potencies with various combinations of preservatives were compared in 4 different types of formulations.

Materials and Methods

The MIC of preservatives is shown in Table 1. However, in our preliminary study, MIC of each preservative was very low for objective skin irritation. Thus, we applied a fivefold concentration of MIC for evaluation, while in sensory skin irritation, we applied a onefold concentration for measuring sensory irritation.

Materials

Part 1. Parabens are esters of 4-hydroxybenzoic acid differing only in the ester group (methyl, ethyl, propyl or butyl). Methylparaben (MP) (>99% purity; Hanbit, Osan, Korea), ethylparaben (EP) (>99% purity; Hanbit), propylparaben (PP) (>99% purity; Hanbit), butylparaben (BP) (>99% purity; Hanbit), PE (>96% purity; Clariant, Muttentz, Swiss) and CPN (>99% purity; Serobilogiques, Pulnoy, France) were prepared at 5× MIC in 20% aqueous ethanol (>99% purity; Sigma, St. Louis, USA) for the evaluation of objective skin irritation. Preservatives tested were prepared at 1× MIC in 0.5% aqueous

carbomer (>60% purity; Noveon, Cleveland, USA) for sensory skin irritation. 0.5% aqueous carbomer was used as negative control for stinging test.

Lactic acid (LA) (>99% purity; Sigma) was prepared at a 5% concentration (w/v) in distilled water (DW) for selection test in stingers.

Part 2: formulations. We formulated 4 different types of emulsion bases as follows.

Emulsion base 1 contains 23.0% oils, 8.0% emollients and 4.0% surfactants.

The ingredients are aqua, hydrogenated polydecene, pentaerythrityl tetraistearate, cyclomethicone, glycerin, diisostearyl malate, squalane, dimethicone, hydrogenated oil, alkyl glucoside, hydrogenated lecithin, palmitic acid, glyceryl stearate, stearic acid, polyacrylamide, isoparaffin, perfume, alkyl acrylate crosspolymer and disodium ethylenediaminetetraacetic acid (EDTA).

Emulsion base 2 contains 18.5% oils, 8% emollients and 1.9% surfactants.

The ingredients are aqua, cetyl octanoate, meadowfoam seed oil, butylene glycol, glycerin, hydrogenated polydecene, hydrogenated oil, alkyl glucoside, dimethicone, cyclomethicone, pentylene glycol, stearic acid, hydrogenated lecithin, cetearyl alcohol, perfume, triethanolamine, carbomer, xanthan gum and disodium EDTA.

Emulsion base 3 contains 15.0% oils, 8.5% emollients and 3.1% surfactants.

The ingredients are aqua, glycerin, cyclomethicone, butylene glycol, hydrogenated oil, pentaerythrityl tetraethylhexanoate, diethylhexylcyclohexane, coconut oil, dimethicone, stearyl alcohol, polyethyleneglycol monostearate, squalane, stearic acid, dimethicone, urea, carbomer, perfume, xanthan gum, sodium methyl stearoyl taurate, triethanolamine, carbomer and disodium EDTA.

Emulsion base 4 contains 6.5% oils, 2% emollients and 1.2% surfactants.

The ingredients are aqua, hydrogenated polydecene, cetyl octanoate, butylene glycol, dimethicone, glyceryl stearate, cetearyl alcohol, hydrogenated lecithin, triethanolamine, carbomer, perfume, sodium methyl stearoyl taurate and disodium EDTA.

Table 1. Minimum inhibitory concentration of *Pseudomonas aeruginosa*

Preservatives	Minimal inhibitory concentration (%), incubation time of 1 and 3 days
Methylparaben	0.16
Ethylparaben	0.08
Propylparaben	0.04
Butylparaben	0.017
Phenoxyethanol	0.40
Chlorphenesin	0.40

Patch test

Part 1: comparison of primary skin irritation potential of preservatives. Subjects: 30 healthy Korean volunteers (20 women and 10 men) participated in this study after informed consent. The mean age was 33.7 years (20–61 years). The exclusion criteria were the following: pregnancy or lactation; alterations of the facial skin, such as wounds, scars or malformations; systemic disorders;

any dermatological disorders; history of major surgery; cosmetic treatment (peeling, biomaterial implant, laser) in the past year and ongoing pharmacological treatment.

Test procedure: 20 µl of test materials was applied to the filter paper discs on IQ test chambers (Chemotechnique Diagnostics, Vellinge, Sweden), and the patches were attached to the forearms of subjects for 1 day. They were removed 1 day after application. Skin reactions were evaluated at 30 min (1 D) and 1 day (2 D) after patch removal. The test reactions were evaluated according to the 4-point grading scale as follows:

- (1) 0: no visible reaction
- (2) +1: mild erythema
- (3) +2: intense erythema
- (4) +3: intense erythema with edema
- (5) +4: intense erythema with edema and vesicle

Part 2: cumulative irritation potential for formulations. Subjects: 15 healthy subjects, 8 women and 7 men, participated in this study after informed consent. The mean age was 29.7 years (22–47 years).

Test procedure: All subjects were patch tested for 21 consecutive days with various formulations using IQ test chambers. 20 µl of test materials was applied to the filter paper discs on IQ test chambers, and the patches were secured to the forearms. Patches were removed 1 day after each application, and irritation grading was performed on the test sites. The same grading scale was used as that in primary skin irritation. Patches were applied 3 times per week during 21 days. Each subject received 9 applications of the test materials to the same sites. At the end of the study, the total irritation score of each test material was determined for each subject by adding up each individual's day score. A mean total score was then determined for the 15 subjects.

Sensory irritation test

Subjects. Selection of stingers: After cleansing the face with water and drying gently with tissue, the subjects rested for 10 min in an environment-controlled room (temperature $24 \pm 4^\circ\text{C}$, relative humidity 40%–45%). Test materials were applied to each side of the nasolabial fold and cheek. Panelists' stinging and burning reactions were recorded at 10 seconds and at each minute for 9 min using a scale from 0 to 3. Stinging reactions were classified on an arbitrary scale as follows: 0 = no stinging, 1 = slight stinging, 2 = moderate stinging and 3 = severe stinging. The test material on soaked cotton swab was rubbed briskly and

then put on the test site for occlusion. With the application of LA, participants were classified as stingers or non-stingers. Classification was performed after 3 repeat tests of a single application of 5% aqueous LA. DW was applied to 1 side and 5% LA to the other. If the average of the responses at these time points was ≥ 0.2 , then the panelists were classified as stingers; if the average of the values was < 0.2 , the panelists were classified as non-stingers.

Stingers: 16 healthy subjects (6 women and 10 men) were selected. They were included in this study after informed consent. The mean age was 28.3 years (25–36 years). All subjects were free of systemic or skin diseases and had not used any topical preparations on the test areas during the study period.

Test procedure: In the same condition of selecting stingers, the same procedure was carried out with several formulations. 0.5 ml of the test materials was applied to each side of nasolabial fold and cheek.

Statistical analysis

Statistical analysis was conducted using SPSS® for Windows computer software (SPSS Science, Chicago, USA). An analysis of variance was performed to determine differences among the test materials. Multiple comparisons were performed using Fisher's least significant difference to evaluate differences in combined test material. The Wilcoxon test was utilized to compare the mean scores of sensory irritation for the test materials. Correlation between skin sensory irritation and an irritation reaction, and between sensory irritation mean scores and concentration of oils and emollients were analyzed by Pearson's correlation coefficient (r). $P < 0.05$ was regarded as statistically significant.

Results

Part 1: irritation potential of several preservatives according to antimicrobial activity MIC

In the first series of 24-hr occlusive patch test, the skin irritation potential of preservatives was compared and summarized in Table 2. It showed that there is no statistical difference in skin objective irritation potentials between different preservatives at $5 \times \text{MIC}$.

The comparison of sensory irritation according to preservatives is shown in Fig. 1. The sensory irritation potential of preservatives was very different at $1 \times \text{MIC}$. The sensory irritation potential of 0.4% CPN was the highest in comparison with the vehicle. Other preservatives

Table 2. 24-hr human patch test results with preservatives at $5\times$ MIC

Test substance	Mean score
20% ethanol solution (vehicle)	0.10 ± 0.55
0.8% methylparaben	0.13 ± 0.43
0.4% ethylparaben	0.23 ± 0.50
0.2% propylparaben	0.23 ± 0.43
0.085% butylparaben	0.10 ± 0.30
2.0% phenoxyethanol	0.16 ± 0.45
2.0% chlorphenesin	0.17 ± 0.38

MIC, minimum inhibitory concentration.

The values represent the mean $\pm$ standard deviation of irritation score ($n = 31$ subjects).

had similar irritant potential at $1\times$ MIC. It also suggested that there is no correlation between skin sensory irritation and an irritation reaction ($r = 0.18$).

Part 2: comparison of various combinations of preservatives in 4 types of formulation

Table 3 summarizes the cumulative irritation data. All formulations showed weak responses. In the objective skin irritation, there was no statistical difference in adverse skin reaction potencies between different combinations of preservatives. It was shown that sensory irritation potential varies according to the change of formulation (Fig. 2). In the same preservative system, sensory irritation in emulsion base 4 type was significantly higher than that in another emulsion base types. In the formulation type containing MP and PP, sensory irritation potential was similar with or without PE, while in the formula containing MP, PP sensory irritation was higher with CPN than without. This means that CPN increased the irritant effect on the MP and PP combined system regardless of the presence of PE, especially in emulsion base types 3 and 4 (Fig. 3).

We also compared changes of sensory irritation according to concentration of oils and emollients

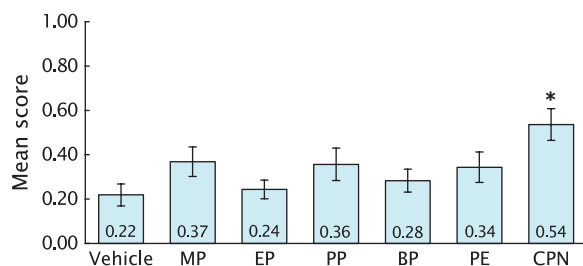


Fig. 1. Comparison of sensory irritation potentials of several preservatives. * $P < 0.05$ versus vehicle. Vehicle, 0.5% carbopol solution; MP, 0.16% methylparaben; EP, 0.08% ethylparaben; PP, 0.04% propylparaben; BP, 0.017% butylparaben; PE, 0.4% phenoxyethanol; CPN, 0.4% chlorphenesin.

Table 3. Comparison of the total cumulative irritation mean score according to formulation types

Test material	Formulation type	Total cumulative irritation mean score
0.2% MP/0.1% PP	Emulsion base 1	0.07 ± 0.26
	Emulsion base 2	0.13 ± 0.35
	Emulsion base 3	0.13 ± 0.35
	Emulsion base 4	0.33 ± 0.49
0.2% MP/0.1% PP/0.3% PE	Emulsion base 1	0.13 ± 0.35
	Emulsion base 2	0.33 ± 0.62
	Emulsion base 3	0.20 ± 0.41
	Emulsion base 4	0.07 ± 0.26
0.2% MP/0.1% PP/0.25% CPN	Emulsion base 1	0.00 ± 0.00
	Emulsion base 2	0.00 ± 0.00
	Emulsion base 3	0.20 ± 0.56
	Emulsion base 4	0.40 ± 0.91
0.2% MP/0.1% PP/0.3% PE/0.25% CPN	Emulsion base 1	0.00 ± 0.00
	Emulsion base 2	0.87 ± 1.19
	Emulsion base 3	0.20 ± 0.56
	Emulsion base 4	0.27 ± 0.46

MP, methylparaben; PP, propylparaben; PE, phenoxyethanol; CPN, chlorphenesin.

in the same preservative systems. There was a significant correlation between susceptibility to sensory irritation and concentration of oils and emollients in the 4 different preservative systems (Fig. 4).

Discussion

Preservatives are antimicrobial chemicals added to cosmetics, pharmaceuticals and foods to protect them against microbial spoilage and infection. The ideal preservative, both effective and devoid of irritant or sensitizing potential, has yet to be found. Parabens are the most commonly used preservatives in cosmetics, and it is non-irritating and non-sensitizing compared with other preservatives such as formaldehyde releasers and isothiazolinones (10). However, application of compounds

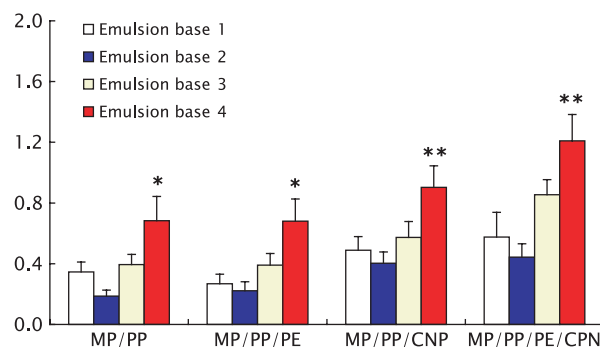


Fig. 2. Comparison of sensory irritation potentials in formulation types according to different preservative systems. * $P < 0.05$ versus base 1 and 2. ** $P < 0.05$ versus base 1, 2 and 3. MP, 0.2% methylparaben; PP, 0.1% propylparaben; PE, 0.3% phenoxyethanol; CPN, 0.25% chlorphenesin.

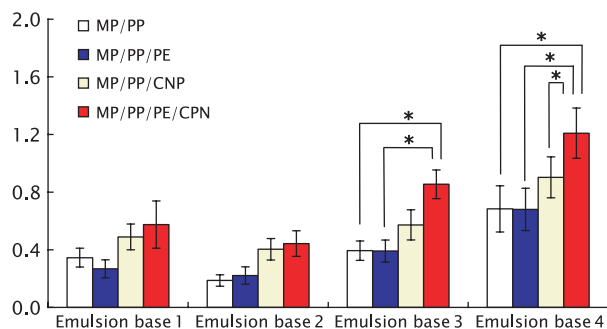


Fig. 3. Comparison of sensory irritation potentials in different preservative systems according to formulation types. * $P < 0.05$. MP, 0.2% methylparaben; PP, 0.1% propylparaben; PE, 0.3% phenoxyethanol; CPN, 0.25% chlorphenesin.

containing parabens to damaged or broken skin can elicit sensitization. PE and CPN are generally used with other preservative in cosmetics.

Several investigators have concluded that parabens, PE and CPN, are safe as cosmetic ingredients. However, in such studies, sensory irritation has not been considered. Sensory skin irritation represents the array of bothersome symptoms such as stinging, burning and itching that occur without visual signs of skin inflammation. This can often lead to consumer dissatisfaction.

In the classic method of sensory irritation test, Frosch and Kligman smeared 10% aqueous LA directly on the skin or smeared 5% aqueous LA on the skin after a 15-min facial sauna (11, 12). In our preliminary study, we found that subjects could not tolerate these 2 methods because of

strong stinging. Therefore, a lower concentration (5% aqueous LA without facial sauna) was used to select stingers in our study. Sensory irritation reaction because of preservatives are not well detected in non-stingers. Therefore, we used only LA stinger panels for this sensory irritation study.

Basketter et al. (13) showed that the response to a 0.3% sodium dodecyl sulfate patch test was assessed in a group of stingers and compared with the response in non-stingers. They concluded that there is no correlation between skin sensory responses and irritation reactions. For comparing skin adverse effect of preservatives according to antimicrobial activity, we evaluated objective skin irritation and sensory irritation potentials at 5× MIC. We found that at conditions of similar antimicrobial activity, similar skin objective irritation potential was observed but not sensory irritation. Elder et al. have shown that as the chain length of the ester group of paraben increases, antimicrobial activity increases (6), while MIC decreases. In our previous research, we found that as MIC of preservatives increased, skin objective irritation potential increased. Toshiro et al. (14) showed that the order of irritation potential was $MP < PP < BP$ (5). We also found that MP, EP, PP, BP, PE and CPN have similar objective skin irritation potentials at the same MIC, but in subjective irritation, CPN potential is higher than other preservatives. Our data indicate that there is a correlation between antimicrobial activity and objective skin irritation potential but not sensory irritation.

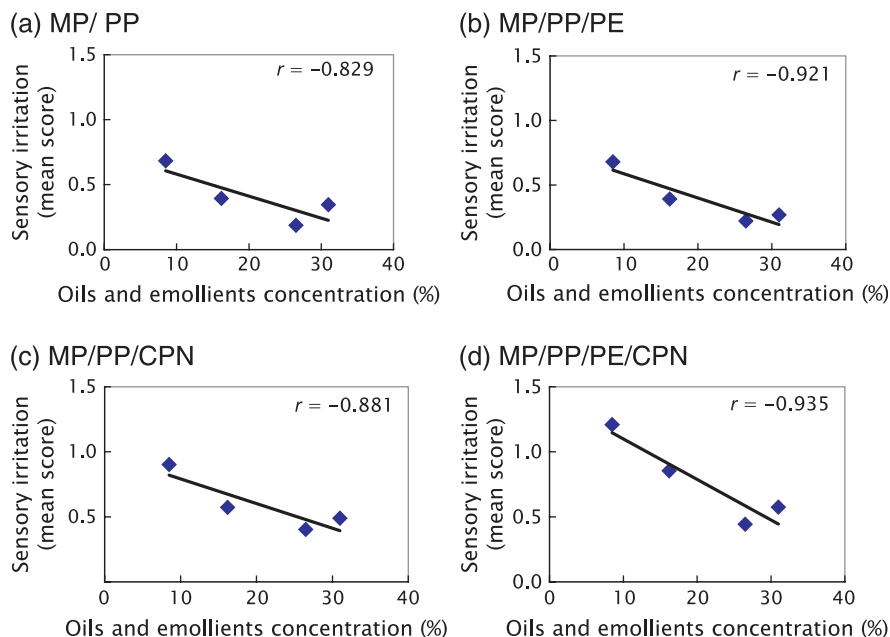


Fig. 4. Correlation between sensory irritation mean scores and concentrations of oils and emollients in 4 different preservative systems. $r \geq 0.8$, strong correlation; $0.6 \leq r < 0.8$, medium correlation; $0.4 \leq r < 0.6$, weak correlation; $r < 0.4$, no correlation. MP, 0.2% methylparaben; PP, 0.1% propylparaben; PE, 0.3% phenoxyethanol; CPN, 0.25% chlorphenesin.

Preservatives are known to elicit sensory irritation in cosmetic products. In our study, we found that sensory irritation potential of PE changed according to formulation types. In general, combining preservatives increases the sensory irritation value equal to their numerical sum. However, we observed that PE combined with CPN increased the irritation value than that of only CPN.

Even in the same preservative system, emulsion base 1 containing higher concentrations of oils and emollients produced lower sensory irritation potential than emulsion base 2. Ashikaga et al. (15) have shown that the mean intensity of sensory irritation by closed application was significantly higher than that of open application (16). This suggests that sensory irritation increased by providing occlusive conditions according to formulation type. Our data also suggest that an increased sensory irritation can occur by combining different preservatives, which is independent to the sensory irritation.

Our data indicate that there is a difference in sensory irritation potential according to the type of formulations. Variation of subjective irritation was not observed so strongly in the toner type, while marked variations were noticed in the lotion and cream types. It was thought that occlusive factors might be related to this phenomenon. In our data, significant correlation was observed between susceptibility of sensory irritation and concentration of oils and emollients in different preservative systems.

As shown in this study, preservative systems of parabens, PE and CPN, widely used as preservatives in cosmetics, are very safe in terms of objective skin irritation. However, there is a variable potency in terms of skin sensory irritation according to formulation types and preservative compositions.

We think that the variable sensory potency of preservatives is related to skin permeability of preservatives and the direct stimulation of nerves. Also, these characteristics can be changed when combining several preservatives. However, further study will be necessary for this question.

Consequently, influence on skin sensory irritation varies with the combination of preservatives in cosmetic products according to the type of formulation; this is independent of objective irritation. Therefore, in developing new preservatives and cosmetics products, it is important to evaluate skin sensory irritation of preservatives in cosmetic products according to the type of formulations.

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