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Original articles

HARMFUL SUBSTANCES IN COSMETIC PREPARATIONS: THE DIFFERENCE BETWEEN DECLARED AND ANALYZED

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Cosmetic preparations contain a large number of ingredients. Some of them may have negative consequences, most often in the form of allergic reactions, skin irritations, hormonal disorders, increased risk of cancer. These chemicals are most often added to protect, soften, or otherwise make the product more usable. The aim of this study was to determine the presence of some potentially harmful substances by qualitative analysis of cosmetic products using the GC-MS method and to compare results with the data stated on the product declaration. A total of 66 samples were analyzed: 10 baby shampoos and bath products, 26 cosmetic products for the axillary area and 30 samples of creams and serums. In 4 samples of baby shampoos and bath products (40%) the presence of potentially harmful ingredients were proven (parabens and phenoxyethanol), while diethyl phtalate was detected in all samples but was not stated in any of the declarations of these samples. Parabens were detected in 7.7% of the products for application in the axillary area, although were most often not declared on products, and diethyl phthalate was detected in 19% of these samples. In 24 samples of creams and serums (80%) the presence of potentially harmful substances buthylhydroxytoluene, cyclopentasiloxane, cyclotetrasyloxan, benzophenone, triethanolamine, phenoxyethanol, diethylphthalate was noted. This study has proven the misleading declarations. Phthalates were most often not declared on products. Better regulation of product declarations is needed, as well as stricter controls on the impact of primary packaging on product composition after packaging.

Keywords: COSMETIC PREPARATIONS, GC-MS METHOD, PARABENS, PHTHALATES, HARMFUL SUBSTANCES

INTRODUCTION

Cosmetic preparations contain a large number of ingredients with possible harmful effects, like butylhydroxyanisole (BHA), buthylhydroxytoluene (BHT), cyclopentasiloxane, cyclotetrasyloxan, benzophenone, triethanolamine, phenoxyethanol, parabens and phthalates. These compounds can have several negative consequences, most often in the form of allergic reactions, skin irritations, hormonal disorders, increased risk of cancer (breast cancer) and reduction in male fertility (1, 2). These chemicals are most often added to protect, soften the packaging (plasticizers) or otherwise make the product more usable (3). The more flexible the plastic packaging is, the higher number of phthalates is in it because they are not chemically bound to plastics so they can easily be released into the cosmetic product and the environment (4). Parabens have been the most widely used preservatives in the cosmetics, pharmaceutical and food industries for decades and their main function is to prevent and slow down the microbiological contamination of these preparations (5, 6). Methylparaben, ethylparaben, propylparaben and butylparaben are the most commonly used (7).

It has been established that parabens act as competitive agonists of estrogen receptors, so-called xenoestrogens, and that they can influence the development of breast cancer (8, 9). The incidence of breast tumors in the upper outer quadrant is 60 percent, suggesting a link between the use of axillary cosmetics and the development of breast tumors (10). The estrogenic potency was shown to increase with the increasing length of the linear alkyl chain and with increased branching of the alkyl chains, resulting in the following potency ranking order: methyl- < ethyl- < propyl- < butyl- < isobutylparaben and aryl group parabens (benzylparaben). Therefore, methylparaben and ethylparaben are considered the least harmful (11).

In some countries only the use of methylparaben and ethylparaben is allowed, while in Croatia the use of parabens, except benzylparaben, as a preservative in cosmetic products is allowed in concentrations of 0.4% for a single ester or 0.8% for a mixture (12). The daily absorption of parabens through the skin is between 0.03 and 4.13 mg/kg (13). However, there are four types of car-

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boxylesterases in the skin and subcutaneous adipose tissue that metabolize parabens and thus prevent complete accumulation in the body (14).

Undesirable substances in cosmetics include phthalates, whose the negative effect on the development of the reproductive system in male laboratory animals has recently become increasingly prominent (15). Many perfumes and air fresheners contain phthalates as a part of artificial fragrances. Most skin lotions, creams, soaps, shampoos, cosmetics contain parabens or phenoxyethanol as preservatives. Due to their lipophilic nature these chemicals are easily and completely absorbed by the body. These chemical compounds are classified as potentially harmful, toxic, and carcinogenic xenoestrogens (16-19). Phthalates are substances that are concealed in cosmetic preparations under the name "fragrances". The law does not require a complete declaration of fragrances, and without a detailed statement on the declaration, it is not possible to know whether the phthalates are present in the product (20).

Due to their physicochemical properties, phthalates are very mobile and easily migrate from plastic primary packing into the space around them, so they are present in water, air, and food, which is why humans are in constant contact with them (21). Some phthalates mimic estradiol and may promote breast cancer (22). The aim of this study was to qualitatively determine the presence of harmful substances in various cosmetic products (shampoos for children, products for axillary application and creams and serums) using the GC-MS method; to compare the results with the declaration on product packaging and compare the frequency of occurrence of products positive for the presence of harmful substances in the price range below the median value (23, 24).

MATERIALS AND METHODS

Materials

Qualitative cross-sectional research was conducted to determine the presence of harmful chemicals in cosmetic preparations. They were randomly selected from local specialty stores, pharmacies, and perfumeries. A total of 66 samples divided into three groups were analyzed. Group 1 (G1) contained 10 baby shampoos and bath products, of which nine were shampoos and one was shampoo/bath combination. Group 2 (G2) included 26 cosmetic products for the axillary area: 5 roll-ons, 4 sticks and 16 sprays, and in Group 3 (G3), 30 creams and serums samples were analyzed.

The aim of this study was to determine the presence of parabens, phthalates (diethylphthalate) and phenoxyethanol in G1; parabens and phthalates in G2 and butylhydroxytoluene (BHT), cyclopentasiloxane, cyclotetrasiloxane, benzophenone, phenoxyethanol, triethanolamine (TEA) and diethyl phthalate in G3. In addition to these analyses, we simultaneously compared the number of declared and detected substances for samples from the G2 and G3 groups.

For GC/MS analysis extraction of the substance was performed using organic solvents chloroform, ethyl acetate and n-hexane (p.a., Merck, Darmstadt, Germany). A total of 0.1 g (100 mg) of each sample was mixed with 2 mL of a mixture: chloroform: ethyl-acetate: n-hexane in the ratios (v/v/v = 1: 1: 1) and vortexed for about 20 seconds. Samples were macerated on a shaker at 30 rpm for 60 minutes. After that, samples were filtered and under a nitrogen stream evaporated to dryness. The residue was dissolved in chloroform and transferred to 1.5 mL vials. The injection was made on refer 0 oC in splitless mode with 1 μL of samples into GC/MS instrument.

Methods

The GC/MS analysis was performed using a Shimadzu GC/MS-QP2010 Ultra with an ion trap mass spectrometer mass selective detector (MSD). The chromatographic column was InterCap 5MS/NP (5% phenyl-95% methyl polysiloxane, length 30 m, diameter 0.25 mm, film thickness 0.25 μm). An initial column temperature of 100°C was held for 0.5 min, then programmed to rise to 220°C at 5 °C min^{-1} and then rise to 275°C and was held for 5 min. The total run time was about 30 min. Ultra-pure grade helium was used as a carrier gas at the flow rate of about 1.0 ml min^{-1}. Samples were qualitatively analysed by gas chromatography-mass spectrometry technique (GC/MS) using full-scan mode, TIC mode, (TIC - Total ion chromatogram) in the range of 40-600 m/z. Data were compared with Wiley 9 Mass spectra and Nist 107 library as well as in-house library containing about 1000 compounds. Each sample was analyzed in duplicate.

RESULTS

In our study 66 cosmetics products, divided into 3 groups, were analyzed. The presence of potentially harmful substances in cosmetic products was investigated. At the same time, the deviation from the results of GC/MS analysis and declaration stated on the product was examined. Qualitative GC/MS analysis was performed in TIC mode. In G1, out of a total of 10 analyzed shampoo and bath samples, in 9 of them the presence of parabens was not confirmed, while methylparaben was detected in only one tested sample. Diethyl phthalate was detected in all samples (Table 1). 2-Phenoxyethanol was detected in 3 samples.

Analysis of samples from G2, out of a total of 26 cosmetic product samples (for application in the axillary area) in 20 of them (77%) the presence of parabens and phthalates was not proven (Table 1).

Table 1.
Number of G1 and G2 samples in which the presence of parabens and/or phthalates was detected and the number of samples in which the same were not detected (free samples)

Substances	G1 (n=10)	G2 (n=26)
Parabens	1	2
Phthalates	10	5
Parabens and phthalates free samples	0	20

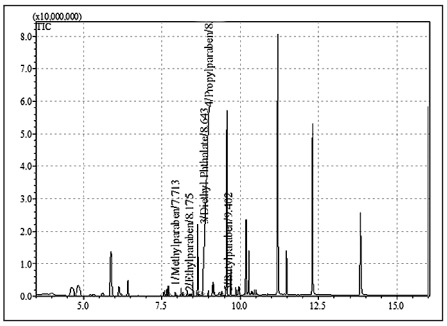

Figure 1.
Total ion chromatogram of sample No.1 from G2, analyzed by GC-MS technique. The x-axis shows the retention time of the sample (retention time, RT) while the y-axis shows the intensity of the substance proportional to the concentration of the substance. The presence of methylparaben (RT = 7.713), ethylparaben (RT = 8.175), propylparaben (RT = 8.798) and butylparaben (RT = 9.402) as well as diethyl phthalate (RT = 8.43) was proved in the tested sample

The presence of parabens was detected in only two samples (No. 1 and 4). In sample No. 1, the presence of methylparaben, ethylparaben, propylparaben and butylparaben was proved (Figure 1), while in sample No. 4 only methylparaben was detected. The presence of diethyl phthalate was proved in 5 samples (No. 1, 9, 17, 22 and 24).

Qualitative analysis of 30 serums and cream in G3 group was performed. In 24 samples, all 7 test compounds were found: butylhydroxytoluene (BHT), cyclopentasiloxane, cyclotetrasiloxane, benzophenone, triethanolamine (TEA), phenoxyethanol and diethyl phthalate. Each was found in at least one test sample. In most samples, 21 of them, the presence of phenoxyethanol was detected, while benzophenone was detected in only one of the 30 analyzed samples. No targeted substances were found in 6 tested samples. Figure 2 shows the results of the detected test substances in all analysed samples in all groups.

DISCUSSION

In recent times, there has been an increase in cosmetic products on the market, so people are often unsure which one to use. The question is what guarantees the quality of the product; is it the price, the brand, the declaration, or something else. Of course, the answers to these questions are not simple because, in general, neither brands nor price is a guarantee of quality.

The ubiquity of various negative connotations about the use of parabens as well as the abundance of advertising signs stating that several personal care products do not contain parabens (paraben-free), gives this experimental procedure of control of methodically selected cosmetic products, a justified purpose. Qualitative GC/MS analysis was performed in TIC mode and therefore significantly more compounds were detected than targeted. The analysis covered only the qualitative aspect to check for possible deviations from declaration. By reviewing the results in G1 group and comparing the identified components with the declared ones, it was noticed that in all samples, except sample number 7, significantly more substances were detected than stated on the declaration (25). Group 1 contained products intended for children care. Considering that young children have an increased sensitivity to the possible toxic effects of some chemicals; particular attention should be paid to the safety of cosmetic products intended for them. Such cosmetic product should contain only the number of ingredients strictly necessary and they all must be declared (26).

Within this research, potentially harmful ingredients were detected in 4 out of 10 samples. Parabens were not declared in any of the 10 analyzed samples. However, methylparaben was detected in one (Table 1 and Figure 2). Previous research has generally shown a high presence of parabens in children's cosmetic products (27). This research shows a significant decrease in the presence of parabens in children's cosmetics, which may be the result of better regulation or awareness of the potential harmfulness of parabens.

There were no declared phthalates in any of the samples, but it is rare to find them on the declaration because they are usually hidden under the name of fragrances. However, diethyl phthalate was detected in all samples, but this does not necessarily mean that diethyl phthalate is an integral component of every product. It is likely that its presence is the result of contamination and/or diffusion from the primary packaging. Sample No. 9, compared to other samples, gave an extremely strong signal, probably associated with its higher concentration. It is followed by samples No. 1 and 3, in which a slightly stronger signal was detected when compared to the other samples.

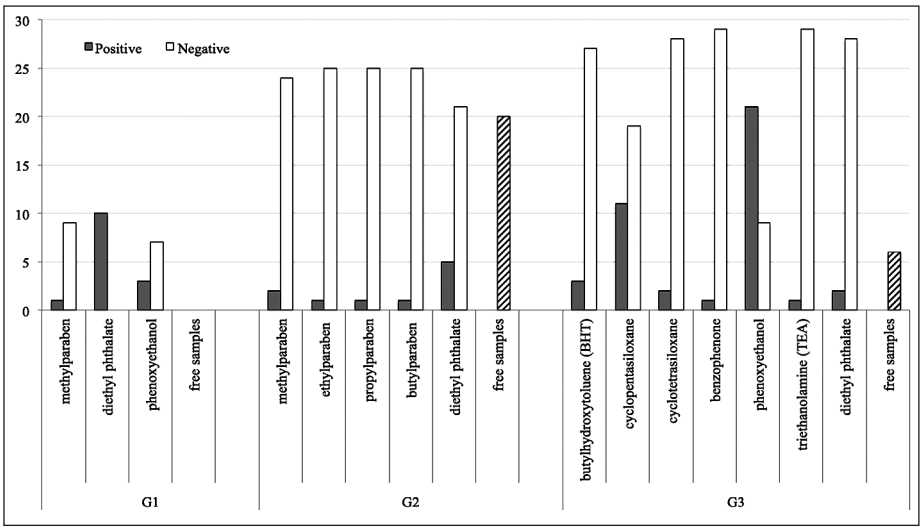

Figure 2.
Graphic representation of analysed and detected harmful substances classified by groups of tested samples and by tested substances in each sample. The number of samples in which the test substance was detected is indicated in gray, and the number of samples in which the presence of test substances has not been proven is indicated in white. Columns with slashes show the number of samples in which no harmful substance was detected, free samples

The authenticity of the declarations concerning the number of substances in shampoo and bath samples proved to be the lowest for products available exclusively in pharmacies and specialized stores, but in these products the least potentially harmful ingredients were detected. In 5 out of 10 samples phenoxyethanol was declared, but 2-phenoxyethanol was only detected in 3 samples. Also, methylisothiazolinone was declared in 1 sample, but it was not confirmed by our analysis. Although to be expected, comparing the regular line with that for atopic dermatitis, no difference was noted between the declared and detected, in the sense that there were fewer of them in products intended for atopic dermatitis, but it depended exclusively on the brand. In the results of samples from the G2 group, a higher percentage of samples negative for the presence of parabens and phthalates was observed. Parabens were identified in 2 samples while phthalates, more precisely diethyl phthalate, were confirmed in 5 samples.

The results of the analysis should be in accordance with the declaration marked on the product. All other possibilities are considered a deviation from the declaration. In sample No. 1 the presence of methylparaben, ethylparaben, propylparaben and butylparaben was proven, but unlike the others, the presence of ethylparaben was not declared. Although the presence of phthalates was confirmed in samples No. 1, 9, 17, 22 and 24, they were not stated in the declaration in any of these products, which was a deviation (28). Diethyl phthalate is widely used and is present in various PVC products such as packaging and plastic accessories. It is important to point out that none of the phthalate-positive samples is stated on the declaration. One of the explanations is that there was a dissusion of diethyl phthalate diffused into the product from the primary packaging of the product (4). Guart et al. in their study, among other things, showed the possibility of migration of phthalates from various plastic materials, while Dey and Das in their study showed that there is a link between septa performance and quality for GC/MS instruments and the release of volatile organic components, including substituted phthalate derivates, at high temperatures (29, 30). Their identification in cosmetic products indicates the need for stricter control of the composition of the product after packaging. "Well-known" brands (those that are largely represented on the market) showed correctness and compliance with the declaration, and those less used and more anonymous showed certain irregularities. The explanation for this observation lies in the fact that large and reputable brands are clearly undergoing stricter quality controls. Qualitative analysis of 30 samples of creams and serums from G3 revealed that in 24 of them all 7 tested ingredients were detected: (butylhydroxytoluene (BHT), cyclopentasiloxane, cyclotetrasiloxane, phenoxyethanol, benzophenone, triethanolamine (TEA) and diethyl phthalate) (31).

To control the declaration stated on the back of the product, the number of declared ingredients of each sample was compared with the number of ingredients detected by the GC/MS method. This study determined the presence of various compounds in the G2 and G3 analysed samples that were not stated on the product label (Figure 3).

This is especially true for samples from the G1 group, baby shampoos and baths. In almost all samples except one, more substances were detected than declared. From a total of 30 G3 samples (creams and serums samples), only in eight samples the number of declared and detected substances was equal. Compared to the number of declared substances, a lower number of substances was detected in 15 samples while a higher number of substances was proven in 7 samples. The obtained results, which indicate a defect and inconsistency with the stated declarations in 43% of products, it is concluded that the products should obviously undergo stricter quality control.

As a possible justification for such a difference in the number of declared and identified ingredients, as a shortcoming of the study, another segment should be considered, the probability of difficult chromatogram reading and inability to identify hydrophilic constituents of samples due to the method of sample preparation for processing (organic solvents) and identification technique. Due to the possible low volatility of some of the tested compounds, the preparation of samples for analysis should contain a larger amount of the sample itself, which is why it is desirable to extend the test to some further studies in the future.

A significant deviation of the detected harmful substances from the declared ones was observed, this is indicating the need for better regulation of product declarations. As this was not the main goal of this study, we did not conduct in-depth research on the deviation from the declaration. We just wanted to mention the importance for future research. In recent decades, there has been a public preoccupation with research into dangerous compounds in children's toys, clothing, and food products, raising awareness of their harmfulness (32). The most interesting harmful ingredient of all analyzed

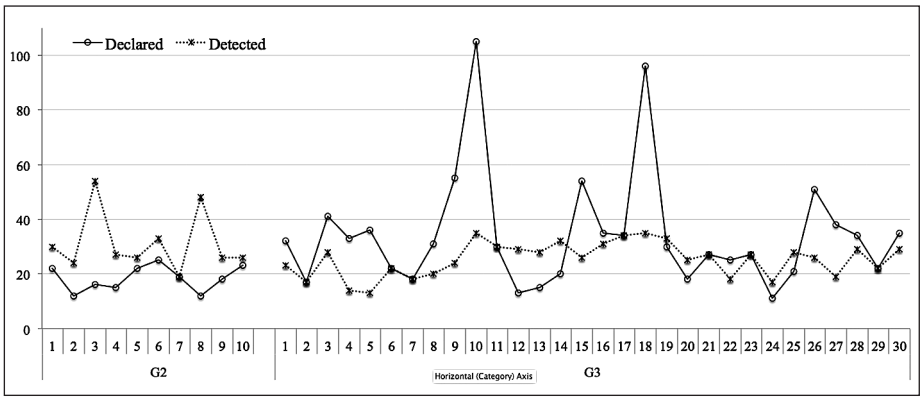

Figure 3.
Graphic representation of declared and detected harmful substances classified by groups and tested samples

samples is diethyl phthalate, known for its negative impact on the human body and the environment, found in 25% of all samples and not declared anywhere (33).

CONCLUSION

A significant deviation was found between the declared and analyzed ingredients in cosmetic products. The number of detected, potentially harmful ingredients was more positively correlated with the brand than with the price of the product. Our research confirms the presence of phenoxyethanol in a high percentage in creams, which is not surprising considering that phenoxyethanol has started replacing parabens. This compound may have deregulatory endocrine function capacity and potential xenoestrogen effect. Furthermore, controlled studies are needed to help detect the negative effects of cosmetic ingredients, which occur due to accumulation in the body, because of frequent and prolonged application to the skin in small concentrations. Potential harmful ingredients are insufficiently researched and can be found regularly in cosmetic products. Our results indicate the need for generally better control of quality and the declaration of cosmetic products and recommends a greater need for stricter controls on the impact of primary packaging on finished products.

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SUKOB INTERESA/*CONFLICT OF INTEREST*
Autori su popunili *the Unified Competing Interest form na www.icmje.org/coi_disclosure.pdf (dostupno na zahtjev)* obrazac i izjavljuju: nemaju potporu niti jedne organizacije za objavljeni rad; nemaju financijsku potporu niti jedne organizacije koja bi mogla imati interes za objavu ovog rada u posljednje 3 godine; nemaju drugih veza ili aktivnosti koje bi mogle utjecati na objavljeni rad./ *All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.*

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Sažetak

ŠTETNE TVARI U KOZMETIČKIM PRIPRAVCIMA: RAZLIKA IZMEĐU DEKLARIRANIH I ANALIZIRANIH

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Kozmetički pripravci sadrže veliki broj sastojaka. Ovi sastojci mogu imati negativne učinke, najčešće u obliku alergijskih reakcija, kožnih iritacija, hormonalnih poremećaja te povećan rizik od malignih oboljenja. Ove supstancije su najčešće dodane kako bi zaštitile, omekšale i učinile proizvod korisnijim. Cilj ove studije bio je otkriti prisutnost nekih potencijalno štetnih supstancija kvalitativnom analizom kozmetičkih proizvoda koristeći GC-MS metodu i usporediti ih sa podacima navedenim na deklaracijama. Analizirano je 66 uzoraka: 10 dječjih šampona i kupki, 26 kozmetičkih proizvoda za pazušno područje te 30 uzoraka krema i seruma. U 4 uzorka dječjih šampona i kupki (40%) je dokazana prisutnost potencijalno štetnih sastojaka (parabeni i fenoksietanol) dok je dietil ftalat pronađen u svim uzorcima, ali nije naveden ni na jednoj deklaraciji ovih uzoraka. Parabeni su pronađeni u 7,7% proizvoda za pazušno područje iako uglavnom nisu navedeni na delaraciji proizvoda, a dietil ftalat je pronađen u 19% ovih uzoraka.U 24 uzorka krema i seruma (80%) pronađeni su butilhidroksitoluen, ciklopentasiloksan, ciklotetrasiloksan, benzofenon, trietanolamin, fenoksietanol i dietilftalat. Ova studija je dokazala čest slučaj zavaravajućih deklaracija kada se neki potencijalno štetni sastojci ne deklariraju. Potrebna je bolja regulacija deklaracija na proizvodima kao i strože kontrole utjecaja primarne ambalaže na sastav proizvoda nakon pakiranja.

Ključne riječi: KOZMETIČKI PRIPRAVCI, GC-MC METODA, FTALATI, ŠTETNE SUPSTANCE

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