https://doi.org/10.31925/farmacia.2024.6.5 **ORIGINAL ARTICLE**

NICKEL AND CHROMIUM IN COSMETIC PRODUCTS: FROM LABORATORY TO REGULATION AND POSSIBLE HEALTH RISKS

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Abstract

The cosmetic industry is a rapidly growing global market that has raised concerns about the safety of its products, particularly regarding the presence of heavy metals such as nickel (Ni) and chromium (Cr). These metals pose potential health risks, including local and systemic toxicity. This study assesses the levels of Ni and Cr in 25 cosmetic products across six categories: oils, blushes, eyeshadows, lipsticks, eyeliners and eye/brow pencils using wet digestion followed by graphite furnace atomic absorption spectrometry (GFAAS). The results revealed that Ni and Cr concentrations varied significantly among products, with the highest levels detected in liquid lipsticks. Additionally, the study compares the Ni and Cr concentrations in the tested cosmetic products with existing regulatory limits and other published data and addresses possible health risks associated with using these products.

Rezumat

Industria cosmetică este o piață globală în creștere rapidă, care a ridicat îngrijorări cu privire la siguranța produselor, în special în ceea ce privește prezența metalelor grele precum nichelul (Ni) și cromul (Cr). Aceste metale prezintă riscuri potențiale pentru sănătate, inclusiv toxicitate locală și sistemică. Acest studiu evaluează nivelurile de Ni și Cr din 25 de produse cosmetice din șase categorii de uleiuri, farduri de obraz, farduri de pleoape, rujuri, creion de ochi și creioane pentru ochi/sprâncene folosind digestia umedă urmată de spectrometrie de absorbție atomică în cuptor de grafit (GFAAS). Rezultatele au arătat că concentrațiile de Ni și Cr au variat semnificativ între produse, cele mai ridicate niveluri fiind detectate în rujurile lichide. Suplimentar, studiul compară concentrațiile de Ni și Cr din produsele cosmetice testate cu limitele de reglementare existente și alte date publicate și investighează posibilele riscuri pentru sănătate asociate utilizarii acestor produse.

Keywords: nickel, chromium, cosmetic products, GFAAS, safety

Introduction

The cosmetics industry is a global powerhouse, with millions of products sold daily, which has seen impressive growth in recent years. This widespread use underscores the importance of ensuring these products are safe for consumers. Though the safety and quality of cosmetics have come under increasing scrutiny, many cosmetic products still do not comply with regulations, and they represent a safety problem for customers. In Europe, the annexes of Regulation (E.C.) No. 1223/2009 of the European Parliament and the Council on Cosmetic Products (R.1223/2009) are periodically updated with the latest recommendations elaborated by the Scientific Committee on Consumer Safety (SCCS) [15].

In recent years, consumers have become more aware of the potential health risks associated with the ingredients used in cosmetic products, especially for skin care products such as moisturisers, anti-ageing cosmetics, and sunscreens. However, this awareness decreases significantly regarding colour cosmetics such as eyeshadows, blushes, lipsticks (both liquid and solid), eyeliners and brow products used to enhance the appearance of the face.

The main ingredients in these cosmetics are the mineral pigments. Due to their vibrant colours and natural origins, these widely used pigments can be a source of contamination by heavy metals (HMs). Heavy metals in cosmetics are well regulated in Europe, precisely in Annex II of R.1223/2009, "List of substances prohibited in cosmetic products". The Annex mentions that the presence of heavy metals such as antimony (Sb), arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), lead (Pb) and nickel (Ni) is prohibited. While many brands claim to adhere to strict safety standards, detecting HMs in some products [4, 13, 36], even nowadays, suggests gaps in the supply chain and quality control processes. These metals pose potential health risks even in small quantities, including skin irritation, allergic reactions, and long-term repeated exposure toxicity [27].

Of all the HMs, nickel is the most frequent allergen in patch-test reactions [32]. Skin absorption of nickel depends on various factors, such as the integrity of the stratum corneum, the salt's type, certain weak organic acids (*e.g.,* octanoic and lauric acid), and the exposure time [2].

Nickel sensitisation is driven by repeated or prolonged exposure to nickel ions released from metallic or non-metallic nickel-containing objects [21]. When nickel ions penetrate the skin, they can bind to proteins and generate complexes recognised as antigens by the immune system. Thus, T-cells are activated, which plays a central role in developing allergic contact dermatitis [21]. Individuals with a mutation in the filaggrin gene, associated with a compromised skin barrier, are even more susceptible to nickel absorption and subsequent sensitisation [31]. T-cell activation is responsible for the allergic contact dermatitis triggered by chromium in sensitised individuals. Upon exposure, chromium binds to skin proteins, forming haptens recognised by the immune system as foreign antigens [10].

Prolonged exposure and accumulation are associated with immunosuppression and immunotoxicity, which can lead to autoimmune diseases [14].

In terms of systemic toxicity, beyond its carcinogenic effects, nickel can induce respiratory issues, such as asthma and chronic bronchitis. Prolonged exposure to nickel has also been linked to cardiovascular diseases through oxidative stress, which leads to inflammation and endothelial dysfunction, acting as risk factors for atherosclerosis and hypertension [18]. Besides the carcinogenicity of Cr^{VI}, chromium can determine impairments of multiple target organs within the digestive or cardiovascular system or the kidney and liver [39]. Although Cr^{III} is less toxic than its hexavalent counterpart, prolonged exposure through daily cosmetic use can still pose risks. As in the case of nickel, dermal contact with chromium compounds can also lead to immune system disorders and induce DNA damage and metastasis [29]. Due to these significant effects, the continuous monitoring of cosmetic products' quality and safety is necessary.

Thus, in the present study, we evaluated different cosmetic products from the Romanian market for their nickel and chromium content and assessed their safety.

Materials and Methods

Sample collection

The most commonly used cosmetic products were considered and collected for analysis. The selected samples represent the most popular and commonly used product types. The criterion for selecting the tested cosmetic products was questionable quality, which drove to different sample sizes among different cosmetic product categories.

Twenty-seven cosmetic products were sampled into six different categories: oils, blushes, eyeshadows, liquid and solid lipsticks, lip pencils, and eye and/or brow pencils. After purchasing the cosmetic products, they were stored in the laboratory at constant temperature and humidity conditions (22 - 25°C and 40 ± 10%, respectively).

Wet digestion (mineralisation) of the cosmetic product samples

Sample mineralisation was carried out in the Ethos Easy microwave digestion system. Approximately 200 mg of sample, 2 mL of 30% H_2O_2 solution, and 8 mL of 65% HNO_3 of HPLC purity were added to each vial to allow detection of impurities at the ppb (µg/L) level.

The oven has three segments/vials for mineralisation. The sample is weighed on an analytical balance directly into the vial. Then, the volumes of 30% hydrogen peroxide (2 mL) and 65% nitric acid (8 mL) are added, as indicated by the mineralisation method in the library existing in the apparatus operating software. One of the samples of interest is recommended to be placed in segment 1, where the sensor is introduced (a thermocouple temperature sensor for monitoring and controlling the temperature up to 300°C). If it is estimated that an exothermic chemical reaction may occur in one of the samples, that sample should be chosen for segment 1. The first stage of the mineralisation process lasts 15 minutes for the lipstick method, during which the temperature rises to 200°C; for three segments, the power used is set to a maximum of 1800 W. The second stage lasts 30 minutes and corresponds to maintaining the temperature at 200°C. The power is set to 1800 W. Afterwards, the vials (segments) are allowed to cool for approximately 15 minutes.

The mineralised samples are filtered to isolate the heavy metal salt solutions to be analysed next. This is done by applying atomic absorption spectrometry using the graphite furnace method. Graphite furnace atomic absorption spectrometry (GFAAS) is an extremely sensitive technique for analysing elements in small sample volumes. Due to the increased atomisation efficiency and controlled environment of the graphite furnace, GFAAS sensitivity is higher than that of flame atomic absorption spectrometry (FAAS) [11].

Reagents

All solutions were prepared with high-purity deionised water obtained with a Millipore model system. The glassware and polyethylene bottles were cleaned by soaking them in 10% nitric acid (Merck, Suprapur) and rinsing them three times with deionised water. High-purity nitric acid, 65% concentration (Trace Grade analysis), was obtained from Merck.

Preparation of calibration solutions for Cr and Ni

A high-purity Cr stock standard solution containing 1000 mg/L $Cr(NO_3)_3$ in 0.5 M nitric acid was purchased from Merck. From the 1000 mg/L stock solution, successive dilutions were made to obtain a final concentration of 100 µg/L, used for the standards of 1, 5, 10, 20, 40 and 60 µg/L.

A high-purity Ni stock standard solution containing 1000 mg/L $Ni(NO_3)_2$ in 0.5 M nitric acid was purchased from Merck. From the 1000 mg/L stock solution, successive dilutions were made to obtain a final concentration of 100 µg/L, used for the standards of 2, 4, 6, 8, 10, 30 and 60 µg/L.

The calibration curve for Ni is characterised by the equation y = 0.0025x + 0.0063, a correlation coefficient R^2 = 0.9973, an LOD of 6 ppb and an LOQ of 20 ppb.

The calibration curve for Cr is characterised by the equation y = 0.0131x + 0.0164 and a correlation coefficient R^2 = 0.9987. The LOD is 2.50 ppb and the LOQ is 8.40 ppb.

Apparatus and methods

The determinations were performed using a SOLAAR 6M Thermo Electron Inc. atomic absorption spectrometer equipped with a deuterium lamp for background correction. The analysis was performed using the GFAAS technique [26, 30] (with the GF95Z Zeeman graphite furnace system and FS95 autosampler) from Thermo Electron Inc. The manufacturer's recommended spectrometer parameters were a wavelength of 357.9 nm for Cr and 232 nm for Ni; the other parameters were set in the optimisation stages. For the GFAAS technique, a normal electrographite cuvette (Normal Electrographite Cuvette - NEC) was used; 20 µL was injected for both standards and samples.

The autosampler's capillary tip height in the cuvette was adjusted by observing the injection using a camera positioned in the graphite furnace (GFTV). All measurements were performed using the integrated absorbance, with at least two replicate determinations for each sample. Argon was used as a protective gas throughout the determination, with a flow rate of 0.2 L/min. The argon purity was 5.0, namely 99.999%. The temperature programs for the graphite furnace are detailed in Table I and Table II.

Table I

Furnace temperature program for Ni determination

Step	Temperature (°C)	Time (s)	Ramp (°C/s)	Gas Flow
Drying	100	30	10	0.2 L/min
Ashing	1000	20	150	0.2 L/min
Atomisation	2500	3.0	0	off
Cleaning	2600	3.0	0	0.2 L/min

Table II

Furnace temperature program for Cr determination

Step	Temperature (°C)	Time (s)	Ramp (°C/s)	Gas Flow
Drying	100	30	10	0.2 L/min
Ashing	1200	20	150	0.2 L/min
Atomisation	2500	3.0	0	off
Cleaning	2600	3.0	0	0.2 L/min

Data exploration

Ni and Cr concentrations were measured in duplicate for each cosmetic product, applying a dilution as necessary to enable their precise quantification using the GFAAS analytical method. To harmonise the data, all measurements were converted from ug/mL to mg/kg by adjusting to 200 mg with a conversion factor of 0.05. Duplicate samples were averaged before summarising as mean with standard deviation, median, and range of measurements (min, max). Data summaries and figures were obtained using R software version 4.3.1 with R studio version 2024.04.2 Build 764.

Averaged samples were presented by type of cosmetic product and by the sample of the product (corresponding to each analysed cosmetic product) and were subsequently compared to thresholds for Ni and Cr [3, 7] and ranges published in the scientific literature [9]. The results were also compared with the published FDA analysis [17].

Health Risk Assessment

The systemic toxicity was determined using the margin of safety (MoS) principle as explained in the SCCS Notes of Guidance [28], which is calculated as the ratio of No Observed Adverse Effect Level (NOAEL) of the investigated compound by its systemic exposure dosage (SED). The systemic exposure dosages (SED) for Ni and Cr were calculated using SCCS's equation [28]:

$$SED = \{[A(mg/kg\ body\ weight/day) \times C(\%)]/100\} \times [Dap(\%)/100],$$

where, A indicates the estimated daily exposure to a cosmetic product *per* kg body weight, based upon the amount applied and the frequency of application, C indicates the metal concentration in the sample, and Dap represents the dermal absorption of the compound under study, respectively.

Since no precise dermal absorption data is available, the conservative default value of 50% was used for both inorganic elements. This approach aligns with SCCS recommendations when no experimental data is available [28].

Regarding Cr exposure, SED is calculated in a worst-case scenario. As the GFAAS results are expressed as total Cr, without distinguishing between Cr^{III} and Cr^{VI}, for SED and MoS, we considered that the exposure was to the most toxic compound (Cr^{VI}). This approach triggers better protection for the consumer.

Results and Discussion

Data characteristics

Using the analytical methods described above, different concentrations of Ni and Cr were identified in the analysed products. Table III shows that the highest concentrations of Ni were present in eye pencils and long-lasting liquid lipsticks, with mean values of 16 mg/kg and 13.8 mg/kg, respectively. In contrast, the lowest concentrations were found in oils, with a mean of 0.761 mg/kg. The Ni concentrations obtained for the different cosmetic types are consistent with previously published data [9], except for oils that were not reported. Nonetheless, the reported values are highly variable for all cosmetic types.

Similarly, to what has been observed for Ni, Cr concentrations are highest in the same type of cosmetic products – eye pencils and long-lasting liquid lipsticks, with mean values of 41 mg/kg and 38.4 mg/kg. The lowest levels were observed in blush powders. Also, in the case of Cr, the obtained values for the different cosmetic types are consistent with previously published data [9].

Table III

Concentrations of Ni and Cr measured in cosmetic products (mg/kg)

	Ni concentration (mg/kg)	**Cr concentration (mg/kg)**
Blush powder (N = 3)	Mean 12.0 [Min, Max]: [7.34, 19.1]	Mean: 4.11 [Min, Max]: [0.176, 8.08]
Eye pencil (Ni: N = 4, Cr: N = 10)	Mean 16.0 [Min, Max]: [9.07, 22.1]	MMean (S.D.): 41.0 (31.5) Median [Min, Max]: 32.3 [5.52, 89.8]
Eyeshadow powder (Ni: N = 3, Cr: N = 6)	Mean: 7.01 [Min, Max]: [1.86, 10.1]	MMean (S.D.): 29.3 (18.1) Median [Min, Max]: 24.5 [11.3, 51.6]
Lip pencil (Ni: N = 5, Cr: N = 10)	Mean: 21.3 [Min, Max]: [3.51, 57.2]	MMean (S.D.): 30.0 (28.1) Median [Min, Max]: 11.3 [6.40, 69.8]
Long-lasting Liquid lipstick (N = 6)	Mean (S.D.): 13.8 (0.664) Median [Min, Max]: 13.8 [13.0, 14.5]	MMean (S.D.): 38.4 (40.8) Median [Min, Max]: 36.6 [0.703, 79.1]
Long-lasting solid lipstick (N = 3)	Mean: 6.21 [Min, Max]: [5.22, 7.81]	MMean: 8.66 [Min, Max]: [5.82, 14.0]
Oil (N = 3)	Mean: 0.761 [Min, Max]: [0.116, 1.56]	NNA
Overall (N = 27)	Mean (S.D.): 12.3 (11.5) Median [Min, Max]: 9.56 [0.116, 57.2]	MMean (S.D.): 28.8 (28.5) Median [Min, Max]: 13.5 [0.176, 89.8]

Legend: N: number of samples; N.A.: not applicable; S.D.: standard deviation; Min: minimum; Max: maximum; For sample sizes of N < 6, data was summarised as mean with a range of observations [Min, Max].

Figure 1A and Figure 1B compare the analysed cosmetic products with Italy's maximum accepted limit for Ni (10 mg/kg) [3]. While oils, long-lasting solid lipstick and eyeshadow powder all have measured values below this threshold, some products (blush powder, eye pencil and lip pencil) can have Ni concentrations above the threshold, with the long-lasting liquid lipstick having all measured values above 10 mg/kg.

Figure 2A and Figure 2B compared the analysed cosmetic products with the maximum accepted limits for Cr^{III} (5 mg/kg) and Cr^{VI} (1 mg/kg) [3]. None of the analysed cosmetic types had measurements below the 1 mg/kg limit. All cosmetic types except blush powders had all or most Cr measurements above the 5 mg/kg threshold. More variability was observed in eye pencils, eyeshadow powder and lip pencils. The data characterisation in Table III also supports this, where the standard deviation is the largest for these three categories.

In Figure 3A, the obtained Cr levels in the blushes are compared to those tested by the FDA from various brands. Two values obtained by the FDA were excluded from the analysis: one with a Cr level of 400 ppm and another where only trace amounts of Cr were found. Notably, the Cr levels in our samples range between 0.176 ppm and 8.08 ppm, while those tested by the FDA range between 0.43 ppm and 400 ppm. Figure 3B shows the levels of Ni found in the blushes from our study compared to those tested by the FDA. The FDA results range between 0.64 and 23 ppm.

The Cr levels in the six eyeshadow powders from the analysis are shown in Figure 4A, which also includes a comparison to FDA-tested samples from different brands. It is important to note that values exceeding 1000 ppm have been excluded from the graph. The FDA reported three elevated Cr levels of 22 000, 3 500 and 1 100 ppm. However, our tested products had Cr levels between 12.00 and 51.50 ppm, whereas the FDA's results were between 11 and 22000 ppm.

Regarding nickel, its levels can be seen in the Figure 4B. One value tested by the FDA was excluded from the graph: 1600 ppm. The Ni concentrations obtained by our method ranged from 1.86 to 10.07 ppm, while FDA levels were between 10 and 1600 ppm.

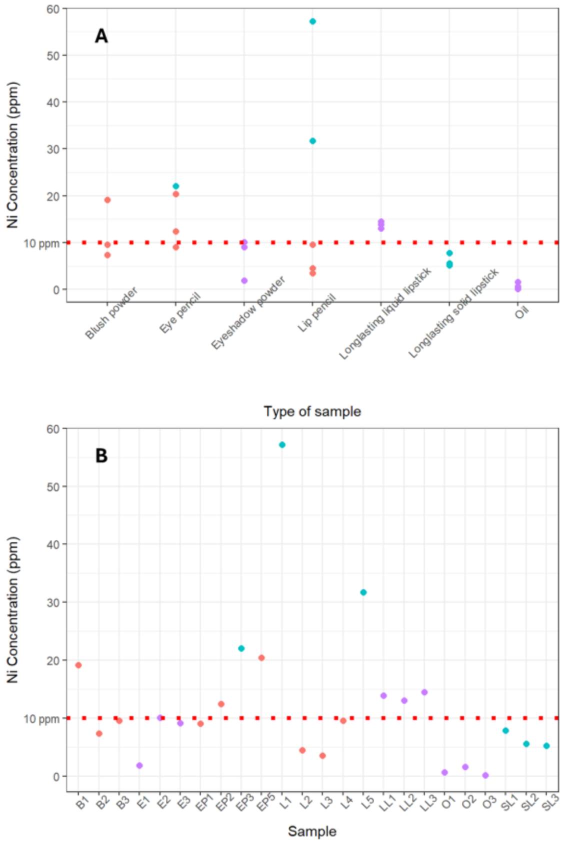


Figure 1.
Comparison of the measured levels of Ni (represented as dots) displayed by (A) cosmetic type or (B) samples of cosmetic products with the maximum accepted limit (dotted red line)

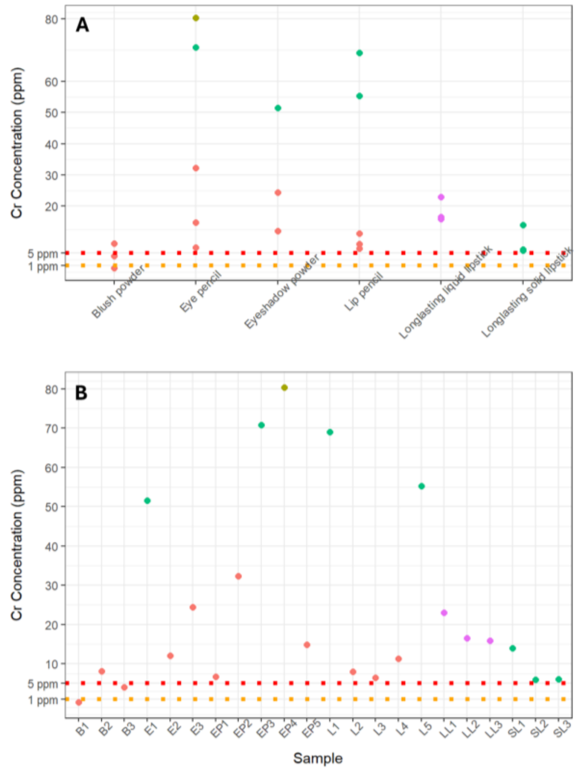
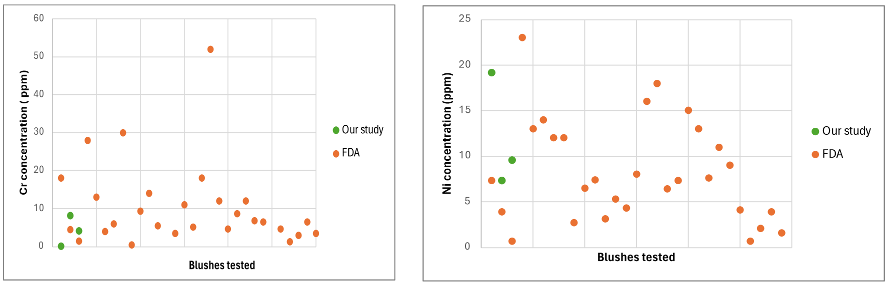
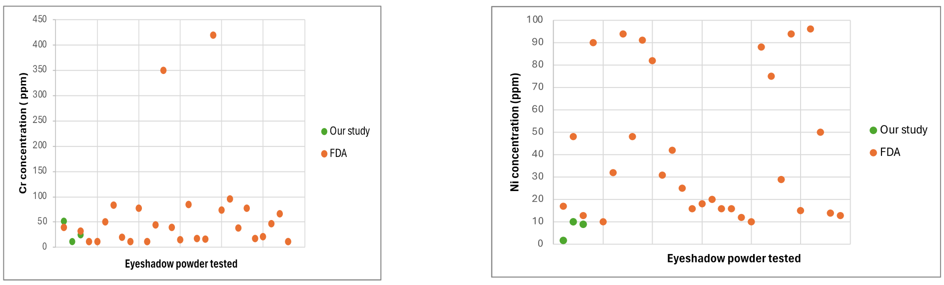


Figure 2.
Comparison of measured Cr levels displayed by (A) cosmetic type or (B) samples of cosmetic products with the maximum allowed limits (dotted lines: yellow - reference at 1 mg/kg; red line - reference at 5 mg/kg)



A B

Figure 3.
Comparison of (A) Cr levels and (B) Ni levels in blushes tested (green) *versus* FDA values (orange)

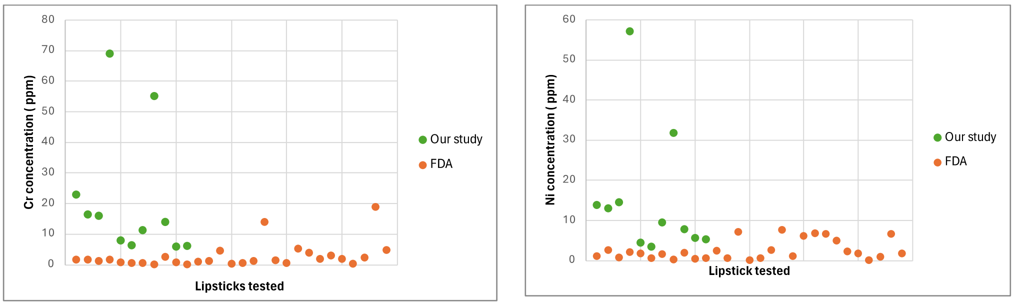


A B

Figure 4.
Comparison of (A) Cr levels and (B) Ni levels in eyeshadow powders tested (green) *versus* FDA values (orange)

The lip products group includes three different product types: lip pencils, long-lasting liquid lipstick, and long-lasting solid lipstick. The Cr levels in these products can be seen in Figure 5A. Interestingly, compared with the published FDA values, the Cr levels found in the tested samples were significantly higher, ranging from 6.12 to 69.05 ppm. The FDA obtained a maximum value of 19 ppm, with most of their results falling within the 0 - 6 ppm range.

A similar finding is in Figure 5B, considering the Ni levels.



A B

Figure 5.

Comparison of (A) Cr levels and (B) Ni levels in lipstick products tested (green) *versus* FDA values (orange)

HMs may be present in cosmetic products as impurities from the primary materials or the production flow or as ingredients mainly used as pigments. Our experiments focused on the quantitative determination of Cr and Ni levels in the selected products belonging to the following categories: blush powders, eye pencils, eyeshadow powders, lip pencils, long-lasting liquid lipsticks and long-lasting solid lipsticks.

Experimental data vs. regulatory recommendations

The presence of chromium in cosmetics varies globally, with some regions imposing strict limits and others offering more general guidelines. For example, R.1223/2009 stipulates that the intentional use of Cr^{VI} in cosmetics is banned due to its toxic, carcinogenic, and allergenic properties [16]. Italy has national recommendations for traces of Cr^{III} and Cr^{VI} in cosmetic products, namely 5 and 1 mg/kg, respectively [3]. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) classifies Cr^{VI} as a hazardous substance in Australia and follows the EU's restriction [6].

In the United States, the FDA has no specific limit for Cr in cosmetics. The Federal Food, Drug, and Cosmetic Act (FD&C) mentions that cosmetic products should not be adulterated or misbranded. While there are no explicit chromium limits, the FDA has issued guidance on the safety of chromium oxide greens (mainly Cr_2O_3), which is considered safe when used as colourant for externally applied products, including those intended for use in the eye area [5, 34]. Chromium hydroxide green is safe for topical use, including in products dedicated to the eye area [35].

The lip area is considered excluded from the permitted use of chromium colour additives. According to R.1223/2009, chromium colour additives can be used in cosmetic products if they are free from chromate ions [16]. In Canada, “chromic acid and its salts” and “chromium” are included in the Cosmetic Ingredient Hotlist as prohibited for use in cosmetic products [19]. No limits for other Cr compounds are regulated.

In our experiments, all tested cosmetic products intended for the lips area (lip pencils, long-lasting liquid lipsticks, and solid lipsticks) proved to have a chromium content of more than the maximum allowed limit of 5 ppm.

The EU regulation for Ni in products that get in contact with the skin refers to a maximum concentration of delivered nickel of 0.5 μg/cm^2/week for short-term contact and 0.2 μg/cm^2/week for prolonged contact, as mentioned in REACH Regulation (EC) 1907/2006 [15]. According to R.1223/2009, metallic nickel and nine nickel compounds are prohibited from being used as cosmetic ingredients and are included in Annex II [16]. On the other hand, the EU legislation introduced the concept of “technically unavoidable in good manufacturing practice” associated with HMs, including Ni; in this direction of trace presence, the Istituto Superiore di Sanità in Italy recommends the maximum nickel impurity limit to 10 mg/kg in cosmetic products, except for toothpaste, where the limit of concentration is 1 mg/kg [3]. The same 10 mg/kg trace limit for Ni was set in Germany, although the limits have been significantly reduced for other HMs such as lead, mercury, or arsenic [22].

South Korean legislation lists Ni as an ingredient that must not be included on purpose in the composition of cosmetic products. However, Ni is sometimes unintentionally derived and technically impossible to remove. The maximum limit allowed for Ni in eye makeup products corresponds to 35 ppm, for makeup products 30 ppm, and for other cosmetic products 10 ppm [25]. Other countries do not state impurity limits but warn about nickel toxicity, including allergic dermatitis.

Experimental data versus other reported data

There has been an increasing interest in the presence of HMs in cosmetic products, and the studies regarding their quantification in these samples have multiplied, especially after the elaboration and implementation of R.1223/2009. One of the latest assessments of the level of HMs in different categories of cosmetic products manufactured in various places in the world was published by Abed *et al*. [1]. Regarding the presence of Ni in lipsticks, the highest levels were reported by Abed *et al*. for Pakistani and Australian manufactured products, 6.48 mg/kg and 6.69 mg/kg, respectively. The range of Ni concentrations for the lipsticks included in our study was more extensive, 5.22 - 7.81 mg/kg for the solid lipsticks and 13 - 14.5 mg/kg for the liquid ones. This indicates that liquid lipsticks contain almost twice as much Ni as solid ones. On the other hand, lip pencils were not reported in the cited paper; however, the samples we tested revealed an extensive range of Ni amounts, 3.51 - 57.2 mg/kg. As the estimated daily exposure to lipsticks is significantly higher than other products, we consider that they deserve particular attention as they are long-stay products and can be ingested, leading to systemic toxicity in addition to local toxicity.

In the published literature, Cr was most abundant in UK-manufactured lipsticks, 2.02 mg/kg, followed by 0.9 - 2.5 mg/kg in American and 0.8 mg/kg in Korean products [38]. Cr concentrations in 15 different Malaysian-marketed lipsticks ranged between 0.48 and 2.50 mg/kg, and the highest values were attributed to the most expensive products tested [38]. Surprisingly, higher Cr levels were determined in our experiments, such as 5.82 - 13 mg/kg for solid lipsticks; in the case of liquid lipsticks and lip pencils, Cr concentration values were primarily spread between 0.703 and 79.1 mg/kg and between 6.4 and 69.8 mg/kg, respectively.

Eye makeup should be as "clean" as possible, as the skin in the eye area (periorbital) has a thin structure and can be easily irritated, which might lead to increased permeation and absorption of different chemical compounds, including HMs [2]. In the published literature, dangerously high levels of Ni were reported in Chinese eyeshadows, with a range of 77.2 - 359.4 mg/kg. Increased Ni concentrations were identified in Italy and USA-manufactured eyeshadows, 28.6 - 134 and 21.8 - 56.5 mg/kg, respectively [1]. According to the same published literature, Cr was most abundant in Chinese and American samples (16.7 - 150 mg/kg and 15 - 280.2 mg/kg, respectively), while the range for the Italian products was much narrower, 38.7 - 55.4 mg/kg [1].

The eyeshadow samples we tested had much lower levels of Ni, 1.86 - 10.1 mg/kg, while the obtained results for Cr are closer to the literature values, varying from 11.3 to 51.6 mg/kg.

Kohl or eye pencils are used worldwide, in many places having cultural or social importance. Cr was quantified in kohl samples produced in Spain and Germany, with a range of concentrations of 0.0011 - 19.62 mg/kg; surprisingly, higher amounts of Cr were found in eye pencils manufactured in Belgium, France, Spain, Switzerland, and the UK, with a maximum of 983 mg/kg [1].

Our tests observed an extensive range of Cr levels, with intermediate values between the mentioned studies, namely 5.52 - 89.8 mg/kg. The Ni levels we found in the oil samples ranged from 0.116 to 1.56 mg/kg, with the upper limit 10 times higher than the range obtained for body creams by Bocca *et al*. [9].

FDA published the results obtained by Hepp *et al*. for Cr and Ni concentrations in various cosmetic products using the inductively coupled plasma-mass spectrometry (ICP-MS) method [20]. Among all the cosmetic products, 30 blushes, 30 eyeshadow powders, and 30 lipsticks from different brands were analysed. As for the Cr and Ni levels, out of the 30 blushes, traces of Cr and Ni were found in only one product, while another had a high Cr content of 400 ppm. Regarding the eyeshadow powders, high Cr levels, exceeding 1000 ppm, were identified in three different products, according to the FDA report. Surprisingly, in the case of lipsticks, the highest levels of Cr and Ni were found in our study. The cause of these results may be that our study included three types of lip products: lip pencils, long-lasting liquid lipstick and long-lasting solid lipstick, while the FDA does not mention the kind of lipstick tested.

Health Risk Assessment

As the estimated daily exposure to the lipsticks is significantly higher compared with other groups of products, we used this category as a "worst-case" scenario to assess whether there is any risk associated with their daily use. We also assessed the systemic and local toxicity of these cosmetic products.

The concentrations of Ni in tested liquid lipstick are in the range of 0.0013 to 0.00145% (w/w) and lower in solid lipstick, in the range of 0.00052 to 0.00078 (w/w). As the calculated relative daily exposure level for lipstick corresponds to 0.9 mg/ kg body weight *per* day, SED values correspond to 0.0000117 to 0.000013 mg/kg body weight/day for two applications *per* day for liquid lipstick and up to 0.000007 mg/kg body weight/day for solid lipsticks. The NOAEL for Ni used to calculate MoS corresponds to 2.2 mg/kg body weight/day, derived from a 2-generation rat study [37]. For two product applications *per* day, the calculated MoS values correspond to 188,034 and 169,230 for liquid lipsticks and even higher for solid lipsticks. As these values are significantly higher than 100, there is no toxicological concern for systemic toxicity following exposure to tested lipsticks.

Regarding Cr exposure, SED is calculated considering the total Cr measured in samples without distinguishing between Cr^{III} and Cr^{VI}. The concentrations of Cr in tested liquid lipstick are in the range of 0.00007 to

0.00791 % (w/w) and lower in solid lipstick, in the range of 0.00058 to 0.0014 (w/w). SED values calculated for the highest Cr concentrations correspond to 0.000071 mg/kg body weight/day for two applications *per* day for liquid lipstick and to 0.000012 mg/kg body weight/ day for solid lipsticks. The most conservative NOAEL value found for Cr corresponds to 2.5 mg/kg body weight/day, retrieved from a 1-year study where rats were exposed to chromate in drinking water [34]. For two daily applications, the calculated MoS values correspond to 35,211 for liquid lipsticks and 384,615 and 208,333 for solid lipsticks, respectively. Also, there is no systemic toxicity concerning the daily application of the tested lipsticks.

In the case of the other category of products, considering the daily applied amount [28], MoS values for Ni and Cr are significantly lower than those obtained for the lipsticks.

As Cr and Ni are well-known sensitisers, the safety assessment implied the local toxicity of both elements. For topical Ni-exposure, a human threshold value of 0.2 µg/cm^2/week for dermal exposure is available for consumer products intended to be in direct and prolonged contact with the skin; for short-term contact, 0.5 µg/cm^2/week is applied [15]. Liquid or solid lipsticks come in contact with the lip's surface for a long time (4.8 cm^2); *per* application, an amount of 0.0057 g is estimated [28]. Assuming that all Ni present in the product would come in contact with the skin (worst case scenario), two applications *per* day to liquid lipsticks would give Ni exposures in the range of 0.01543 µg/cm^2/week to 0.01721 µg/cm^2/week, which is at least 11 times lower than the set threshold. When exposure is twice daily, 7 times *per* week, maximum Ni exposure would be 0.1205 µg/cm^2/week, which is still lower than the threshold.

Although lipsticks are leave-on products with prolonged skin contact, it seems reasonable to assume that sensitising effects will not occur on healthy consumers, knowing that traces in personal care products of the same magnitude are not the primary cause of sensitisation [24]. However, an already Ni-sensitised consumer may react and consequently suffer from allergic contact dermatitis [33].

The threshold for Cr^{VI} sensitisation is approximately 10 ppm [12], but Cr-sensitised people react to concentrations as low as 7 ppm [23]. Only the total sum of Cr^{III} and Cr^{VI} is available in the analysed samples. Assuming the worst-case scenario that the total concentration corresponds to Cr^{VI}, the maximum concentrations found in tested liquid and solid lipsticks are higher than the threshold for sensitisation (10 ppm). As Cr^{VI} is a certified carcinogenic (IARC Group 1), ultra-high sensitive quantification methods were used in previous studies, such as IC-ICP-MS [3]. As the analytical method applied in the present research was GFAAS, a moderate sensitivity quantification technique, the worst-case scenario of translating all the chromium into Cr^{VI} was considered.

This preliminary finding suggests sensitising effects may occur on healthy consumers when using the tested products. Furthermore, this assumption is plausible considering the aggregate exposure, as the consumer may use multiple products.

Conclusions

Chromium and nickel are found in various cosmetic products, which raises significant concerns regarding local and systemic toxicity. The existing regulations on Cr and Ni limits of concentration in cosmetics vary widely across different regions. Our experimental results revealed that certain cosmetic products, especially lipsticks and lip pencils, contain higher levels of Cr and Ni than reported data from the other areas. Liquid lipsticks contained almost double the Ni concentration of solid lipsticks. The variation in Cr levels was also notable, with high concentrations being identified, particularly in lip and eye makeup products. Although the high levels of Cr and Ni found in cosmetics, especially those applied to the lips, pose potential health risks**,** the calculated MoS indicate no toxicological concern for systemic toxicity.

Furthermore, as Cr and Ni are well-known sensitisers, the assessment of local toxicity revealed that the Ni sensitising effects would not occur on healthy consumers when using the tested products. However, it is possible to happen in the case of Chrome, as the maximum concentrations found in tested liquid and solid lipsticks are higher than the threshold for sensitisation. This finding underscores the need for stringent regulation and consistent monitoring of HM content in cosmetics, as it is crucial to minimise exposure and protect consumers from potential adverse health effects.

Conflict of interest

The authors declare no conflict of interest.

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