Evaluation of heavy metal content of some lipsticks in Iran market

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Abstract
This study quantitatively estimated heavy metals as cadmium, lead, chromium and nickel (Cd, Pb, Cr and Ni) using flame atomic absorption spectrometry (FAAS) in ten lipstick products sold by local markets in Sari, Mazandaran State, Iran. All the samples were digested in HNO₃ and HClO₄ and then evaluated for heavy metal contents. All the concentrations are mean value of triplicate experiments for each sample. Analysis of variance (ANOVA) and Tukey post-hoc tests were used to determine significant variations in heavy metal contents and p < 0.05 was considered as significant. The determined range of cadmium, lead, chromium and nickel were 0.01-0.05 µg/g, 0.18-0.80 µg/g, 0.06-0.75 µg/g, and 0.00-0.34 µg/g, respectively. These results showed that nickel and cadmium contents were less than WHO and US FDA safe limit, while for chromium only one samples (No.5) showed higher concentration than safe limit. The content of lead in all samples were lower than safe limit unless sample 6 which showed high level of lead.

Keywords: Cadmium, lead, chromium, nickel, lipstick

Introduction
Cosmetics have been widely used by people all over the world since ancient times for cleaning, improving or changing the skin, hair, nails and teeth (1). These products may have various exposure frameworks (2,3). Some of cosmetics are rinsed-off (e.g. shampoos and toothpaste), other cases the products (e.g. body lotion, deodorant, lipsticks) may remain in contact with the skin over several hours, some of them may be applied over a large surface of the body (e.g. body emulsions) and some others are applied via spray, presenting the possibility of aspiration (4). Among the cosmetic products, lipsticks have the higher risk of direct oral ingestion, aggravating the negative effects of their ingredients. Therefore, people’s concern about cosmetics toxicity has become an important issue. The early lipsticks presented in the market consist of beeswax, fat and pigment as the main ingredients. The worldwide use of lipsticks has been increasing due to the willing of individual beautification (5). Lipsticks are usually contain heavy metals including lead, nickel, aluminum, arsenic, cadmium, antimony, and chromium (6). Lip products are presented in a wide variety of colors, that are produced by addition of pigments. These pigments may be mineral or organic and may contain heavy metals as impurities in the pigment formulation (7-9). It is believed that at certain specified limits, some heavy metals could be of biological importance to human (10). However, heavy metals such as lead, nickel, cadmium and chromium have been reported not to have any known biological importance and can be very toxic at very low concentration (11-17). Cadmium is toxic at very low levels. Exposures to the cadmium may cause renal dysfunction and long term exposure to cadmium can lead to obstructive lung disease (10). Cadmium is one of the heavy metals that if it is directly subjected to the human body it would decrease the blood pressure (18). It is also connected with the diabetes (19). Absorption of lead by the body causes inhibition of haemoglobin synthesis, kidney dysfunction, reproductive and cardiovascular systems dysfunction (20-
The toxic effects of cadmium and lead are shown via bonding to sulfydryl groups of proteins and depletion of glutathione. Lead is described as one of the most dangerous contaminants to arise in human civilization due to its distribution in environment as polluting element (23,24). Exposure of lead at high levels is well known to be toxic, but exposure to relatively low levels may entail adverse health effect. High levels of lead exposure may cause serious health damages including both acute and chronic poising, pathological change of organs and disease related to cardiovascular, kidney, bone, and liver and it can even cause cancer owing to excessive accumulation in human body (24,25). Chromium and cobalt may undergo cycling reactions to generate reactive radicals in the body (26).

Breathing high levels can cause irritation to the lining of the nose, asthma and shortness of breath or wheezing. Skin contact can cause skin ulcers. Allergic reactions consisting of severe redness and swelling of the skin have been noted. Long term exposure can cause damage to liver, kidney circulatory and nerve tissues, as well as skin irritation (27). In addition, Ni, Co and Cr are well known that causing of allergic contact dermatitis (28). Small amounts of nickel are needed by the human body to produce red blood cells, but in excessive amounts, can become mildly toxic. Short-term over exposure to nickel is not known to cause any health problems, but long-term exposure can cause decreased body weight, heart and liver damage, and skin irritation (29). Therefore, it is very important to determine the concentration of these metals in lipsticks, which are used by millions of people.

Due to wide use of lipsticks in Iran, lack of data on their toxicity or their safety, the goal of this study was to assess the public health risk from certain brands of lipsticks sold at Sari region in Iran. In this context, 10 different brands of lipsticks were randomly purchased from local markets in Sari, Mazandaran State, Iran and the concentration of heavy metals such as cadmium, lead, chromium and nickel were analyzed using Atomic Absorption Spectrometry.

Materials and methods

Chemicals and instruments

All chemical compounds were pure and used without further purification. The following acids of the highest purity for heavy metal analysis were provided from Merck Company: HNO$_3$ (67% ) and HClO$_4$ (60%). Stock standard solutions of Cd, Cr, Pb and Ni ions were purchased from Merck Company. Working standard solutions were freshly prepared by stepwise dilution of the stock solution with deionized water.

The concentration of metal ions was determined by atomic absorption spectrophotometry using a Rayleigh-WFX-130 Atomic Absorption Spectrophotometer.

Collection of samples

Ten samples of different brands of lipsticks in mostly used colors were collected randomly from retail shops in Sari local markets in Mazandaran State, Iran. The samples were of different qualities and popular brands with different price ranges. The lipstick samples were assigned to codes 1 to 10 instead of their brand names as shown in table 1.

Methods

Lipsticks are present in the semi solid form, so pretreatment of samples are required before analysis. For this purpose, 3.0 g of lipstick samples were exactly

<table>
<thead>
<tr>
<th>sample code</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Color</strong></td>
<td>gold</td>
<td>pink</td>
<td>violet</td>
<td>blue</td>
<td>red</td>
<td>green</td>
<td>purple</td>
<td>yellow</td>
<td>beige</td>
<td>brown</td>
</tr>
</tbody>
</table>

Table 1: The color of investigated lipsticks
Heavy metal contamination in some lipsticks

Weighted with electrical analytical balance and put into digestion flask. Lipstick samples were digested by using wet digestion method by repeatedly addition of 10.0 ml of 4:1 HNO₃/HClO₄ at interval of 3 minutes for 5 times using the same ratio at atmospheric pressure under open system on hot plate at 100 °C and heated until the white fumes started evolving, which showed the completion of digestion process. The resulting digestate was filtered with filter paper and transferred to a 100.0 mL capacity volumetric flask and made up to the mark by the addition of doubly distilled deionized water and the concentration of the studied metal ions was then determined by flame atomic absorption spectrometry. Calibration solutions of heavy metals studied in this article were prepared as follows: for Cd ion, 0.0, 0.4, 0.6, 0.8, 1.0 mg L⁻¹, for Pb ion, 0.0, 2.0, 6.0, 8.0, 10.0 mg L⁻¹, for Cr ion, 0.0, 0.05, 1.0, 1.5, 2.0, 2.5 mg L⁻¹ and for Ni ion, 0.0, 0.5, 1.0, 3.0, 5.0 mg L⁻¹.

Statistical analysis
Analysis of variance (ANOVA) and Tukey post-hoc tests were performed on each brand of lipsticks to find out if there were significant variations in the concentrations of heavy metals in different colors of each brand. The statistical significance was determined as 0.05 (p < 0.05) alpha levels. SPSS 15.0 version (SPSS Inc., Chicago, IL, USA) statistical package was used for the statistically analyses. All the concentrations are mean value of triplicate experiments for each sample.

Results
Table 1, shows the color of the investigated lipsticks in this project. The samples numbered from 1-10. The content of cadmium (µg/g) in the lipstick samples 1-10, using Flame Atomic Absorption Spectrophotometry method, is depicted in figure 1 and figure 2, presents the lead concentration (µg/g) of lipstick samples used in this study. Figure 3, shows chromium content (µg/g) in the samples and concentration of nickel in the samples is given in figure 4.

All the concentrations are mean value of triplicate experiments for each sample.

Discussion
The contents of cadmium, lead, chromium and nickel in lipstick samples are presented in figures 1-4 respectively. As it is clear from these figures, all the samples contain variable concentration of Cd, Pb, Cr and Ni ions, some within the acceptable limits and some out of the safe range. The limited value for cadmium reported in the literature is about 0.9-3 µg/g (18, 19). All the samples showed cadmium in their formulation in the range of 0.01-0.05 µg/g (Fig. 1). So trace amount of cadmium in all tested lipsticks is safe.

The USFDA (US Food and Drug Administration) limit for lead as color additive in cosmetics is 20 g/g (30). The campaign for safe cosmetics has given the 0.1 µg/g lead level in candy (31). Safe level of lead according to EPA lead safe level is 0.5 µg/g (32). The lead content of samples measured by Flame Atomic Absorption (FAA) Spectrophotometer in this study was in the range of 0.18-0.80 µg/g (Fig. 3). This result shows that all the samples have lead concentration below FDA limit but according to EPA lead safe level, only sample 6 showed a higher concentration.

According to EPA the safe level of chromium is 1 µg/g (33). The content of of chromium in the lipstick samples investigated in this study by Flame Atomic Absorption spectrophotometer is depicted in figure 3. As it is clear in this figure, only one sample (sample 5) shows higher concentration than safe limit (1 µg/g), while the others show chromium concentration in the range of 0.06-0.75 µg/g lower than safe level.

Investigation for safe levels of nickel in household products reported that it should not contain more than 5 µg/g and that the ultimate target level should be 1 µg/g (29, 34). The FAA spectrophotometry measurement of nickel content in the lipstick samples in this study is shown in figure 4. As it is seen in figure 4, the concentration of nickel in samples is in the range of 0.0-0.34 µg/g which is well below the safe level. However, samples 7 and 8 do not show any detectable nickel by FAA spectrophotometer. The possible influence of colors on heavy metal contents was investigated also for different colors of each brand. The one-tailed Student’s t-test was performed to investigate for safe levels of nickel in household products.
Figure 1 Cd concentration (µg/g) in the lipstick samples

Figure 2 Pb Concentration (µg/g) in the lipstick samples

Figure 3 Cr concentration (µg/g) in the lipstick samples
applied to compare the mean heavy metal contents of different colors. Results showed that the mean value of lead for green lipsticks (0.8 ± 0.46 μg/g) was statistically higher than that for purple lipsticks (0.16 ± 0.22 μg/g) at 95% probability. Even though this seemed to suggest a tendency of green lipsticks to contain more lead than the other colors, and particularly than the purple ones, statistically the average lead content for the various colors did not show any significant difference at 95% probability, except for the green. Similar results were obtained comparing the average chromium content of different colors. The chromium level (2.1±0.8 μg/g) was significantly higher in red materials. The results in the case of lead and chromium showed that the type of pigment used in lipsticks contributes to its heavy metal content.

In the cases of nickel and cadmium, the one-tailed Student’s t-test did not show any statistically significant difference among the various color categories at 95% probability, thus it has to be remembered that the type of pigment used in lipsticks has no relation to its heavy metal content.

**Conclusion**
Ten samples of lipsticks mostly used in Mazandaran state, Iran, were collected from local markets in Sari, were evaluated for heavy metals (Cd, Pb, Cr and Ni) levels by flame atomic absorption spectrophotometer. Cadmium concentration was lower than safe limit. The lead content of the samples were lower than FDA safe limit, but only one sample was higher than EPA lead safe limit (sample 6). All the samples showed Chromium content less than 1g μg/g unless one sample (sample 5) showed higher than safe limit. None of the samples showed nickel content higher than safe limit (1 μg/g). Although content of heavy metals in the tested lipsticks was reasonable, but the continous use of lipsticks can increase the absorption of heavy metals into the body by swallowing or through dermal absorption of these products. Therefore, informing the users of lipsticks specially teenagers, of the harmful consequences of lip products should be a big concern.

**Conflict of Interest**
The authors declared no conflict of interest.
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References