Elemental impurities in lipsticks: Results from a survey of the Portuguese and Brazilian markets

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A B S T R A C T

For safety reasons, European regulations prohibit the use of a long list of metal(loid)s as ingredients of cosmetic products. However, their presence as impurities in finished products is virtually unavoidable, even under GMP conditions. This study aimed at determining the elemental profile of lipsticks available in the Portuguese and Brazilian markets. A total of 96 lipsticks were purchased in Brazil (n = 53; 9 brands) and Portugal (n = 43; 7 brands) and the content of 44 elements was determined. Results ranged from < 1 μg/g to several tens of μg/g (e.g., Sn, Mn, Zn). Significant differences were found between Portuguese and Brazilian products for several elements, particularly for Pb. For the elements of major toxicological concern (Pb, Cd, As, Sb, Hg), mean values were always below the current limits set by the German competent authority. However, a significant percentage of exceedances were observed for Pb (24%) and Cd (21%). A safety assessment was carried out for the toxicologically relevant elements. Results showed that, except for Pb, the systemic exposure resulting from lipstick use represents less than 0.2% (ca. 3% for Pb) of the respective permitted daily exposure even in the worst-case scenario (i.e., ingestion of the total amount of product applied).

Keywords: Metals Lipsticks ICP-MS Safety assessment Limits European regulation

1. Introduction

Cosmetics are highly regulated products in most developed countries. Lipsticks, in particular, because of the potential for systemic exposure through oral ingestion beyond the exposure due to dermal contact, are subject to special attention (European Regulation, 2009; Food and Drugs Act, 2017; U.S Government Publishing Office, 1974).

Regarding elemental species, several metallic elements (e.g., Fe, Zn, Ti, Bi, Cu, Mn) may be present in important amounts in cosmetic products, including lipsticks, since they enter in the composition of ingredients commonly used in the manufacturing of these products. Many others metals and metalloids, on the contrary, due to their well-known toxicity, are strictly prohibited by international regulations. According to the European Regulation No. 1223/2009, a long list of substances (listed in the Annex II) are prohibited in cosmetic products, which includes, in the case of lipsticks, all the compounds of Sb, As, Be, Cd, Cr, Pb, Au, Nd, Te, Tl, Hg and Se (European Regulation, 2009).

However, due to their ubiquitous and persistent nature, the presence of trace amounts of those elements in cosmetic products is virtually unavoidable even under conditions of good manufacturing practices (GMP). In accordance with article 17 of the European Regulation No. 1223/2009, the non-intended presence of small quantities of the prohibited substances (stemming from impurities of natural or synthetic ingredients, the manufacturing process, migration from packaging, etc.) must be limited to the amounts that are technically unavoidable under GMP and, according to article 3 of the same Regulation, cannot compromise the product safety for human health under normal or reasonably foreseeable conditions of use. The safety of each individual cosmetic product has to be demonstrated in the Safety Report, a mandatory component of the respective Product Information File (European Regulation, 2009).

Lead is the element that gave rise to these concerns and has received most of the attention. In 2007, the Campaign for Safe Cosmetics, a coalition of different US entities that works to eliminate substances linked to adverse health impacts from the cosmetics, raised a warning flag when Pb at levels up to 0.65 ppm were found in 61% of the 33 most popular lipstick brands available in the US market (Campaign for Safe Cosmetics, 2007). In 2009, Al-Saleh et al. evaluated the Pb content in lipsticks (n = 26) available in Saudi Arabia market but imported from countries with a low level of regulatory oversight, and identified several products containing very high amounts of Pb (three brands with several thousand of ppm). Also in 2009, FDA chemists published the development and validation of a method for the determination of total Pb levels in lipsticks, combining microwave-assisted acid digestion and inductively coupled plasma–mass spectrometry (ICP–MS), and the results of a small survey (20 samples from the USA market) were reported. The average Pb content found was 1.07 µg/g (range: 0.09–3.06 µg/g) (Hepp et al., 2009). Latter, in 2012, the FDA released the results of an expanded survey of the US market, where the levels of Pb were determined in 400 lipsticks, including a variety of shades, manufacturers and price ranges (Hepp et al., 2012). The average Pb content was 1.11 µg/g (range: 0.03–7.19 µg/g), very close to the results found in the initial study. In 2013, a European survey on the content of Pb in 223 lip products (lipsticks and lip glosses from 55 brands; purchased in 15 different European Union countries) was performed, and a mean Pb content of 0.75 µg/g was found (Piccinini et al., 2013). More recently, Brazilian researchers also gave attention to this question and evaluated the Pb content in 21 lipstick samples, reporting values ranging from 0.27 to 4.54 µg/g (Soares and Nascentes, 2013).

However, as mentioned above, in addition to Pb, there is a long list of other elements whose intentional use as ingredients of cosmetic products is prohibited and must be kept at residual levels in the finished products in order to ensure their safety for the consumers.

For the elements of higher toxicological concern (Pb, As, Cd, Hg and Sb) German and Canadian competent authorities conducted specific studies to determine the levels that could be technically achievable is cosmetic products. Based on these studies, the following limits were set: a) Germany (1985) – Pb: 20 µg/g; As: 5 µg/g; Cd: 5 µg/g; Hg: 1 µg/g; Sb: 10 µg/g (BGA, 1985); b) Canada (2012) – Pb: 10 µg/g; As: 3 µg/g; Cd: 3 µg/g; Hg: 1 µg/g; Sb: 5 µg/g (Health Canada, 2012). Last March 2017, German Consumer Protection and Food Safety authority (BVL) have published an up-to-date overview of the technically preventable contents of heavy metals in cosmetic products, and the previous limits were significantly reduced. The new limits were set at Pb: 2 µg/g; As: 0.5 µg/g; Cd: 0.1 µg/g; Hg: 0.1 µg/g; and Sb: 0.5 µg/g, which are 10–50 times lower than the previous ones. These limits were based on the 90^{th} percentile of the dataset obtained in the German Monitoring Scheme (BVL, 2017).

This paper presents the results of two studies on the elemental impurities of lipsticks. The first one corresponds to a study performed on products manufactured in Brazil and commercially available in that country (study BR). The second study was performed in lipsticks available in the Portuguese market, but not necessarily manufactured in Portugal (study PT). The initial idea was also to limit the study to products manufactured in Portugal, but since only two manufacturers were identified, it was decided to include also low cost products (not included in the 2013 European survey), commercially available in "Chinese stores" and low-cost youth fashion and beauty stores. Besides Pb, a wide panel of other elements (43) was determined, including those prohibited by the European Regulation. A comparison was made with the limits set by the German and Canadian authorities. Additionally, a safety assessment was performed to evaluate the potential systemic exposure that may result from the lipsticks use in the worst-case scenario, i.e., assuming the total ingestion of the amount of product applied.

2. Materials and methods

2.1. Samples

The main idea of this study was to determine the elemental impurities in lipsticks actually manufactured in Brazil and Portugal (and not just sold in these markets). Thus, two studies were carried out: (1) Brazilian study – A total of 53 lipsticks were purchased in May/June 2013 at local stores (city of Vitória, Espírito Santo), representing nine brands (from nine different Brazilian manufacturers); (2) Portuguese study – a total of 43 lipsticks were purchased in March/April 2014 at local stores (city of Porto). Since only two Portuguese manufacturers were identified (n = 10 and n = 5 products), it was decided to extend the study to the lower cost products available in the market, independently of the country of origin. These samples include five brands from Chinese manufacture (n = 22 products) and one brand from Turkish manufacture (n = 6 products). These products were obtained at "Chinese stores" and low cost youth fashion and beauty stores. Lipsticks were grouped according to their color in: (1) light pink, (2) dark pink, (3) light brown, (4) dark brown, (5) red and (6) berry. General information about lipsticks in provided in supplementary material (Table S1).

2.2. Sample pretreatment

Lipstick samples were mineralized by closed-vessel microwave-assisted acid digestion in a MLS-1200 Mega (Milestone, Sorisole, Italy) microwave oven equipped with an HPR-1000/10 S rotor and pressure and temperature probes, following the two-step digestion procedure developed and validated by Hepp et al. (2009). Approximately 300 mg of sample was directly weighed into the microwave oven PTFE vessels and 7 mL of concentrated (≥69% w/w) HNO_3 (Trace*SELECT*®; Fluka, France) and 2 mL of concentrated (47–51% w/w) HF (Trace*SELECT*®, Fluka, Germany) were added. Then the vessels were sealed, heated to 130 °C over 15 min and held at this temperature for 3 min. After that, the temperature was ramped to 200 °C over 15 min and held at this temperature for 30 min. After cooling below 50 °C, 30 mL of 4% (w/v) H_3BO_3 (99.999% trace metal basis, Aldrich, St. Louis, MO) were added and the vessels were heated again to 170 °C over 15 min and held for 10 min, to neutralize the remaining HF. Finally, after cooling, the sample solutions were transferred into 50 mL polypropylene volumetric flasks and the volume was adjusted with ultrapure water (resistivity > 18.2 MΩ cm at 25 °C) produced by a Sartorius (Goettingen, Germany) Arium® pro water purification system. One sample blank was included in each microwave-assisted acid digestion run (10 samples).

2.3. Sample analysis

Sample analysis was performed by ICP-MS using an iCAP™ Q (Thermo Fisher Scientific, Bremen, Germany) instrument equipped with a concentric glass nebulizer, a baffled cyclonic spray chamber (Peltier-cooled), a standard quartz torch and a two-cone design (skimmer and sample nickel cones). High-purity (99.9997%) argon (Gasin II, Leça da Palmeira, Portugal) was used as the nebulizer and plasma gas. The equipment control and data acquisition were made through the Qtegra software (Thermo Fisher Scientific, Bremen, Germany). The ICP-MS analysis was carried out under the following conditions: RF power, 1550 W; argon flow rate, 14 L/min; auxiliary argon flow rate, 0.8 L/min; nebulizer flow rate, 1.02 L/min. The following elemental isotopes (*m/z* ratios) were monitored for analytical determination: ^{7}Li, ^{9}Be, ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{73}Ge, ^{75}As, ^{82}Se, ^{85}Rb, ^{88}Sr, ^{90}Zr, ^{93}Nb, ^{98}Mo, ^{107}Ag, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{125}Te, ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{153}Eu, ^{157}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{171}Yb, ^{175}Lu, ^{178}Hf, ^{181}Ta, ^{182}W, ^{185}Re, ^{202}Hg, ^{205}Tl, ^{208}Pb and ^{238}U. Indium (^{115}In) was monitored as internal standard.

The instrument was tuned daily for maximum signal sensitivity and stability and low oxide and doubly charged ions formation using a Tune B iCAP Q solution (1 µg/L of Ba, Bi, Ce, Co, In, Li and U in 2% HNO_3 and 0.5% HCl). Calibration standards were prepared by serial dilution of four commercial multi-element standard solutions: *Plasma*CAL SCP-33-MS from SCP Science (Baie-d'Urfé, Quebec, Canada); "Transition metal mix 2", "metalloid and non-metal mix" and "periodic table mix 3" all TraceCERT® from Fluka, Germany. An internal standard solution was prepared by dilution of a 1000 mg/L indium standard solution (TraceCERT®, Sigma-Aldrich, New Haven, CT), which was then added

Table 1
Summary of the results (μg/g) obtained for the elements prohibited under the European Regulation No. 1223/2009.

Element	< LoD (%)[a]		Median		Mean		SD		Max		Limits[b]		Nº Samples > BVL limit	
	PT	*BR*	*PT*	*BR*	*PT*	*BR*	*PT*	*BR*	*PT*	*BR*	Health Canada	BVL (Germany)	*PT*	*BR*
Pb*	7	2	1.47	0.73	1.92	0.95	1.57	0.87	4.89	4.24	10	2	18	5
Sb	40	8	0.09	0.09	0.24	0.30	0.41	0.69	1.97	4.15	5	0.5	5	6
As	86	70	0.08	0.08	0.23	0.23	0.56	0.38	2.95	1.76	3	0.5	3	6
Cd	21	17	0.02	0.02	0.09	0.08	0.30	0.18	1.97	0.96	3	0.1	15	5
Hg	100	100	–	–	–	–	–	–	–	–	1	0.1	–	–
Cr	0	6	1.06	0.83	2.26	2.80	3.62	5.06	16.7	28.3	–	–	–	
Zr*	7	0	0.35	0.72	1.99	3.21	5.29	7.68	30.6	40.1	–	–	–	
Nd*	53	28	0.01	0.05	0.19	0.81	0.80	2.13	5.28	9.60	–	–	–	
Be	5	26	0.04	0.13	0.33	0.18	0.39	0.19	1.17	0.75	–	–	–	
Tl	14	17	0.01	0.06	0.06	0.08	0.07	0.09	0.20	0.33	–	–	–	
Te	100	100	–	–	–	–	–	–	–	–	–	–	–	
Se	100	100	–	–	–	–	–	–	–	–	–	–	–	

*Significant differences were found between the mean content of these elements in the Portuguese and Brazilian samples.
[a] Percentage of samples with a result lower than the LoD (limit of detection). *PT* = samples acquired in the Portuguese market (n = 43). Country of manufacture: China (n = 22), Turkey (n = 6), Portugal (n = 15). *BR* = samples acquired in the Brazilian market and manufactured in Brazil (n = 53).
[b] Element cncentrations in cosmetic products are seen to be technically avoidable when they exceed these limits.

to all samples and standards solutions to obtain a 10 μg/L final concentration. The ICP-MS system was washed using a solution of 2% HNO_3 containing 200 μg/L Au. The limits of detection (LoD) were calculated as the concentration corresponding to three times the standard deviation of 10 sample blanks and are presented in supplementary material (Table S2). Results were expressed as μg/g wet weight.

2.4. Analytical quality control

Because lipstick is not available as a certified reference material (CRM) for metal determination, fish protein (DORM-3) and dogfish liver (DOLT-4), both from the National Research Council (Ontario, Canada), and estuarine sediment (BCR-667, from Institute for Reference Materials and Measurements, Geel, Belgium) were used for analytical quality control purposes. The CRMs were subjected to the same sample pre-treatment as the lipstick samples. The values obtained proved the accuracy of the analytical procedure and are presented in supplementary material (Table S3).

2.5. Safety assessment

A safety assessment was performed based on a two-step calculation:

1 Calculation of the estimated daily intake (EDI), as follows:

$$EDI\left(\frac{\mu g}{day}\right) = metal\ content\ in\ lipstick\left(\frac{ug}{g}\right) x\ \ mass\ of\ ingested\ product\left(\frac{g}{day}\right)$$

where the mass of lipstick that can be ingested is 40 mg day^{-1}, considering an amount of lipstick used per application of 10 mg and an application frequency of 4 ×/day, as proposed by the Dutch National Institute for Public Health and the Environment (RIVM) (RIVM, 2006). For this purpose, it was assumed that the total amount of the element present in the product is readily available for gastrointestinal absorption (i.e., bioaccessibility of 100%).

2 Calculation of the relative intake indices (RIIs), as follows:

$$RII\ (\%) = \frac{EDI}{PDE}\ x\ 100$$

where the permitted daily exposure (PDE) values were retrieved from the ICH Q3D Guideline for Elemental Impurities published by the European Medicines Agency (EMA) (EMA, 2016). This guideline was designed for drug products but its principles of safety assessment following oral administration can be reasonably applied to lipsticks considering the potential for oral exposure resulting from the use of these products.

2.6. Data analysis

Statistical analysis was performed using IBM (New York, NY) SPSS Statistics 24 software. For the statistics calculation, results that fall below the LoD were assumed as the LoD divided by the square root of 2, a commonly used procedure for data imputation (Rebelo et al., 2015). A Shapiro-Wilk's test was performed to assess the normality of data. The assumption of homogeneity of variance was tested using Levene's test. The differences in the elements content between Portuguese and Brazilian samples were tested with the non-parametric Mann-Whitney test. The differences between lipsticks of different colors were tested by the Kruskal-Wallis, followed by post-hoc Dunn's test for multiple comparison. Statistical significance was considered for $p < 0.05$.

3. Results and discussion

3.1. Elements content in lipsticks

A total of 96 lipsticks were analyzed for their total content in 44 elements (n = 53 in the study of Brazilian products; n = 43 in the study with products from the Portuguese market). Two groups of elements were considered according to their regulatory status: group 1–elements prohibited under the European Regulation No. 1223/2009; group 2–other elements.

The results for group 1 elements are shown in Table 1. The content of **Hg, Te** and **Se** was < LoD in all the samples. Many samples also presented contents < LoD for As and Nd. Lipsticks from the Portuguese market presented a significantly higher mean content of **Pb** than those from Brazil (1.92 vs. 0.95 μg/g). On the contrary, Brazilian lipsticks showed a significantly higher mean content of **Zr** (1.99 vs. 3.21 μg/g) and **Nd** (0.19 vs. 0.81 μg/g). For the other prohibited elements, no significant differences were observed between the mean contents.

For **Pb**, and as mentioned before, several studies exist about its content in lipsticks. Although there are some studies reporting higher Pb values – e.g., a small study (n = 15 samples) conducted by Zakaria and Ho (2015) where a mean Pb content of 3.21 μg/g was found – the mean Pb content obtained in our study (1.92 and 0.95 μg/g in Portuguese and Brazilian samples, respectively) is in good agreement with most of the available studies: 1.11 μg/g in the study by Hepp et al.

Table 2
Detailed results (µg/g) obtained for the elements of major toxicological concern in the lipsticks samples studied (n = 96).

Sample ID	Brand	Country of manufacture	Pb	Cd	Sb	As
1	B1	China	0.10	0.002	0.14	n.d.
2			0.62	0.003	0.02	n.d.
3			0.13	0.003	0.30	0.21
4			n.d.	**0.114**	n.d.	n.d.
5			0.14	0.005	0.32	n.d.
6			**2.14**	0.001	n.d.	n.d.
7	B2	China	n.d.	n.d.	n.d.	n.d.
8			n.d.	**0.108**	n.d.	n.d.
9			1.68	**1.972**	**1.51**	**2.95**
10	B3	China	**2.76**	0.063	**0.65**	**1.51**
11			1.47	0.055	**0.83**	**2.10**
12			**3.92**	0.001	n.d.	n.d.
13			1.26	0.003	0.17	0.19
14			1.17	**0.152**	0.31	n.d.
15			1.24	**0.128**	0.11	n.d.
16	B4	China	1.64	0.018	0.32	n.d.
17			1.48	0.016	0.22	n.d.
18			1.08	**0.163**	0.28	n.d.
19			0.38	**0.136**	n.d.	n.d.
20	B5	China	**3.55**	n.d.	**1.08**	n.d.
21			**3.98**	0.005	0.27	n.d.
22			**3.22**	0.004	**1.97**	n.d.
23	B6	Turkey	**3.59**	n.d.	n.d.	n.d.
24			**3.24**	0.041	n.d.	n.d.
25			**3.15**	0.003	0.11	0.17
26			**3.63**	0.010	0.02	n.d.
27			**3.96**	0.018	n.d.	n.d.
28			**3.69**	n.d.	n.d.	n.d.
29	B7	Portugal	0.26	**0.153**	n.d.	n.d.
30			0.78	**0.142**	0.08	n.d.
31			1.20	**0.141**	0.24	n.d.
32			0.10	**0.134**	n.d.	n.d.
33			0.13	**0.125**	n.d.	n.d.
34			0.15	0.095	n.d.	n.d.
35			1.01	**0.131**	0.29	n.d.
36			1.15	**0.123**	0.17	n.d.
37			**4.19**	0.003	0.27	n.d.
38			0.41	**0.135**	0.03	n.d.
39	B8	Portugal	**3.56**	n.d.	n.d.	n.d.
40			**3.66**	n.d.	n.d.	n.d.
41			**4.89**	n.d.	0.09	n.d.
42			**4.63**	n.d.	0.37	n.d.
43			**2.99**	n.d.	n.d.	n.d.
44	B9	Brazil	1.35	0.029	0.02	n.d.
45			1.17	0.001	0.06	n.d.
46			1.05	n.d.	0.01	n.d.
47			4.24	0.001	0.26	**1.50**
48			1.78	0.019	0.26	n.d.
49			1.24	0.023	0.10	n.d.
50	B10	Brazil	0.14	n.d.	0.01	0.22
51			2.33	0.018	0.11	n.d.
52			1.24	n.d.	0.09	n.d.
53			1.37	0.027	0.13	n.d.
54			1.01	n.d.	0.26	0.24
55	B11	Brazil	1.00	0.003	**4.15**	0.24
56			0.96	0.013	0.27	n.d.
57			0.02	n.d.	0.03	0.19
58			1.11	0.010	0.42	n.d.
59			0.05	0.022	0.06	n.d.
60			0.69	0.071	0.26	n.d.
61	B12	Brazil	0.69	0.776	**0.53**	**0.92**
62			0.16	0.452	0.33	n.d.
63			0.61	0.094	**2.21**	0.48
64			0.74	0.475	**2.19**	**1.51**
65			0.32	0.082	**0.76**	**1.76**
66			0.57	0.962	0.38	**0.75**
67	B13	Brazil	0.05	0.020	n.d.	n.d.
68			1.71	n.d.	0.02	0.16
69			1.07	0.067	0.05	n.d.
70			n.d.	0.021	n.d.	n.d.
71			0.15	0.077	0.24	n.d.
72			0.73	0.032	n.d.	n.d.
73	B14	Brazil	1.37	0.097	0.27	n.d.
74			0.36	0.129	0.18	n.d.
75			0.14	0.027	n.d.	0.17
76			0.67	0.064	0.04	n.d.
77			2.20	0.065	0.45	n.d.
78			2.57	0.070	**0.53**	n.d.
79	B15	Brazil	0.18	n.d.	0.01	n.d.
80			1.73	n.d.	0.14	n.d.
81			0.91	0.023	0.04	n.d.
82			0.73	0.019	0.05	0.20
83			1.39	0.005	0.31	**0.58**
84			1.66	0.019	0.08	n.d.
85	B16	Brazil	1.12	0.043	0.03	n.d.
86			0.30	0.010	0.03	n.d.
87			0.30	0.011	0.04	0.40
88			0.36	0.019	0.03	n.d.
89			0.10	0.010	0.01	n.d.
90			0.39	0.009	0.04	n.d.
91	B17	Brazil	0.18	n.d.	0.01	n.d.
92			1.13	0.016	0.11	n.d.
93			0.43	0.035	0.02	n.d.
94			0.49	0.018	0.33	0.16
95			0.32	0.016	0.09	n.d.
96			3.62	0.010	0.03	n.d.

n.d. = < Lod (Pb: 0.008 µg/g; Cd: 0.001 µg/g; Sb: 0.009 µg/g; As: 0.110 µg/g).
Figures in bold are results above the German BVL limits.

(2012) with 400 lipstick samples from the US market; 0.75 µg/g (n = 150) in the European survey (Piccinini et al., 2013); 0.96 µg/g (n = 48) in the study by Atkins (2012); and 0.69 µg/g (n = 28) in the study by Al-Saleh and Al-Enazi (2011). The mean Pb content obtained in this study was clearly below the limit set by the Canadian health authority (20 µg/g) and even below the recently revised limit set by the German Federal Office of Consumer Protection and Food Safety (BVL) of 2 µg/g. However, a significant number of samples (23 out of 96 samples) exceeded the BVL limit. Most of these samples (n = 18) were from the Portuguese market, manufactured in China (n = 6), Turkey (n = 6) and Portugal (n = 6) (Table 2). For the other elements prohibited under the European Regulation and for which limit values as impurities is cosmetics have been established by some competent authority (i.e., Sb, As, Cd and Hg), their mean values were well below the limits set by both Health Canada and BVL (Table 1). The percentage of samples exceeding the BVL limits was 21% (20/96) for Cd, 11% (11/96) for Sb and 9% (9/96) for As. Most of the samples exceeding the BVL limit for Cd (15 out of 20) were from the Portuguese market, as observed for Pb (Table 2). However, the Cd and Pb contamination did not overlap, i.e. the exceedances were not observed in the same samples/brand (Brand 7 for Cd and Brand 5, 6 and 8 for Pb). These results show that contents lower than 2 µg/g for Pb, 0.5 µg/g for Sb and As, and 0.1 µg/g for Cd and Hg in lipsticks are technically feasible under GMP, but there is still a long way to improve, especially for Pb and Cd.

Few studies exist on the content of Sb, As, Cd and Hg in lipsticks. For **Sb**, its mean content in Portuguese and Brazilian samples (0.24 and 0.30 µg/g, respectively) was in good agreement with the data from Atkins (2012) (0.33 µg/g; n = 48 lipsticks), but significantly higher than the mean content found by Al-Saleh and Al-Enazi (2011) (0.052 µg/g; n = 28 lipsticks). For **As**, the mean content (0.23 µg/g in both Portuguese and Brazilian samples) was in good agreement with the literature data. In one study (Atkins, 2012), the mean As content was 0.3 µg/g (n = 48) and in another study (Al-Saleh and Al-Enazi, 2011) it was 0.51 µg/g (n = 28). It must be noted that most samples (74 out of 96) gave results < LoD (0.11 µg/g). A survey of cosmetics for As, Cd, Cr, Co, Pb, Hg, and Ni content published by Hepp et al. (2014) also found similar results. From a total of 30 lipstick samples (19 different manufacturers), 21 presented results < LoD (0.048 µg/g). For the nine quantifiable samples, a mean As content of 0.29 µg/g, a value quite similar to ours, was obtained. For **Cd**, the mean content found (0.09 and 0.08 µg/g in Portuguese and Brazilian samples, respectively) was in close agreement with the results from Zakaria and Ho (2015), who found a mean Cd content of 0.09 µg/g (n = 15 lipsticks). However, other studies have found significantly lower values: 0.014 µg/g (n = 28 lipsticks) in the study by Al-Saleh and Al-Enazi (2011); and < 0.018 µg/g in all the samples (n = 30 lipsticks) in the study by Hepp et al. (2014). The **Hg** content was always < LoD (0.032 µg/g). In their study, Hepp et al. (2014) reported values < LoD (0.0010 µg/g) in all the samples (n = 30).

Regarding the remaining elements prohibited under the European Regulation, **Cr** mean content (2.26 and 2.80 µg/g in Portuguese and Brazilian samples, respectively) was in good agreement with some published data: 2.77 µg/g (n = 30), in the study by Hepp et al. (2014), and 2.44 µg/g (n = 48) in the study by Atkins (2012). Al-Saleh and Al-Enazi (2011) and Zakaria and Ho (2015) also determined the Cr content in lipsticks but significantly lower mean contents were found: 1.06 µg/g (n = 28) and 1.01 µg/g (n = 15), respectively. These lower values are in good agreement with our median Cr values (1.06 and 0.83 µg/g, in Portuguese and Brazilian samples, respectively).

The mean **Zr** content obtained in our study (1.99 and 3.21 µg/g, for Portuguese and Brazilian samples, respectively) was significantly lower than the value obtained by Atkins (2012): 8.1 µg/g (n = 48). Some samples presented a significantly higher Zr content. This will certainly be due to the use of some particular colorants, which may contain that element. In fact, although Zr and its compounds are generically prohibited as ingredients in cosmetics, there are a few exceptions (Zr lakes, pigments or salts of permitted coloring agents) (European Regulation, 2009). It must be highlighted that the maximum Zr content found in this study (40.1 µg/g, in a Brazilian sample) was quite similar to the value found by Atkins (2012): 39.4 µg/g.

For **Nd**, the mean content was significantly different between Portuguese and Brazilian samples (0.19 vs. 0.81 µg/g, respectively) and significantly higher than the mean content found by Atkins (2012): 0.07 µg/g (n = 48).

The content of **Be** and **Tl** was very low in all the samples (mean values: 0.33 and 0.18 µg/g, for Be; 0.06 and 0.08 µg/g, for Tl, in Portuguese and Brazilian samples, respectively). The only study available on these elements was carried out be Atkins (2012), who found a higher mean Be content (0.49 µg/g) and a similar Tl content (0.08 µg/g).

Table 3 presents the results for group 2 elements (i.e., other elements determined in the lipstick samples in addition to those prohibited by the European Regulation). Most elements (20 out of 32) presented mean contents in the sub-ppb range. The **Re** content was always < LoD (0.0001 µg/g). The 12 most abundant elements (with mean content above ppb level) were Sn > Zn > Sr > Mn > Rb > Cu > Li > Ni > Nb > V > Ce > W.

Significant differences were observed between Portuguese and Brazilian samples for **Sr, V, Eu** and **Ag** (higher contents in Portuguese samples), and for **Ce, Dy, Er, Pr, Sm, Yb, Ho** and **Lu** (higher contents in Brazilian samples).

The difference in **Sr** content between Portuguese and Brazilian samples (68.6 vs. 19.3 µg/g) was particularly noticeable. The European Regulation No. 1223/2009 prohibits or restricts the use of the most common Sr compounds. However, this element may be found in several colorants (particularly red colorants) allowed in cosmetics, which may justify the results obtained, including the great difference between the different samples (SD = 118.9 µg/g for the Portuguese samples).

The elements **Sn, Zn, Sr, Mn** and **Rb** were found at mean contents > 10 µg/g in the analyzed samples. Except for Sn, our results were in good agreement with the literature data. Liu et al. (2013) analyzed the content of several metals in lip products and found a mean **Mn** content in lipsticks (17.8 µg/g; n = 6) quite similar to our values (19.0 and 26.3 µg/g, for Portuguese and Brazilian samples, respectively). In another study (Atkins, 2012), a mean **Mn** content of 20.5 µg/g was obtained. For Sn, Zn, Sr and Rb the work by Atkins (2012) is the only one reporting their content in lipsticks, as follows: 40.1 µg/g for **Zn** (vs. 51.0 and 60.3 µg/g in our study; for Portuguese and Brazilian samples, respectively); 30.3 µg/g for **Sr** (vs. 68.6 and 19.3 µg/g); 22.9 µg/g for **Rb** (vs. 20.6 and 15.1 µg/g) and 21.8 µg/g for **Sn** (vs. 76.8 and 59.4 µg/g).

The mean **Cu** content (8.23 and 5.78 µg/g, for Portuguese and Brazilian samples, respectively) was significantly higher than the content found by Atkins (2012) (1.47 µg/g, n = 48) and Liu et al. (2013) (< 0.01 µg/g, n = 6). The mean **Ni** content (1.64 and 3.29 µg/g, for Portuguese and Brazilian samples, respectively) was in good agreement with the literature data: a mean Ni content of 2.88 µg/g (n = 48) in the study by Atkins (2012); 2.58 µg/g (n = 30) in the study by Hepp et al. (2014); and 1.11 µg/g (n = 28) in the study Al-Saleh and Al-Enazi (2011). For the elements **Li**, **V** and **W**, only the work by Atkins (2012) provides data on their content in lipsticks, with significantly higher mean values: 19.51 µg/g for **Li**, 4.68 µg/g for **V** and 3.04 µg/g for **W**. No study is available on the content of **Nb** in lipsticks. For **Co**, the mean content found (0.39 and 0.48 µg/g, for Portuguese and Brazilian samples, respectively) was in the middle of reported values: 0.44 µg/g (n = 48) in the study by Atkins (2012); 0.28 µg/g (n = 6) in the study by Liu et al. (2013); 0.86 µg/g (n = 30) in the study by Hepp et al. (2014).

For the remaining elements determined (Table 3), very limited or no information exists on their content in lipsticks. Only one study (Atkins, 2012) provides data on the content of some of these elements (La, Mo, Gd, Pr, Sm, Yb, Ge and Hf) in lipsticks. Significantly lower mean values (compared to the results obtained in the present study) were reported: 0.4 µg/g for **La**; 0.27 µg/g for **Mo**; 0.03 µg/g for **Gd**; 0.01 µg/g for **Pr**; 0.03 µg/g for **Sm** and 0.01 µg/g for **Yb**. Similar values were reported for **Ge** (0.25 µg/g) and **Hf** (0.15 µg/g).

The relationship between the lipsticks color and elemental contents was also studied. For the 44 elements determined, significant differences between colors were found only for **Mn, Co, Cu** and **Zn**. Dark brown lipsticks showed a significantly higher content of **Mn** and **Co** than both dark and light pink lipsticks, while light pink lipsticks showed a significantly higher content of **Cu** and **Zn** than red lipsticks (data not shown).

3.2. *Safety assessment*

A safety assessment was carried out for the elements considered in the ICH Q3D Guideline for Elemental Impurities (EMA, 2016). The results are presented in Table 4. For this purpose, the content (µg/g) corresponding to the 90th percentile was used, following the same methodology of BVL (BVL, 2017). The highest estimated daily intake (EDI) was observed for Sn (8.30 µg/day) and the lowest for Ag (0.002 µg/day). The relative intake index (RII) values were below 0.2% for all the studied elements (except for Pb: 2.9%), considering the permitted daily exposure (PDE) set in the ICH guideline.

4. Conclusions

The results of these surveys show that the current levels of elemental impurities in lipsticks, particularly those elements prohibited under the European Regulation (EC) No. 1223/2009, are quite low and do not pose safety concerns regarding consumers' health. Most of the analyzed samples presented contents of Pb, Cd, As, Sb and Hg below the most recent and strict limits set by the German competent authority (BVL) – used as reference because both Brazilian and European legislation (applied in Portugal) do not establish limits for these elemental impurities – confirming that they are technically achievable through adequate raw material choices and good manufacturing practices. However, a significant percentage of samples/brands showed Pb and Cd contents higher than the German BVL limits, showing that the manufacturing conditions of these products can be further improved.

Table 3
Summary of the results (μg/g) obtained for the other elements analyzed in lipsticks.

Element	< LOD (%)[a]		Median		Mean		SD		Max	
	PT	*BR*	*PT*	*BR*	*PT*	*BR*	*PT*	*BR*	*PT*	*BR*
Sn	7	9	8.81	2.81	76.8	59.4	157.6	223.3	665.4	1598
Zn	5	8	8.72	11.4	51.0	60.3	137.9	305.5	663.7	2235
Sr*	5	2	23.6	2.0	68.6	19.3	118.9	35.8	480.0	134.6
Mn	7	6	6.26	11.9	19.0	26.3	26.2	46.8	87.4	277.8
Rb	26	9	1.28	9.87	20.6	15.1	25.5	17.4	76.8	65.1
Cu	12	13	4.16	4.69	8.23	5.78	12.3	6.06	66.6	33.4
Li	33	19	2.25	1.04	4.19	2.37	4.94	2.81	14.6	11.4
Ni	23	17	0.55	0.60	1.64	3.29	4.01	8.01	25.6	45.5
Nb	2	0	0.96	1.65	1.86	2.39	2.55	3.10	13.0	19.6
V*	0	40	1.53	0.33	1.97	1.40	1.61	3.60	10.3	19.0
Ce*	91	47	0.13	0.20	0.34	1.52	1.14	2.91	7.56	10.9
W	9	42	0.06	0.11	1.07	0.46	2.27	0.73	13.1	2.71
La	28	49	0.44	0.11	0.81	0.84	1.81	1.66	11.3	7.59
Eu*	5	6	0.46	0.05	0.78	0.49	0.87	0.84	3.95	4.50
Mo	21	57	0.10	0.03	0.58	0.51	1.31	0.90	6.09	5.22
Co	0	8	0.10	0.14	0.39	0.48	0.68	0.93	3.50	5.12
Gd	14	15	0.14	0.04	0.48	0.27	0.86	0.46	5.00	2.01
Ge	70	58	0.07	0.07	0.32	0.23	0.65	0.30	3.44	1.33
Ta	5	0	0.20	0.16	0.22	0.20	0.34	0.18	2.19	0.99
Hf	5	0	0.025	0.046	0.075	0.112	0.152	0.217	0.880	1.174
Ag*	19	17	0.012	0.003	0.037	0.012	0.053	0.028	0.250	0.139
Dy*	40	11	0.004	0.012	0.032	0.089	0.123	0.202	0.808	0.868
Er*	9	0	0.004	0.009	0.023	0.052	0.090	0.114	0.591	0.523
Pr*	63	34	0.004	0.015	0.053	0.228	0.235	0.607	1.542	2.793
Sm*	70	43	0.005	0.021	0.036	0.167	0.148	0.385	0.969	1.731
U	7	0	0.012	0.010	0.014	0.033	0.014	0.060	0.052	0.304
Yb*	5	0	0.007	0.014	0.032	0.057	0.112	0.119	0.740	0.553
Tb	23	30	0.006	0.003	0.009	0.019	0.020	0.041	0.136	0.174
Ho*	30	17	0.001	0.002	0.007	0.017	0.026	0.038	0.171	0.172
Lu*	12	2	0.001	0.002	0.005	0.009	0.020	0.019	0.130	0.089
Tm	16	2	0.001	0.002	0.004	0.008	0.015	0.017	0.100	0.081
Re	100	100	–	–	–	–	–	–	–	–

*Significant differences were found between the mean content of these elements in the Portuguese and Brazilian samples.
[a] Percentage of samples with a result lower than the LoD (limit of detection). *PT* = samples acquired in the Portuguese market (n = 43). Country of manufacture: China (n = 22). Turkey (n = 6). Portugal (n = 15). *BR* = samples acquired in the Brazilian market and manufactured in Brazil (n = 53).

Table 4
Estimated daily intake (EDI) and relative intake index (RII) of selected elements.

Element	90th percentile (μg/g)	Daily intake (g)	EDI (μg/day)	PDE[a] (μg/day)	RII (%)
Ag	0.06	0.04	0.002	167	0.001
As	0.51		0.02	15	0.14
Cd	0.14		0.01	5	0.11
Co	1.34		0.05	50	0.11
Cr	7.49		0.30	10700	0.003
Cu	15.3		0.61	3400	0.02
Li	10.3		0.41	560	0.07
Mo	1.49		0.06	3400	0.002
Ni	5.93		0.24	220	0.11
Pb	3.63		0.15	5	2.91
Sb	0.56		0.02	1200	0.002
Sn	207		8.30	6400	0.13
Tl	0.20		0.008	8	0.10
V	3.30		0.13	120	0.11

[a] PDE (permitted daily xposure) values retrieved from the ICH Q3D Guideline (EMA, 2016).

For the most toxicologically relevant elements, the safety assessment carried out showed that, even in the worst-case scenario, i.e., considering the total ingestion of the amount of lipstick usually applied per day (40 mg), lipstick use would represent less than 0.2% of the PDE limits established by the European Medicines Agency for drug products, except for Pb, for which it can represent around 3% (still a very low contribution for the total daily intake).

Despite the limited number of samples analyzed, this study included representative products from both markets, particularly low cost products, thus confirming that when lipsticks are manufactured with good quality raw materials and according to current good manufacturing practices, it is possible to obtain a product with very low elemental impurities levels, which pose no risk for human health. However, a continuous surveillance of these products must be maintained in order to ensure the compliance with the current regulations and guidelines.

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