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DETERMINATION OF TOTAL AND BIOACCESSIBLE ELEMENTS IN TEMPORARY ADHESIVE TATTOOS USED BY CHILDREN AND ADULTS

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The presence of bioaccessible potentially-toxic elements in five different brands of temporary tattoos (packaged with bubble gums) aimed at children and three product brands used by young and adult audiences were analyzed in accordance with the Brazilian Organization of Technical Standards (ABNT) and International Organization for Standardization (ISO) for toy's safety standards. Bioaccessible barium, copper and strontium were found in the concentration range between 1.78–11.1 $\mu g\ g^{-1}$, 0.0364–0.875 $\mu g\ g^{-1}$, 0.155–9.9 $\mu g\ g^{-1}$, respectively. Bioaccessible lead ($2.6 \pm 0.1\ \mu g\ g^{-1}$ – $4.36 \pm 0.06\ \mu g\ g^{-1}$) and boron ($2.14 \pm 0.07$ – $3.54 \pm 0.07\ \mu g\ g^{-1}$) were found in two samples whereas chromium ($0.403 \pm 0.004\ \mu g\ g^{-1}$) in one sample aimed at children. Bioaccessible aluminum was found in six samples, mostly at products used by children. Other elements whose bioaccessibility was evaluated were Mo (< LOD), Cd (< LOD), V ($0.148 \pm 0.003\ \mu g\ g^{-1}$) and Co (0.003 – 1.756 $\mu g\ g^{-1}$). All samples met the upper limits permitted by the national toy safety standard, however, chromium, lead, cobalt and barium presented higher bioaccessibility levels than the allowed values by cosmetic regulation that controls products applied directly to the skin.

Keywords: toys; temporary adhesive tattoos; heavy metal; bioaccessibility; ICP-MS.

INTRODUCTION

The trend to marking the body with a tattoo was adopted by different ancient cultures many years ago in Japan, China, Greece, Egypt, among others.[1] This practice can take on different meanings depending on the region or country, being used to show an elegant body ornament elevating self-esteem, but also participation in a specific socio-cultural group or to express a feeling.[1-4] In recent years, the procedure of marking the body with a tattoo has been gaining more followers. A recent study performed in Brazil found that around 37% of the population have at least one tattoo, while in other countries this percentage can be higher, for instance in Sweden (47%) and USA (46%).[1]

In general, tattoos can be divided into two categories: permanent and temporary tattoos.[1] The permanent tattoo consists of applying colored pigments into the dermal layer using a needle, and this ink cannot be removed from the skin.[2,5-8] The temporary tattoo consists of an image attached to a support, usually paper or plastic. This support is moistened with water and applied directly to the skin.[9] After a few seconds the support is removed and the image remains fixed on the application site. Some advantages of a temporary tattoo are: it costs less than a permanent tattoo, there is no pain to apply it into the skin, no risk of infection (HIV and hepatitis, for instance), and allows you to apply it on the body without a long-term commitment.[10] In most cases, temporary tattoos can be removed at any time from the skin just using water or ethanol. However, although the low permanence into the skin, the use of temporary tattoo has fascinated both children and adults for a long time.[1,11-13]

All different types of tattoos contain several substances that can expose the skin to chemical compounds in a short or long period of time.[1-4,6,14] Depending on the concentration, the exposition can cause a slight allergy, damage to human epithelial cells or carcinogenic effects.[5,7,15]

The main problem is due to the presence of dyes or other toxic elements that are not approved by FDA (Food and Drug Administration) and can be harmful for the skin[9]. Children are more sensitive to the effects of toxic compounds caused by this exposition, which can cause skin irritation such as redness and swelling.[1,16,17] Furthermore, there are several reports in the literature showing allergic reactions, local infections or granulomas caused by the different compounds present in temporary tattoos.[9,13,18-24]

Although the several problems previously mentioned earlier, the consumer does not know the composition of temporary tattoos due to inadequate labeling on the packaging.[1] Moreover, these products can be easily found in markets with various shapes, figures and are also packaged with bubble gum. Dyes are the main constituents of these temporary tattoos and, therefore, impurities from the manufacturing process may also be present.[11] Temporary tattoo inks are protected with plastic films and substances such as phthalates may migrate to the inks and, consequently, adhere to the skin when using the product. In addition to these components, aromatic amines, polycyclic compounds with or without azo group, nanoparticles, hydrocarbons and toxic metals can be found in these temporary adhesive tattoos and lead to skin irritation.[1] The quality control of these products is not well established and is confusing. According to Rubio *et al.*,[1] temporary tattoos must be correctly labeled and the chemical composition must comply with the Safety Standards of Toys and, because the product is in direct contact with the skin, the product must comply with the laws about the Cosmetics Regulation.

Rastogi and Johansen[11] analyzed 36 synthetic adhesive tattoos in order to investigate the presence of different dyes using a method based on solvent extraction and analyzed the samples by high performance liquid chromatography (HPLC) with spectrophotometric

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detection (UV-Vis). At least 11 compounds were identified in concentrations more than 4479 mg kg^{-1}. The red dye (barium bis[4-[(2-hydroxy-1-naphthyl)azo]-2-methylbenzenesulphonate) was present in 94% of the temporary tattoos analyzed in a concentration of up to 2391 mg kg^{-1}. According to the authors, this dye has already been reported as allergenic due to impurities present in the pigment.

Sukuroglu *et al.*[25] determined phenylenediamine (PPD) by high performance liquid chromatography and Co, Ni, Pb, Cr by inductively coupled plasma mass spectrometry (ICP – MS) in samples of commercial temporary black henna tattoo from Turkey. The authors found the presence of the allergenic additive (PPD) in all samples in the range between 3.37 and 51.6% (w/w).

At least 23 samples showed PPD concentration above the limit of 6% (w/w) stipulated by the European Cosmetics Regulation.[26] In the United States, the FDA has banned the use of PPD in products applied directly to the skin including temporary tattoos.[9] In this study, the authors used the term bioaccessible as a soluble metal fraction that form hydrate ions in aqueous solution and can potentially be absorbed by the human body or skin.[27] The values of bioaccessible metals in deionized water were in the range between 0.15-0.18 µg L^{-1}, 0.32-0.42 µg L^{-1}, 0.55-0.67 µg L^{-1}, and 0.13-0.38 µg L^{-1} for Co, Ni, Pb and Cr, respectively. The authors stressed that due to the high toxicity, the presence of these elements in cosmetics is prohibited in several countries of the European Union according to the current legislation.[26] Similarly, these metals are also prohibited in cosmetic formulations in Brazil according to RDC n° 83 of the National Health Surveillance Agency (ANVISA).[28] Moreover, the presence of metals in these tattoos may be related to the presence of inorganic and organometallic pigments, paint impurities, additives or mineral adulterants that was added to the tattoo, in order to give a metallic and shiny appearance, when the product is applied into the skin.[1]

Besides the effects of toxic elements in the literature being well-known, data related to these contaminants is still scarce in synthetic temporary tattoos, especially those aimed at children. Children can be exposed to chemicals through oral and dermal exposure, and the contact of metallic ions with the skin induces dermatitis and rashes that can be absorbed into the bloodstream.[16,29-34] In this context, the investigation of the bioaccessibility of potentially toxic elements in temporary tattoos is necessary due to the unknown composition of the ingredients and the consequent possibility of dermal contamination and adverse effects related to the health of children and adults using this product.

Thus, the aim of the current study is to verify the bioaccessibility of B, Al, V, Cr, Co, Cu, Sr, Mo, Ba, Cd, and Pb in synthetic temporary tattoos available in the Brazilian market. For this purpose, samples of different brands of temporary tattoos packaged with bubble gums, whose target audience is children, and samples of temporary tattoos sold on cards used mainly by young people and adults were analyzed. The determination was performed by ICP-MS after an extraction of the elements with a diluted HCl solution. The sample preparation procedure and the results obtained were performed and compared according to the Brazilian Safety of toys - Part 3: Migration of certain elements of Brazilian Association of Technical Standards (ABNT),[35] International Organization for Standardization (ISO),[36] and Brazilian standards of permitted and prohibited substances in National Health Surveillance Agency (ANVISA).[37] The use of HCl solution is recommended by the methodologies imposed by safety standards as they simulate dermal and oral exposure conditions in which some fraction of elements present in toys (temporary tattoos) is solubilized in body fluids. Although these temporary tattoos are not considered toys, they are widely used by children and eventually can be ingested accidentally by children. Considering the absence of regulatory agencies for such temporary tattoos, we compare the obtained bioaccessible results with upper limits established by toy safety and cosmetic regulations.

EXPERIMENTAL

All solutions were prepared using high-purity water obtained from a Milli-Q system (Millipore, Germany), resulting in water with resistivity equal to 18.2 MΩ cm. The analytical reference solutions were prepared in 0.1% (v/v) of HNO_3 by successive dilutions of the 1000 mg L^{-1} multi-element solution (Quimlab, Brazil). All solutions were stored in polypropylene bottles.

Kid's temporary tattoos were purchased in supermarkets and candy stores. In total, 5 samples of different brands (A, B, C, D and E) were acquired containing bubble gums packed together with temporary tattoos. These samples are manufactured in Brazil, in addition, they are sold in sealed boxes containing 100 units each or are sold individually in smaller quantities. In this specific work, all the samples mentioned were acquired in a sealed box. The temporary tattoo is between the bubble gum and the outer packaging (label) and the drawing containing the inks is protected with a plastic film to prevent contact of the gum with the tattoo ink. The temporary tattoo designs are supported on a paper or plastic substrate, depending on the brand. The dimensions of the applicable area containing the adhesive tattoo inks are variable and depend on the size of the design and the brand. Temporary tattoos from each sample were selected for analysis in such a way that most dyes were present and, in this way, different elements could be extracted and detected.

Other 3 samples (F, G and H) of different brands of temporary tattoos were purchased at the local and online stores. In this case, these tattoos are sold separately and not attached to a product like the ones mentioned above. These samples were acquired as cards containing one or more figures of different sizes and areas containing the applicable ink. In the samples in which the cards contained several drawings, the temporary tattoos were cut to remove the excess of unprinted paper with the aid of scissors. All temporary tattoos are printed on a paper substrate and the inks are protected by a plastic film to prevent direct contact with air. The application is similar to those aimed at children; however, the durability is greater, that is, the contact of the ink with the skin remains for a longer period. The target audience of these tattoos is diverse; it can include children, youth and adults depending on the visual aspect and parental authorization when underage. Figure 1 shows some examples of samples/tattoos packaged together with bubble gums and adhesive tattoos on cards used at work. In total, 9 temporary tattoos were analyzed in this study and classified in A, B, C, D and E as being present in bubble gums aimed at children and F, G and H as temporary tattoos of cards that are most used young and adult audiences.

Bioaccessibility of elements in temporary tattoos and HCl extraction

The procedure for preparing temporary tattoo samples consisted of extracting potentially toxic elements using a solution of 0.07 mol L^{-1} HCl according to the Brazilian safety standard of ABNT NBR NM 300-3:2011[35] and ISO 8124-3:2020[36] on element migration in toys. This method is based on the bioaccessibility of certain elements (metals) after the use of toys whose focus is oral exposure due to suction, swallowing or licking. The use of HCl solution during extractions is recommended by the methodologies imposed by safety standards as they simulate dermal and oral exposure conditions in which some fraction of elements present in toys (temporary tattoos) is solubilized in body fluids. In this way, the extracted bioaccessible elements can potentially be absorbed by cell membranes and cause

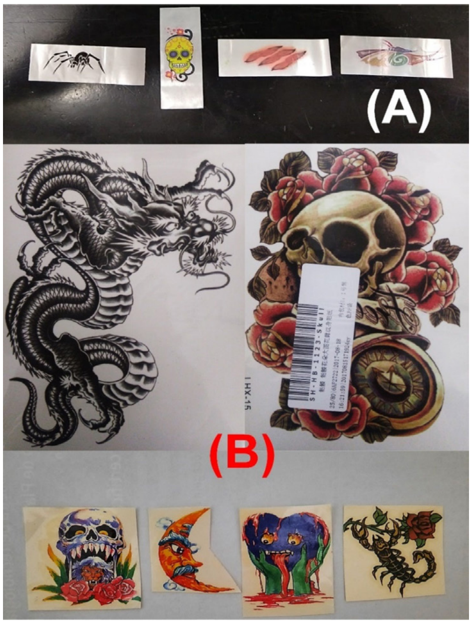

Figure 1. *Examples of the analyzed adhesive tattoos:* ***(A)*** *temporary tattoos present in bubble gum and* ***(B)*** *temporary adhesive tattoos on cards*

health damage depending on the concentration.[35,36] The 0.07 mol L^{-1} HCl solution is responsible for extracting only the bioaccessible fraction. The total content of elements is obtained after procedures of total digestion of the temporary tattoo samples. Therefore, the bioaccessible fraction of the elements present in the analyzed samples is necessarily smaller than the total concentration. For comparison, complete digestion of temporary tattoo samples was performed as a way to demonstrate the percentage of bioaccessible elements in relation to the total concentration. The bubble gums of each brand were unpacked and the adhesive tattoo separated. Then, the plastic film that protects the paint was removed, leaving the substrate containing the figure. Each temporary tattoo from 5 different bubble gum brands has figures with different areas, so they have different mass. In this way, the mass used in the extraction procedure was standardized. Approximately 0.6 g of the samples (A, B, C, D and E), which consisted of the respective tattoo attached to the paper substrate was used. According to the standard safety of toys, the substrate must also be part of the extraction. Temporary tattoos for each brand were added in 5 different beaker flasks. Then, 14 mL of HCl (0.07 mol L^{-1}) solution was added over the tattoos contained in the tubes and stirred at 37 ± 2 ºC for 1 h using a magnetic stirrer. After, the solution remained at rest for one more hour. The care with adding the solution ensured that all temporary tattoos accommodated inside the tube were in direct contact with the liquid, ensuring maximum efficiency during extraction. The volume of the solution used in this procedure followed the recommendation of ABNT NBR NM 300-3:2011[35] and ISO 8124-3:2020.[36] Then samples were mixed with HCl (0.07 mol L^{-1}) using a mass of extracting solution that was 50-fold higher than the mass of sample. The solutions were filtered through a PTFE membrane with a pore diameter of 0.45 µm, stabilized with ultrapure hydrochloric acid to the final concentration of 1.0 mol L^{-1} and stored in a polypropylene tube. The same procedure was applied to temporary card tattoos. However, the plastic film, that was covering the figures, was removed before the experiment.

For large adhesive tattoos, the substrate was cut into smaller parts with a plastic ruler. Initially, the plastic film covering the figure's paint was removed. Each sample was transferred to a beaker flask and the masses were related to the total weight of the card containing the temporary tattoo and the paper substrate. Then, each sample was mixed with a solution of HCl (0.07 mol L^{-1}) using a mass proportion of 1:50 (HCl: sample) as mentioned before. The mixture was stirred for 1h at 37 ± 2 ºC and remained for another 1h of rest. The solutions were filtered, stabilized with HCl and stored in polypropylene tubes for further analysis. The tattoo masses used in the samples (A, B, C, D, E, F, G and H) extracted with HCl were comprised in the range between 0.4 and 0.7 g.

Microwave-assisted digestion of temporary tattoos

The total concentration of all elements determined in the temporary tattoos required a complete sample digestion procedure. The preparation of temporary tattoos prior to the digestion procedure followed the same steps reported for the extraction of the bioaccessible fraction. Approximately 0.6 g of the samples (A, B, C, D and E) was initially removed from the plastic film that protects the painting, leaving only the tattoo adhered to the paper substrate. Each tattoo sample of different brands was packaged in 5 different polypropylene tubes. Similarly, samples F, G and H were separated from the film that covers the tattoo ink and added to three other different tubes. It should be noted that the mass of the samples was the same used in the experiments to determine the fraction of bioaccessible elements and the total concentration.

The digestion procedure was performed in an ETHOS ONE (Milestone, Sorisole, BG, Italy) microwave oven, using a diluted acid mixture (2.0 mL of HNO_3 + 1.0 mL of H_2O_2 + 7 mL of H_2O), and the heating program was executed in three steps (temperature [80 °C, 140 °C and 190 °C], ramp [5 °C min^{-1}] and hold [2 min, 2 min and 10 min]) as follows, respectively temperature/ramp/hold: 1st step (140/5/1), 2nd step (180/4/5) and 3rd step (200/4/10).

Instrumentation

After the procedure of extracting temporary tattoos with HCl and the complete digestion, the solutions obtained were analyzed by inductively coupled plasma mass spectrometry (ICP - MS), model iCAP Q from Thermo Fisher Scientific (Cambridge, England), equipped with a quadrupole mass analyzer and a collision reaction cell set to kinetic energy discrimination (KED) mode, using 99.999% He as collision gas and 99.999% Ar gas was used to carry out the plasma formation and maintenance process. The instrumental parameters used in the operation of the equipment are described in Table 1.

Table 1 Instrumental parameters used in the operation of ICP-MS

Parameter	Operational Condition
Radio frequency power (W)	1550
Nebulization chamber temperature (°C)	2.7
Peristaltic pump speed (rpm)	40
Sampling depth (mm)	5
Auxiliary gas flow (mL min^{-1})	0.8
Nebulizer gas flow (mL min^{-1})	0.975
Nebulizer	Concentric
Spray chamber	Cyclonic
Number of replicates	3
Dwell time (s)	0.05
Operation mode	KED (Kinetic Energy Discrimination)
Analyzed isotopes	^{11}B, ^{27}Al, ^{51}V, ^{53}Cr, ^{59}Co, ^{63}Cu, ^{88}Sr, ^{98}Mo, ^{138}Ba, ^{114}Cd, ^{208}Pb
Internal standard	^{115}In

Analyses were performed using the optimized instrumental parameters shown in Table 1. All analytical reference solutions were prepared using concentrations of multi-element standards in a range from 0 to 500 µg L^{-1}, in order to perform the instrument calibration.

RESULTS AND DISCUSSION

The concentration of elements in tattoos was determined using an ICP-MS method that had the following figures of merit evaluated: linear range, limits of detection (LOD) and quantification (LOQ). The values obtained for each element are listed in Table 2.

Table 2. Analytical parameters of the inductively coupled plasma mass spectrometry method

Element	LOD (µg g^{-1})	LOQ (µg g^{-1})	Linear range (µg g^{-1})	R^2
B	0.19	0.64	0.64-52.1	0.999
Al	0.32	1.1	1.1-52.1	0.999
V	0.029	0.097	0.097-111.3	0.999
Cr	0.093	0.31	0.31-111.3	0.999
Co	0.0003	0.001	0.001-5.17	0.999
Cu	0.009	0.032	0.032-5.17	0.999
Sr	0.014	0.046	0.046-111.3	0.999
Mo	0.003	0.011	0.011-111.3	0.998
Cd	0.06	0.2	0.2-111.3	0.999
Ba	0.06	0.2	0.22-111.3	0.999
Pb	0.64	2.1	2.1-111.3	0.999

The limits of detection (LOD) and quantification (LOQ) for the determination of elements in temporary tattoo samples were calculated as 3 × SD/*S* and 10 × SD/*S*, respectively, where SD is the standard deviation of 10 readings of blank and *S* the slope (sensitivity) of the analytical curve for each element. The LOD and LOQ calculations were performed as described by the ABNT NBR NM 300-3:2011 standard. The low LODs and LOQs obtained in this study demonstrate the high sensitivity of the method for elemental determination in tattoos samples. The linear range obtained for each metal was wide, with a linear correlation coefficient (R^2) greater than 0.998 for all the quantified elements.

In order to evaluate the presence of polyatomic interferences, at least 2 isotopes of each element were monitored and the results was compared using Student's t-test. No significant differences ($p > 0.05$) were observed between the results of ^{11}B and ^{10}B, ^{63}Cu and ^{65}Cu, ^{86}Sr and ^{88}Sr, ^{137}Ba and ^{138}Ba, ^{206}Pb and ^{208}Pb. However, this evaluation was not allowed to some elements. Aluminum and cobalt are monoisotopic elements, vanadium has only one alternative isotope to be compared (^{50}V), which has an isobaric interference of ^{50}Cr and all the results of Mo and Cd were below the LOQ. When ^{52}Cr and ^{53}Cr results were compared, significant differences ($p > 0.05$) were observed. Therefore, polyatomic interferences, such as $^{40}Ar^{12}C^+$, can occur. Thus, ^{53}Cr was chosen to chromium measurements, although it is less abundant.

Total elementary determination in tattoo samples after digestion

The focus of this investigation is to evaluate the bioaccessible elements in temporary tattoos. However, the knowledge of the total concentration of the elements present in the samples can provide information on the percentage of bioaccessible elements that will be discussed further in the text. Table 3 lists the total concentration of elements in the temporary tattoos after their microwave-assisted digestion. Temporary tattoos can be considered as toys as they can be ingested accidentally by children. Considering the limit values established by ABNT and ISO for toys, alarming levels of Cr (sample A), Ba (sample F) and Pb (sample C) were found. Other elements that do not present limit values but are prohibited at any concentration can be mentioned, such as V and Co. Nevertheless, it is crucial to evaluate the bioaccessible fraction of all elements because they may be not released to biological fluids by the direct contact between temporary tattoos and fluids (sweat, saliva or gastric fluids). Next section presents the results obtained for the bioaccessible elements in the same analyzed samples.

Table 3. Total concentration of elements after digestion of temporary tattoo samples

Samples ↓ / Elements→	B (µg/g)	Al (µg/g)	V (µg/g)	Cr (µg/g)	Co (µg/g)	Cu (µg/g)	Sr (µg/g)	Mo (µg/g)	Cd (µg/g)	Ba (µg/g)	Pb (µg/g)
A	2.16 ± 0.09	155 ± 38	5.4 ± 0.1	343 ± 6	< LOD	118 ± 9	18.0 ± 0.8	8.8 ± 0.6	< LOD	171 ± 24	172 ± 2
B	3.22 ± 0.06	893 ± 42	0.574 ± 0.005	53.3 ± 0.9	< LOD	100 ± 8	19 ± 2	94 ±3	< LOD	243 ± 4	5.0 ± 0.2
C	6.3 ± 0.1	236 ± 3	0.53 ± 0.02	7.2 ± 0.2	< LOD	544 ±33	3.3 ± 0.2	69 ± 4	< LOD	255 ± 39	920 ± 19
D	14.1 ± 0.8	558 ± 191	< LOD	< LOD	12.8 ± 0.6	274 ± 15	18.7 ± 0.9	2.0 ± 0.8	< LOD	139 ± 25	1.4 ± 0.4
E	1.6 ± 0.4	22412 ± 511	0.45 ± 0.06	< LOD	68 ± 11	517 ± 32	11 ± 2	0.34 ± 0.08	< LOD	345 ± 107	< LOD
F	11.1 ± 0.9	6399 ± 1588	< LOD	< LOD	97 ± 27	932 ± 242	68.5 ± 0.9	0.97 ± 0.08	< LOD	1507 ± 151	< LOD
G	< LOD	153 ± 11	1.5 ± 0.2	3.4 ± 0.7	19 ± 2	51 ± 3	53 ± 2	< LOD	< LOD	147 ± 27	1.00 ± 0.05
H	16 ± 1	835 ± 133	0.70 ± 0.04	1.2 ± 0.2	67 ± 8	484 ± 36	13 ± 3	0.6 ± 0.1	< LOD	239 ± 16	0.8 ± 0.2
Toy limit (ABNT)	NS*	NS*	NS*	60	NS*	NS*	NS*	NS*	75	1000	90
Toy limit (ISO)	NS*	NS*	NS*	60	NS*	NS*	NS*	NS*	75	1000	90
Cosmetic limit	max. 180000[a]	Allowed	Prohibited	Prohibited	Prohibited	Allowed[b]	35000[c]	NS*	Prohibited	Allowed[d]	Prohibited

*NS = Not specified; [a] Maximum permitted concentration in the form of boric acid or boron salts used in products applied directly to the skin.[37] [b] Permitted in the form of dyes Cl 74160 (blue), Cl 74260 (green), Cl 77400 (brown). The maximum limits allowed are not mentioned.[42] [c] Concentration allowed in the form of strontium chloride hexahydrate. Strontium lactate and nitrate are prohibited substances for use in cosmetics.[37] [d] Barium compounds are prohibited with the exception of insoluble barium salts such as barium sulfate, barium sulfide, lacquers, and pigments prepared under conditions that do not release barium ions.[37]

Determination of bioaccessible elements in tattoo samples

The ABNT NBR NM 300-3:2011 and ISO 8124-3:2020 standards focuses on assessing the chemical safety of toys from oral exposure to bioaccessible harmful elements as the relevant route of contamination, as the oral and gastrointestinal absorption of elements is greater than cutaneous absorption.[38] Thus, as the temporary tattoo is in direct contact with the user's skin for varying periods of time, the results obtained for the elements analyzed in this work were compared with the maximum limits allowed for the same elements found in cosmetic products in Brazil[28,37] and national or international toys safety standards.[35,36]

Table 4 shows the concentration of bioaccessible elements in tattoos and the respective limits allowed in cosmetic products and toys in accordance with Brazilian standards. Among the 11 elements determined in the 8 adhesive tattoo samples, only Mo and Cd were not detected in all samples (< LOD). Cd is a toxic element and is classified as a carcinogen.[15] It can be seen that the LOD for Cd (Table 3) is below the maximum limit allowed for this element according to the toy safety rules. Therefore, the methodology used in this work allows monitoring low levels of Cd concentration in temporary tattoo samples. There is no report about the presence of Cd and Mo in synthetic adhesive or henna-based temporary tattoos. On the other hand, a study showed the presence of Cd in cosmetic hair dyes based on henna.[39] Brazilian regulations prohibit the presence of cadmium compounds in cosmetic products,[28] while the toy's safety standards (ABNT and ISO) establish a limit of 75 mg kg^{-1} of bioaccessible Cd.[35,36]

According to Table 4, two samples of temporary bubble gum tattoos (A and C) showed concentrations of bioacessible Pb. The concentration values were of 2.6 ± 0.1 µg g^{-1} in A and 4.36 ± 0.06 µg g^{-1} in C. These values are below the limits established by the toys safety standards, which stipulates a maximum lead value of 90 µg g^{-1}.[35,36] In all other samples, Pb was detected but it was not possible to detect because they are below the LOD of the method. According to cosmetics regulations, the presence of lead and its compounds in products applied on the skin is prohibited.[28] Lead is a highly toxic metal and exposure can cause headaches, memory loss, abdominal pain, problems related to the male and female reproductive system, kidney and cardiovascular problems and in children can cause impaired cognitive development. Studies have shown the presence of lead in hair dyes[39,41] and temporary henna tattoos.[25,42] Ozbek and Askman[39] determined the concentration of lead in two hair dyes based on henna (green and black) by high-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GFAAS). The concentrations obtained were 0.93 ± 0.05 and 0.60 ± 0.05 µg g^{-1} for green and black henna tatoos, respectively. Sukuroglu *et al.*[25] analyzed by ICP-MS the presence of bioaccessible lead in 25 temporary henna tattoos using deionized water as extractor solution. The samples showed that lead concentration was in range between 1.59-17.7 µg g^{-1}. With respect to skin absorption, a study realized by Sauber *et al.*[43] showed that inorganic lead compounds, such as $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$, are easily absorbed by human skin and detected in sweat, blood and urine after 6 h of contact with the solutions of these salts on the skin. The authors revealed that in 5 mg of Pb^{2+} applied to the skin, 1.3 mg had been absorbed within 24 hours.

The presence of chromium was found only in sample A, which refers to a temporary tattoo packaged together with bubble gums. In this sample, which is used mainly by children, the obtained concentration was 0.403 ± 0.004 µg g^{-1}. Cr was detected in samples E, F and H, however, the values were found below the LOD. Sukuroglu *et al.*[25] obtained concentrations of chromium between 0.13-0.38 µg g^{-1} in temporary henna tattoos. Chromium compounds are well known for their negative health effects, and Cr(VI) species (such as chromate and dichromate ions) shows high toxicity and carcinogenicity.[44] In contact with the skin, chromium can cause allergic dermatitis,[31] in addition, *in vitro* studies with human skin have shown percutaneous penetration of different chromium species, such as Cr^{3+}, CrO_4^{2-} and $Cr_2O_7^{2-}$ which can cause negative health effects.[30,31,45] Salts and other chromium species are prohibited in the composition of any cosmetic product according to the Brazilian resolution.[28] On the other hand, the permitted limit of bioaccessible chromium in toys is 60 mg kg^{-1} according to the toys safety standards.[35,36] The chromium value obtained in the adhesive bubble gum tattoo is below the limit established by the toy's safety standards. However, considering the regulation on cosmetics for

Table 4. Concentration of bioaccessible elements after extraction with 0.07 mol L^{-1} HCl

Samples ↓ / Elements→	B (µg/g)	Al (µg/g)	V (µg/g)	Cr (µg/g)	Co (µg/g)	Cu (µg/g)	Sr (µg/g)	Mo (µg/g)	Cd (µg/g)	Ba (µg/g)	Pb (µg/g)
A	< LOQ	3.1 ± 0.1	< LOD	0.403 ± 0.004	< LOD	0.120 ± 0.002	0.215 ± 0.004	< LOD	< LOD	1.779 ± 0.009	2.6 ± 0.1
B	< LOQ	2.92 ± 0.07	< LOD	< LOD	< LOD	0.182 ± 0.002	0.680 ± 0.003	< LOD	< LOD	2.16 ± 0.02	< LOD
C	< LOQ	1.42 ± 0.06	< LOD	< LOD	< LOD	0.219 ± 0.002	0.155 ± 0.005	< LOD	< LOD	5.14 ± 0.02	4.36 ± 0.06
D	< LOQ	520 ± 12	< LOD	< LOD	0.01935 ± 0.00008	0.354 ± 0.003	0.481 ± 0.005	< LOD	< LOD	5.95 ± 0.02	< LOD
E	<LOQ	525± 12	<LOD	< LOD	0.0161 ± 0.0003	0.385 ± 0.002	0.565 ± 0.005	< LOD	< LOD	7.711 ± 0.008	< LOD
F	2.14 ± 0.07	392 ± 10	0.148 ± 0.003	< LOD	1.76 ± 0.02	0.875 ± 0.005	9.9 ± 0.2	< LOD	< LOD	11.1 ± 0.1	< LOD
G	<LOQ	1.97 ± 0.02	< LOD	< LOD	0.0321 ± 0.0002	0.0364 ± 0.0003	4.63 ± 0.07	< LOD	< LOD	1.299 ± 0.003	< LOD
H	3.54 ± 0.07	37 ± 1	0.295 ± 0.004	< LOD	0.910 ± 0.002	0.192 ± 0.002	1.49 ± 0.01	< LOD	< LOD	6.35 ± 0.02	< LOD
Toy limit (ABNT)	NS*	NS*	NS*	60	NS*	NS*	NS*	NS*	75	1000	90
Toy limit (ISO)	NS*	NS*	NS*	60	NS*	NS*	NS*	NS*	75	1000	90
Cosmetic limit	max. 180000[a]	Allowed	Prohibited	Prohibited	Prohibited	Allowed[b]	35000[c]	NS*	Prohibited	Allowed[d]	Prohibited

*NS = Not specified. [a] Maximum permitted concentration in the form of boric acid or boron salts used in products applied directly to the skin.[37] [b] Permitted in the form of dyes Cl 74160 (blue), Cl 74260 (green), Cl 77400 (brown). The maximum limits allowed are not mentioned.[40] [c] Concentration allowed in the form of strontium chloride hexahydrate. Strontium lactate and nitrate are prohibited substances for use in cosmetics.[37] [d] Barium compounds are prohibited with the exception of insoluble barium salts such as barium sulfate, barium sulfide, lacquers, and pigments prepared under conditions that do not release barium ions.[37]

prohibits any form of free chromium, the tattoo sample is not adequate according to Brazilian resolutions.

Vanadium was found only in a sample that corresponds to the H adhesive tattoo. The found concentration was 0.295 ± 0.004μg g^{-1}. The cosmetics regulation prohibits the use of divanadium pentoxide in cosmetic products.[28] The toys safety standards do not mention or stipulate limit values for vanadium ionic compounds.[35,36] For boron, two samples of adhesive tattoos (F and H) presented relatively large concentrations, 2.14 ± 0.07 and 3.54 ± 0.07 μg g^{-1}, respectively. No acceptable boron limits are reported in the toy's safety standards. On the other hand, for cosmetics, only compounds such as boric acid, borates and tetraborates are allowed with maximum permitted limits of 0.1-18% in the preparation of the products.[37] In the other samples, boron was detected, but it was not quantified because the concentrations were below the LOQ value.

Copper and strontium were detected in 100% of the temporary tattoo samples. For copper, the concentrations were in the range between 0.037-0.875 μg g^{-1} while for strontium it varied between 0.155-9.9 μg g^{-1}, with emphasis to the sample F that presented the highest concentration. Some strontium salts like lactate, nitrate and polycarboxylate are prohibited by the resolution of cosmetic products.[28] However, strontium chloride, acetate, peroxide and hydroxide are accepted with restrictions for use in cosmetics.[37] The toy's safety standards do not report limit values for the presence of strontium and copper in toys.[35,36] Copper-based dyes, such as Cl 74160 (blue), Cl 74260 (green), and Cl 77400 (brown), are permitted in cosmetic formulations.[40]

The presence of cobalt was detected in 5 of the 9 samples analyzed according to Table 4. The concentration range obtained for this element varied between 0.002-1.756 μg g^{-1}, and the sample F, corresponding to the adhesive tattoo, presented the highest value. Cobalt is an element known to cause allergic reactions in contact with the skin.[29,30] In fact, Kang and Lee[46] determined by flame atomic absorption spectroscopy (F AAS) the bioaccessible presence of cobalt in 15 henna tattoos. The authors found the presence of cobalt in 4 different samples with concentrations in the range of 2.96-3.54 μg g^{-1}. In addition, it was found in one patient, positive allergic reactions to cobalt caused by skin exposure with henna tattoo.[46] In another study, the concentration of bioavailable cobalt in henna tattoos was obtained in the range between 0.15 and 0.18 μg g^{-1}.[25] No limits for cobalt are mentioned in the toy's safety standards. The dye Cl 77346 (green), which is cobalt-based dye, is allowed in cosmetic formulations[40] while other compounds, such as cobalt chlorides, sulfates and benzosulfonates, are prohibited.[28]

Bioaccessible barium was found in all analyzed tattoo samples. The obtained concentrations remained in the range between 1.779-11.116 μg g^{-1}. The adhesive tattoos directed to the child audience A, B, C, D and E, and to the young and adult audience F, G and H presented bioaccessible barium concentrations in the order of magnitude of parts per million (ppm). The maximum acceptable limit of bioaccessible barium is 1000 μg g^{-1} according to toy's safety standards.[35,36] In cosmetic product resolutions, barium compounds are allowed only in their insoluble form.[28] That is, it can be seen from Table 4 that the barium compounds present in the tattoo samples are in the soluble form as they were extracted by 0.07 mol L^{-1} HCl. Several dyes based on insoluble barium salts are allowed, such as Cl 12085 (red) Cl 15510 (orange), Cl 15540 (red), Cl 15630 (red), Cl 15850 (red), Cl 15865 (red), Cl 15985 (red), Cl 16255 (red), Cl 17200 (red), Cl 19140 (yellow), Cl 42051 (blue), Cl 45370 (orange), Cl 45380 (red), Cl 45410 (red), Cl 45430 (red), Cl 77120 (white).[40] The toxicity of barium depends on the solubility of its salts. Barium sulfate is known to be insoluble, however, impurities such as the presence of barium carbonate which is more soluble causes the release of barium ions. In 2003 in Brazil, about 20 people died of poisoning after ingesting a pharmaceutical product containing barium sulfate (Celobar®) used as a radiological contrast agent. Experts pointed out the presence of barium carbonate (13% w/w), which is soluble in the hydrochloric acid of the stomach and releases barium ions.[47] Barium intoxication can cause damage to the gastrointestinal tract, respiratory and cardiac paralysis, in addition, it is related to skin allergies as long as compounds containing this metal.[48,49] According to the Agency for Toxic Substances and Disease Registry (ATSDR), there are no reliable data regarding the health effects of humans after direct exposure of the skin with barium ions.[50]

Bioaccessible aluminum was found in all analyzed temporary tattoos. The found concentration range was wide and comprised between 1.972 – 553.279 μg g^{-1}. According to Table 4, the sample E (aimed at children) showed an incredible 553.279 μg g^{-1} of aluminum. Aluminum poisoning can cause anemia, bone disease and impair kidney function. Studies have shown the great capacity of the skin to absorb aluminum present in the cosmetic composition of antiperspirants that acts by blocking the secretion of sweat.[51,52] In another study, the use of scanning electron microscopy and energy dispersive X-ray microanalysis allowed the authors to report the presence of granular tumors in the skin induced by hypersensitivity to aluminum in a permanent tattoo.[53] The toy's safety standard do not mention or establish any maximum permitted limits for aluminum in toy samples.[35,36] Cosmetic regulations allow the use of a series of aluminum-based substances and dyes.[37,40]

Percentage of bioaccessible elements

The percentage of bioaccessible elements was calculated using the percentage ratio between the concentration of analytes after extraction with 0.07 mol L^{-1} HCl and total elemental analysis. The calculated values are listed in Table 5. Figure 2 shows an illustrative plot with the data of Table 5 in order to highlight the most bioaccessible elements in the analyzed samples.

Overall, high bioaccessibility for some samples was reached for the determination of Al and Sr as compared to other elements. Sample H presented different behaviour in comparison with other samples in which a much higher bioaccessibility was verified for B, Al, V, Co, Sr and Ba (up to 42.1%). This sample was a temporary adhesive tatoo with a different nature compared with the others as shown in Figure 1B. In addition, for all samples, the percentage of bioaccessibility of Cd cannot be calculated (identified as ND - not determined) because both total and bioaccessible concentrations were below LOD or LOQ; the same occurred for other elements in some samples. In some cases, the bioaccessibility is very low reaching values of 0%, which means that the bioaccessible concentration of these elements were below LOQ or LOD although the same samples can present some elements in a concentration range quantified as shown in Table 3. This is the case for B, V, Cr, Mo and Pb, which confirms that these elements are in a chemical form that is poorly absorbed by the human body or fluids.

Thus, the results attained in the current work indicate that the presence of toxic elements in most of the analyzed samples is clear. The use of temporary adhesive tattoos in Brazil and in the world is a common practice mainly among children, but it also covers a part of young people and adults who are willing to change the look of the body. Considering the toy's safety standard, the metal concentrations in all tattoo samples analyzed are within the required standards. On the other hand, when compared to the regulation of cosmetics, most samples, including those used by children, present metallic ions that are prohibited in the composition of cosmetics, such as Pb, Cr and especially Ba. Although Al is an accepted substance, high levels were

Table 5. Percentage of bioaccessible elements in temporary tattoos compared to total concentration

Samples ↓ / Elements→	B (%)	Al (%)	V (%)	Cr (%)	Co (%)	Cu (%)	Sr (%)	Mo (%)	Cd (%)	Ba (%)	Pb (%)
A	0%	2.0%	0%	0.11%	ND	0.10%	1.19%	0%	ND	1.04%	1.51%
B	0%	0.32%	0%	0%	ND	0.18%	3.57%	0%	ND	0.88%	0%
C	0%	0.6%	0%	0%	ND	0.04%	4.69%	0%	ND	2.01%	0.47%
D	0%	ND	ND	ND	0%	0.12%	2.57%	0%	ND	4.28%	0%
E	0%	2.46%	0%	ND	0.02%	0.07%	5.13%	0%	ND	2.23%	ND
F	0%	4.89%	ND	ND	1.81%	0.09%	14.4%	0%	ND	0.73%	ND
G	ND	1.28%	0%	0%	0.16%	0.07%	8.73%	ND	ND	0.88%	0%
H	22.1%	4.19%	42.1%	0%	1.35%	0.04%	11.5%	0%	ND	2.65%	0%

ND = not determined; in the cases that total and bioaccessible concentration values were < LOQ or < LOD.

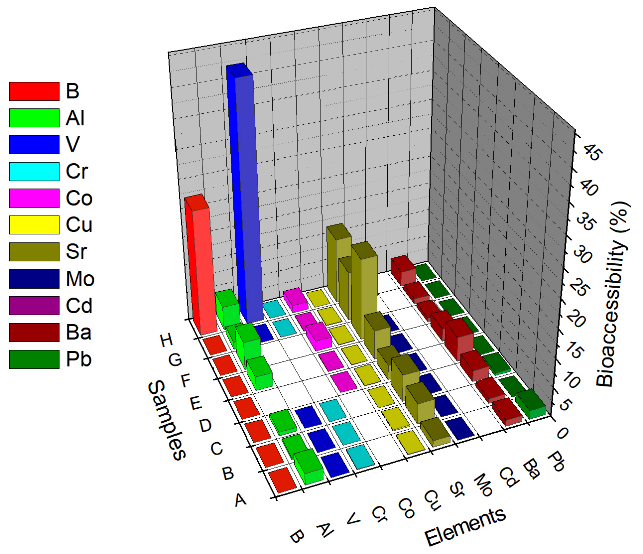

Figure 2. *3D chart illustration the bioaccessible elements (%) in the analyzed samples (A to H)*

obtained in most samples. It is well known that gastric absorption is more efficient than dermal absorption, however, it is necessary to consider that excessive dermal exposure of children and adults with the ingredients of temporary tattoos can lead to allergies and dermatitis. In addition, as has been shown, the skin is able to absorb and transport elements to the bloodstream. It is also likely that the substrate containing the temporary tattoo inks can be taken to the mouth by the children and therefore be ingested. Thus, strict quality control of this type of product is necessary because these products do not present labeled values and the element composition is unknown. The poisoning effects of these elements in certain concentrations and their absorption through skin or by oral ingestion can lead to future health-related problems.

CONCLUSIONS

In this study, the levels of bioaccessible elements were evaluated (B, Al, V, Cr, Co, Cu, Sr, Mo, Ba, Cd and Pb) in temporary tattoos used by children and youth/adults. The evaluation was carried out by simply extracting the potentially toxic elements from the tattoos with a 0.07 mol^{-1} HCl solution and analyzed by ICP – MS as described by the national and international toy's safety standards. The experimental analytical parameters, such as LOD, LOQ and linear range, confirmed the adequate performance of the methodology and enabled the comparison with the limits of the standards. Cd (bioaccessible and total content) and Mo (bioaccessible) were not detected in all analyzed samples (<LOD). On the other hand, bioaccessible barium was obtained in all 8 samples tested whose concentration varied from 2.001-11.116 µg g^{-1}. Chromium was found in one of the tattoo samples used by the child audience, while lead and vanadium were found in two samples, with lead being detected in tattoo samples for children. High concentrations of aluminum were obtained mainly in children's tattoos. Cobalt, a metal known to be allergenic in contact with the skin, was detected in 7 samples. Copper and strontium were found in all tested samples. The concentrations of bioaccessible elements obtained in this study did not exceed the maximum limits established by the Brazilian toy's safety standards. However, bioaccessible species of chromium, lead, barium and cobalt have been detected which are prohibited in cosmetic products applied directly to the skin due to toxic health effects. Thus, it can be concluded that although temporary tattoos are in accordance with toy safety, some toxic elements found in tattoos are in direct contact with the skin of those using the product, especially children. It is then necessary to alert the community and regulatory bodies about the risks involving the use of temporary tattoos due to the unknown composition of the inks. In future works, the identification and determination of the concentration of dyes present in these products may bring more information about the composition of the inks of this type of product.

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