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Determination of Heavy Metals in Tattoo Ink

Kimia Eghbali[1], Zahra Mousavi[2] and Parisa Ziarati[3]*

[1]Pharmacy School, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran.
[2]Department of Pharmacology and Toxicology, Pharmaceutical Sciences Branch, Islamic Azad University, (IAUPS), Tehran, Iran.
[3] Department of Medicinal Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran.

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In recent years, tattoos have become very popular worldwide and millions of people have black or colored tattoos. Despite the increasing number of tattooed individuals, presently there are few requirements, legislation and criteria for the safety of tattoos and permanent make-up. The aim of the survey is to assess whether the common or commercially purchased tattoo permanent inks in Iran market comply with maximum concentrations of heavy metals in the EPA's guidelines and find out the relation of colours by Zinc, Lead and Cadmium contents. 100 samples of 12 different permanent make –up ink tattoo brands in different available colours were randomly purchased from cosmetic stores and market in Tehran in main seven colours.: black, White ,yellow, brown ,red ,green and brown. Lead, Cadmium and Zinc contents were analysed by a Flame Emission Spectrophotometer. Analysis of variance (ANOVA) was done on each brand of tattoo ink to find out if there is significant variation in the concentrations of heavy metals in different colours of each brand. This result reveals that the type of pigment used in tattoo inks contributes to its heavy metal content. All the tattoo ink samples monitored in this study contained detectable contents of lead and cadmium. Cadmium contents in all group colours in Chinese and USA brands (probably fake brands) samples was much higher than maximum limited 0.2 mg/kg set by EPA and the highest one related to white colour 2.1473 mg/kg. In black and white colour the highest and lowest level of lead were observed respectively. White , yellow and orange ink samples showed the highest level of zinc content, while all samples had less content of this metal comparing by Maximum concentrations of zinc 50 mg/kg in tattoo and permanent make-up substances given in the EPA's *Guidelines* in 2012.

Key words: Tattoo ink, Heavy metals, Cadmium, Pigment, Colour.

In recent years, tattoos have become very popular worldwide and millions of people have black or colored tattoos[1] and tattooing practice is adopted widely in youth and women in Iran. In New Zealand, 20 percent of the population has a tattoo, including one in three people under the age of 30[2, 3]. Clinical observations, case reports and surveys show that tattoos are often associated with a variety of adverse skin reactions[4-13] whereas the causation of tattoo inks and the documented occurrence of cancer in tattooed skin is still controversially discussed[14]. The medical literature offers numerous case reports on dermatological diseases caused by tattoos, which includes pseudolymphoma, allergic or granulomatous skin reactions[15-19]. Despite the increasing number of tattooed individuals, presently there are few requirements, legislation and criteria for the safety of tattoos and permanent make-up. The list of ingredients on tattoo inks is usually missing or incomplete[20] and tattoo inks are not comprised by

* To whom all correspondence should be addressed.
Tel: +98-21-22600037; Fax: +98-21-22633986;
Email : ziarati.p@iaups.ac.ir

legislation on cosmetic products or on medicine but in November 2011, the Environmental Protection Authority (EPA) approved a new standard, the *Tattoo and Permanent Make-up Substances Group Standard*, to better manage the risks associated with chemical composition of tattoo inks and permanent make-up substances[21].

Titanium, barium, aluminum and copper are often used as colorants in tattoos; inks using nonmetal colorants may include traces of antimony, arsenic, cadmium, chromium, cobalt, lead and nickel. Some metal oxides (aluminum oxide, titanium oxide) in the Nano scale size are also utilized to reach the desired color, transparency or fluorescence[18]. Common ingredients of red-coloured inks are mercury and cadmium; Yellow-coloured inks commonly contain lead, cadmium and zinc[2]. Orange-coloured inks commonly contain cadmium[2]. Common ingredients of green-coloured inks are lead, chromium and copper and White inks commonly contain lead, zinc and barium[2].

Tattoo inks contain many components. Frequently, tattoo inks are pigment mixtures and may contain components such as titanium dioxide for lightening the ink shade. Precursors and by products of pigment synthesis may also be present. In addition, diluents used to suspend pigments may be complex mixtures[22, 23]. Besides adverse reactions, there is another risk factor regarding the colorants used for tattooing. Since these compounds are predominantly not produced for tattooing but are also ingredients of paints and varnishes, there are no specific declarations on the ingredients. The colorants consist of starting material and byproducts of the synthesis, titanium dioxide for the lightening of the colorant, and other unspecified compounds in different concentrations. On one hand there are regulations that relate to ingredients in paints and varnishes, but these regulations are different from those regulating cosmetics, foods, and drugs. In Europe, many of the azo pigments used in tattoos such as PR 22 are not allowed in cosmetics since they can be cleaved, yielding carcinogenic amines[24]. In the United States, the FDA considers the inks used in intradermal tattoos, including permanent make-up, to be cosmetics and considers the pigments used in the inks to be color additives requiring premarket approval under the Federal Food, Drug, and Cosmetic Act. However, because of other public health priorities and a previous lack of evidence of safety concerns, FDA has not traditionally regulated tattoo inks or the pigments used in them[24, 25].

The aim of the survey is to assess whether the common or commercially purchased tattoo permanent inks in Iran market comply with maximum concentrations of heavy metals in the EPA's guidelines and find out the relation of colours by Zinc, Lead and Cadmium contents.

MATERIAL AND METHOD

Sampling method

100 samples of 12 different permanent make –up ink tattoo brands mostly Chinese or USA fake brands (Batch number/Barcode**:** Unknown and Net weight:0.5 oz. /Bottle) in different available colours were randomly purchased from cosmetic stores and market in Tehran in main seven colours.: black, White ,yellow, brown ,red ,green and brown.

Estimation of Cd, Zn and Pb

All glassware and plastic containers used were washed with liquid soap, rinsed with water, soaked in 10% volume/volume nitric acid for 24hrs, cleaned thoroughly with distilled water and dried in such a manner to ensure that any contamination does not occur. Blanks and samples were also processed and analyzed simultaneously. All the chemicals used were of analytical grade (AR). For heavy metal analyses 2 gram of each sample was weighed on electronic balance (Shimadzu LIBROR AEX 200G). The samples were analyzed according to standardized international protocols by wet digestion method, Using HNO3 (65% Merck) , concentrated HClO4 and H_2SO_4 and H2O2 (30%) (3:2:1:1), analyzed by a Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene flame for heavy metals in research Analytical Laboratory in Pharmaceutical Sciences Branch , Islamic Azad University , using at least five standard solutions for each metal. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines[26, 27, 28, 29].

RESULTS

The concentrations of 3 metals (lead, Zinc and Cadmium) were determined in 100 samples from 12 different brands of imported permanent

make-up tattoo ink. Results were determined as mean ± SD of dry weight from three replicates in each test.

All the tattoo ink samples monitored in this study contained detectable contents of lead and cadmium. Figures 1 and 2 show the mean content of Pb and Cd (mg/kg DW) in 100 studied samples in all colour studied. In black and white colour the highest and lowest level of lead were observed respectively.

Results also showed that even in the same colour ink tattoo samples there was a wide range of Lead and Cadmium contents in different brands. Our finding showed no significant differences of the level of zinc content in the cheap brands of USA and Chinese brand sold in Tehran markets .

Analysis of variance (ANOVA) was done on each brand of tattoo ink to find out if there is significant variation in the concentrations of heavy metals in different colours of each brand. In all brands there is a significant difference in the levels of lead in entire seven main colour group analysed and black colour samples in 12 of 14 monitoring samples had lead contents much higher than the other groups of colours ($p<0.003$). This result reveals that the type of pigment used in

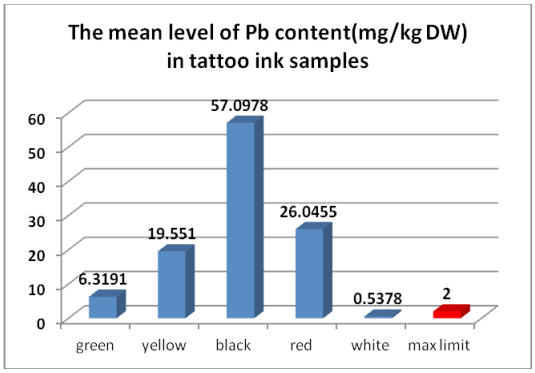

Fig. 1. The mean level of Lead content (mg/Kg DW) in different colour tattoo permanent ink samples.

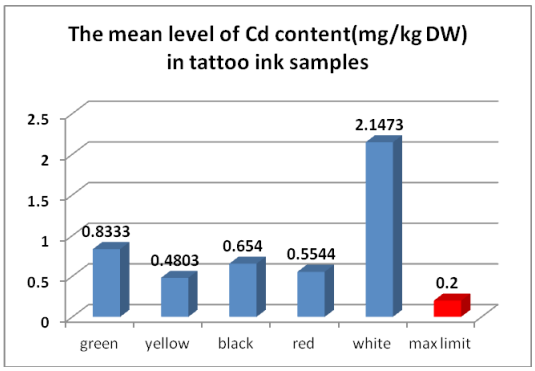

Fig. 2. The mean level of Cadmium content (mg/Kg DW) in tattoo permanent ink samples.

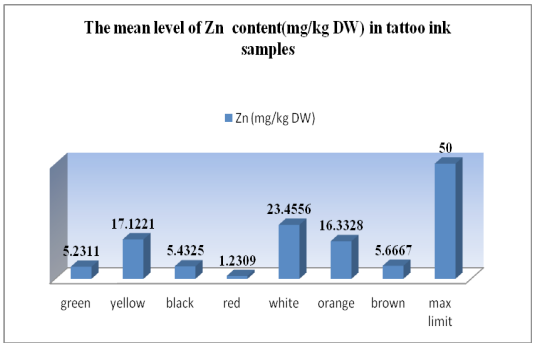

Fig. 3. The mean level of Zinc content (mg/Kg DW) in tattoo permanent ink samples.

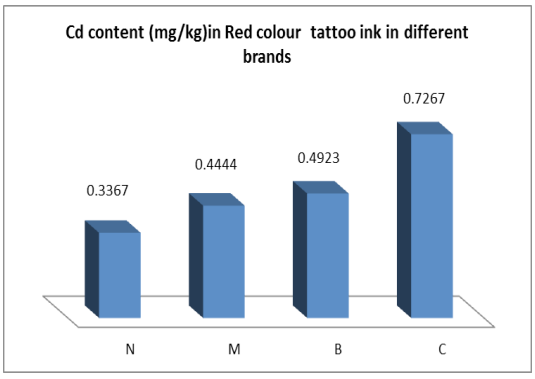

Fig. 4. Cadmium content (mg/kg DW) in red permanent make-up colour tattoo in 4 different studied brands.

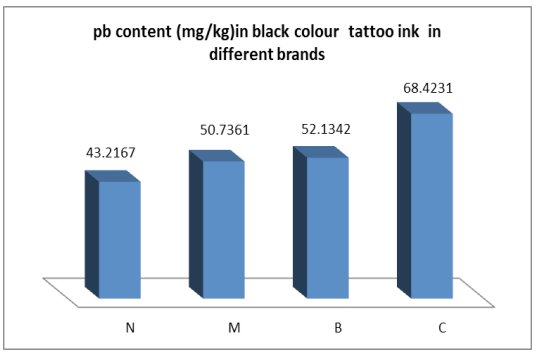

Fig. 5. Lead content (mg/kg DW) in black permanent make-up colour tattoo in 4 main different studied brands.

tattoo inks contributes to its heavy metal content. In Charm colour brand made in China(Code C in figure 5) , the mean contents of lead in dark black colour 68.4231 mg/kg, dark brown colour 56.3321 mg/kg and red colour 26.0455 mg/kg showed the higher lead content than 2 mg/kg which is established as the maximal lead limit by Environmental Protection Authority (EPA) approved a new standard, the *Tattoo and Permanent Make-up Substances Group Standard*[2,21] which means all samples except white colours would be leaded contaminated. Cadmium contents in all group colours in Chinese and USA brands (probably fake brands : Casmara and Biotouch, samples was higher than maximum limited 0.2 mg/kg set by EPA and the highest one related to white colour 2.1473 mg/kg. The concentration of cadmium shows significant differences of this heavy metal in the colour main groups ($p< 0.001$). All monitoring samples from other 7 main group (red, green, white, orange, yellow, brown and black had cadmium contamination and their concentrations were much higher than 0.2 mg/kg cadmium.

In figure 3, Zinc content in different brands and colours was compared regarding to the studied colours. White , yellow and orange ink samples showed the highest level of zinc content , while all samples had less content of this metal comparing by Maximum concentrations of zinc 50 mg/kg in tattoo and permanent make-up substances given in the EPA's *Guidelines* in 2012[21,30].

In figure 4, Cadmium contents in 4 brands were compared, the concentration of Cd shows significant differences of this heavy metal in the different studied brands ($p< 0.005$).

Code N, M ,B and C related to: Mom's Millennium Tattoo , Casmara, Biotouch and Charm Colour respectively.

In figure 5, Lead contents in black colour samples in 4 main studied brands were compared , The concentration of lead shows no significant differences of this heavy metal in the different studied brand samples.

DISCUSSION

Yellow-coloured inks commonly contain lead, cadmium and zinc[2]. Among the 8 colour-variants of yellow analysed, all samples exceeded cadmium and lead; while all yellows had zinc levels that meet the Guidelines. Lead interferes with a variety of body processes and is toxic to many of the body's organs and tissues, including the heart, bones, intestines, and kidneys, nervous and reproductive systems. In severe cases, lead poisoning symptoms can include seizures, coma and death. Other symptoms commonly associated with lead exposure include abdominal pain, confusion, headache, anemia and irritability[31,32,33].

Orange-coloured inks commonly contain cadmium. There were nine colour-variants of orange samples tested and 5 non-compliant metals were recorded. All exceeded the cadmium guideline value. Cadmium is a heavy metal that poses severe risks to human health, including kidney, bone, and pulmonary damage[34,35,36].

Estimating the safety assessment of cosmetic products such as tattoo inks is a complex issue. Our finding is similar to other last studies. In a Swedish report called"Farliga ämnen itatueringsfärger"20 various tattoo inks were tested for mineral elements. Some of the elements were demonstrated in concentrations exceeding the maximum permissible values .The colours were black, red, orange, violet, blue, green and yellow[37]. In a German study from 2009, carried out by Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (carried out in the light of the implementation of the regulation in the field of tattooing in Germany that came into effect on 1 May 2009), the metal content and preservative content was investigated in 148 commercial tattoo inks. The samples showed a large content of copper, iron, chrome and zinc and a smaller amount of tin, lead, manganese, selenium, arsenic, thallium, mercury and uranium[38]. In another American study carried out by FDA in 2004, 7 yellow tattoo inks from commercial suppliers were analysed. The monoazo pigment Yellow 74 appeared frequently. The pigment was subject to a light chemical change when exposed to light, including sunlight of photodecomposition with the creation of a number of new chemical substances[39]. In an Italian study from 2009, 56 samples of tattoo ink from 4 suppliers, including the colours called Starbrite, were analysed for content of metals[40]. An American study from 2001 of 29 tattoo inks from the supplier Huck Spaulding Enterprises Inc. and of China ink (carbon black) especially investigated the content

of inorganic elements by means of the radio diffraction method that determines all substances in the sample with an atomic weight above 11[41]. In a report from the Swiss Federal Office of Public Health (FOPH) from 2009, 152 tattoo ink samples were collected by inspectors from 55 tattooists and they were analysed and assessed with regard to compounds, preservation and microbiology, and only 36 % of the samples were acceptable according to the Swiss standard for compounds in tattoo inks while a large number were criticizable or subject to bans, most frequently due to content of non- permitted type or amount of pigment or preservative[42].

CONCLUSION

Most samples contain metals which are orders of magnitude greater than the Guidelines and may likely pose public health risk. Many people may not be aware that tattoo inks may pose harm from the chemicals from which they're made. Making the results publicly available may provide useful, though limited, information for tattoo artists when deciding to purchase tattoo inks. The public may also find the information useful, though limited, if they decide to request a tattoo.

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