

ORIGINAL ARTICLE

Hexavalent chromium in tattoo inks: Dermal exposure and systemic risk

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Background: The presence of hexavalent chromium (Cr(VI)), which is carcinogenic to humans and a dermal sensitizer, in tattoo inks may represent a serious health concern. The level of this impurity is limited to 0.2 mg/kg in tattoo inks by the European Resolution ResAP(2008)1.**Objectives:** To analyse 29 tattoo inks, produced in Europe and the United States, of different colours and brands, for Cr(VI) to assess their conformity with ResAP(2008)1 and to characterize dermal and systemic risks.**Methods:** Ion chromatography and inductively coupled plasma mass spectrometry were used to determine the levels of Cr(VI) in inks; risk characterization was performed by calculating the systemic exposure dosage (SED) and margin of safety (MoS).**Results:** Ninety per cent of inks contained Cr(VI) (range: 0.22–4.09 mg/kg), ie, above the maximum allowed level, and no information appeared on the label. More than 1 mg/kg Cr(VI) was detected in 27.6% of inks; these might represent a possible cause of dermal adverse reactions. Exposure to Cr(VI) in inks resulted in negligible SED values and MoS values of >100 (safety threshold), indicating no appreciable systemic risk.**Conclusions:** The minimization of Cr(VI) contamination and the use of technology compliant with good manufacturing practices is recommended to increase the safety of tattoo inks.

KEYWORDS

chromium, Cr(VI), exposure assessment, risk characterization, tattoo inks

1 | INTRODUCTION

Chromium (Cr) is a relatively common element, occurring naturally in rock, soil, plants, animals, and volcanic dust and gases. The most stable valence states are trivalent Cr (Cr(III)) and hexavalent Cr (Cr(VI)). Chromium is chiefly found as Cr(III) in nature, whereas Cr(VI) is generally produced by industrial processes.¹ Oxidation of Cr(III) to Cr(VI) in soils and water can also occur, depending on several factors.¹ Many Cr(VI) compounds were, historically, used in different types of paints, inks, and powder coatings, including chromate pigments in the form of PbCrO₄ and Pb₂OCrO₄ (yellow chrome and red chrome, respectively), BaCrO₄ (lemon chrome), and ZnCrO₄ (zinc yellow and zinc green). Other Cr(VI) compounds include the orange potassium dichromate (K₂Cr₂O₇), red potassium trichromate (K₂Cr₃O₁₀), and red chromium trioxide (CrO₃).² In addition, Cr(VI) was found to be present as an impurity of other classes of pigments or generated during the

manufacture of dyes and pigments even if it was not originally present in its hexavalent state.

From the 1980s, considerable evidence accumulated on cancer risks of Cr-exposed workers, leading to the classification of Cr(VI) compounds as human carcinogens (Category 1); Cr(III) compounds were not classifiable regarding their carcinogenicity to humans.³ In addition, Cr(VI) compounds were able to induce allergies at lower exposure levels than most Cr(III) compounds.⁴ Regarding regulatory issues, the only colorants allowed by the Cosmetic Regulation EC 1223/2009 (Annex IV) were those based on Cr(III) (yellow-green colours), namely the Cr oxide (Cr₂O₃) and Cr hydroxide (Cr₂O[OH]₄) free from chromate ions.⁵ Because low-level impurities are often present in cosmetic products, the Regulation (Article 17) tolerated Cr(VI) in technically unavoidable trace amounts only if these trace amounts are evaluated as safe in the final product. The German Federal Institute for Risk Assessment holds the position that 1 mg/kg

Cr(VI) in cosmetics could lead to contact dermatitis.⁶ Whereas consumers affected by cosmetics can easily remove them and avoid the allergen, tattooed individuals are unable to terminate the exposure immediately after the onset of allergic symptoms (redness, swelling, itching, inflammation, etc.).⁷ This difference leads to serious health concerns, and actually points to an inequality of treatment between tattoo inks and cosmetics.⁸ Another important problem is that pigments used in tattoo ink formulation are originally produced for other applications (such as textiles, dyes, car paints, and plastic pigments) because they have good light-fastness properties. However, they generally contain impurities such as Cr(VI) in Cr oxides, nickel, copper and cobalt in iron oxides, aromatic amines in azo-colorants, and polycyclic aromatic hydrocarbons in carbon black.^{9–11} A survey conducted on the Italian market showed a level of total Cr (tCr) exceeding the safe dermatological limit of 1 mg/kg in 62.5% of cases in 56 tattoo inks.¹² Clinical knowledge of tattooing complications is based on case reports. For example, considerable levels of tCr were determined in skin biopsies sampled from the tattooed areas of 3 patients reporting pseudolymphomas of the skin.¹³ However, the observation of dermal reactions, even if supplemented with the quantification of metals in the individual culprit inks, did not give clear evidence of metals being responsible for the allergenic reactions.¹⁴

In terms of health risks, some concern has been raised because of emerging evidence of the occurrence in tattoo inks of particles in the nanosize range (<100 nm).¹⁵ In February 2008, Resolution ResAP (2008)1 on the requirements and criteria for the safety of tattoos and permanent make-up was adopted by the Council of Europe; it recommended that member states adhere to a number of principles, and limit the levels of impurities in such products¹⁶. Concerning Cr(VI), the maximum allowed level as an impurity was set at 0.2 mg/kg. ResAP (2008)1 also recommended that the presence of Cr(VI) in tattoo inks should be mentioned on the package, together with a warning such as "Contains chromium. Can cause allergic reactions". The recommended limit applies to Cr(VI) and not to the tCr level, and this means that analytical methods should allow the speciation of Cr, that is, the separation of Cr(VI) and Cr(III), which does not always apply to information about the Cr levels in products. The speciation analysis of Cr is a challenging task that requires high-quality methodology, mainly with respect to the stability of Cr species (in particular, avoiding the conversion between Cr(VI) and Cr(III)) during sample extraction.¹⁷ At present, the lack of an analytical method to determine Cr(VI) in tattoo inks, at the very low level recommended by ResAP(2008)1, permits ResAP(2008)1 to be bypassed simply by a false declaration that is just not detectable.¹⁸ Because of this, a sensitive, specific and accurate analytical method was developed in this study to determine Cr(VI) levels in tattoo inks, based on a first alkaline extraction step followed by ion chromatographic (IC) separation and on-line detection by inductively coupled plasma mass spectrometry (ICP-MS). This analytical method was then applied to 29 commercially available tattoo inks of different colours and from different manufacturers, in order to test their compliance with the purity limits of ResAP(2008)1 and the dermal reaction threshold suggested by the literature.^{6,16} Moreover, based on a possible exposure scenario, the systemic health risk associated with the presence of Cr(VI) in tattoo inks was investigated by

calculating the systemic exposure dosage (SED) and the margin of safety (MoS).^{19,20}

2 | METHODS

2.1 | Samples and reagents

A total of 29 tattoo inks were purchased from 7 different producers, 4 from the United States and 3 from Europe: 7 "Starbrite Colors" inks; 8 "Intenze Prod." inks; 5 "Millennium Colorworks Inc." inks; 2 "Eternal" inks; 5 "Diabolo by Deep Colours" inks; 1 "Dermagol" ink; and 1 "Micky Sharpz" ink. All inks were liquids. Inks had different colours: blue (9); green (5); orange/red (4); violet (5); brown (2); black (2); grey (1); and white (1).

The alkaline extraction solution (AES) (pH ~ 13) used to extract Cr(VI) from samples was obtained by dissolving 0.28 M Na₂CO₃ and 0.5 M NaOH (Fluka, Buchs, Switzerland) in high-purity deionized water (resistivity of 18 MΩ cm, EASY-pure UV).²¹ The mobile phase used in the chromatographic separation was a buffer solution of 0.25 M (NH₄)₂SO₄ (Panreac Química, Castellar del Vallès, Spain) and 0.1 M NH₄OH (Fluka). Standard solutions of 1000 mg/L (HPS, Charleston, South Carolina) were used for Cr(III) and Cr(VI) quantification. For recovery testing, the certified reference material National Institute of Standards and Technology (NIST) SRM 2701 (NIST, Gaithersburg, Maryland) with a certified content of Cr(VI) (551.2 ± 34.5 mg/kg) in contaminated soils was used.

2.2 | Instrument configuration

The Thermo Fisher Scientific (Bremen, Germany) Dionex ICS-5000+ IC system equipped with the Dionex AG-7 (2 × 50 mm) and AS-7 (2 × 250 mm) anion exchange columns was used to separate the 2 Cr species. This system, because of its completely metal-free solvent pathway, is perfectly suitable for elemental speciation studies at trace levels. As the detector, a Thermo Fisher Scientific ICAP-Q ICP-MS instrument was used, equipped with a PFA concentric nebulizer and a quartz cyclonic spray chamber. Helium at 99.999% purity was used as the collision gas in the QCell collision cell. The QCell and the kinetic energy discrimination (KED) conditions were optimized for Cr by use of a solution containing the IC mobile phase as the blank and the same solution spiked with 1 ng/mL Cr as the optimization standard. With a flow rate of 1.0 mL/min on the peristaltic pump, the system was optimized for the lowest background equivalent concentration while maintaining suitable sensitivity for Cr. The settings used for the IC and ICP-MS systems are shown in Table 1. Fully automated operation of the IC was achieved with CHROMELION software (Thermo Fisher Scientific, version 7.1). All calculations, including background subtraction, peak area integration, and calibrations, were performed with Thermo Fisher Scientific QTEGRA software.

2.3 | Sample processing

The extraction procedure for Cr(VI) was conducted according to the method US EPA 3060A for Cr(VI) determination in soils, sludges, sediments, and waste materials, which has been slightly modified and already successfully applied for the determination of this species in cosmetics.^{21,22} Aliquots of ~0.5 g of inks were transferred into

TABLE 1 Ion chromatography (IC) and inductively coupled plasma mass spectrometry (ICP-MS) operating conditions

IC Dionex ICS-5000+	
Column and precolumn	Dionex AG7 (2 × 50 mm) and AS7 (2 × 250 mm)
Eluent	(NH ₄) ₂ SO ₄ (0.25 M) + NH ₄ OH (0.1 M)
Injection volume	25 µL
Eluent flow	0.4 mL/min
ICAP-Q ICP-MS Thermo Fisher Scientific	
Gas flow	Plasma, 15 L/min; aerosol, 1.05 L/min; auxiliary, 1.2 l/min
Radiofrequency power	1500 W
Cell gas flow	He 4.5 mL/min
Analytical mass	⁵² Cr
Dwell time	50 ms

250-mL glass flasks and, after addition of 25 mL of AES (0.28 M Na₂CO₃ + 0.5 M NaOH, pH ~ 13), were weighed. Subsequently, the solutions were heated, under stirring, at 90 to 95°C for 60 minutes. After the extraction process, the sample was cooled and weighed again, and, in the case of a weight loss, this was corrected by adding AES. The sample was then centrifuged at 2700g for 20 minutes, and the supernatant solution was filtered through a poly(vinylidene difluoride) membrane filter (0.45 µm) and analysed. The same procedure was also performed on samples used for recovery tests. The tCr level in tattoo inks was quantified by ICP-MS after acid-assisted microwave digestion of aliquots of tattoo inks, with a previously published method.¹²

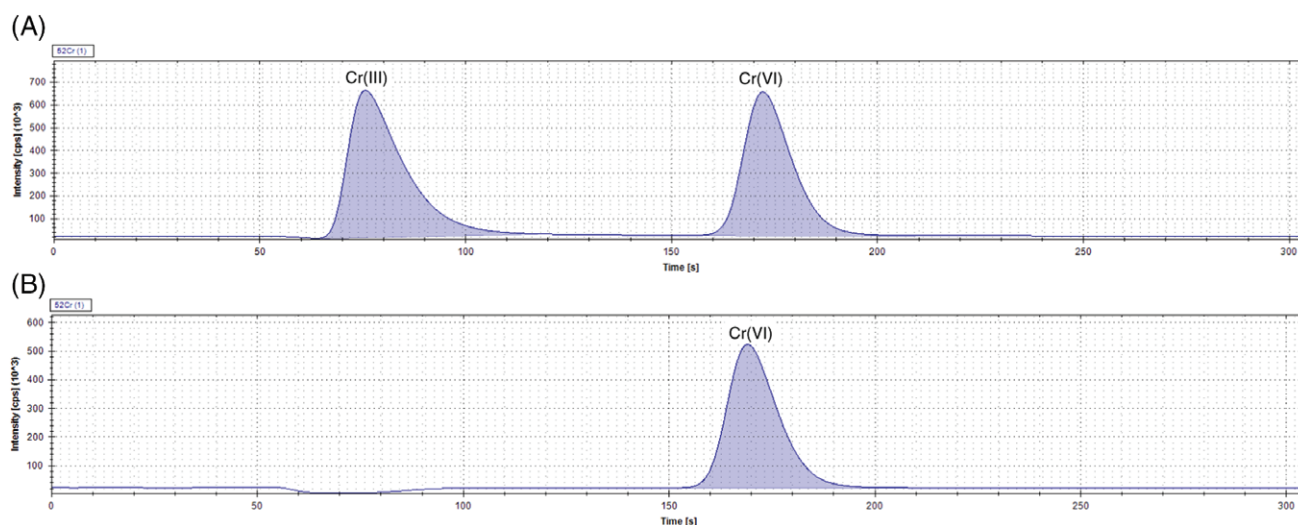
3 | RESULTS

3.1 | Method performances

Chromatographic separations on the ICS-5000 were carried out with the parameters summarized in Table 1. For elution of the Cr species, anion exchange chromatography was used with (NH₄)₂SO₄ (0.25 M) + NH₄OH (0.1 M) as the mobile phase at a constant flow rate of 0.4 mL/min. Figure 1A shows the separation of Cr(III) and Cr(VI) in

water, and Figure 1B shows the same separation in AES; the absence of Cr(III) in AES confirmed that, in the presence of a strong alkaline environment, Cr(III) remained in the solid phase.¹⁷ The accuracy of the EPA 3060A method was evaluated by analysing 2 different recovery samples produced in the laboratory. The first sample was prepared by adding the soil-based NIST SRM 2701 with a certified content of Cr(VI) to a face powder cosmetic sample. The preparation of this mixture was performed through subsequent dilutions and homogenizations in a ball mill of tungsten carbide Vibratory Micro Mill Pulverisette (Fritsch, Idar-Oberstein, Germany). The final mixture, containing 11.94 mg/kg Cr(VI) and ~1000 mg/kg tCr, was used to calculate the method recovery for powder cosmetics and the potential interconversion between Cr(III) and Cr(VI) species. The second test sample was prepared by adding a standard solution of Cr(VI) to an ink sample before the alkaline extraction; the final mixture, containing 10 mg/kg Cr(VI), was used to evaluate the method Cr(VI) recovery for tattoo inks. The results are shown in Table 2. The excellent recovery data (99%) for both test samples showed that Cr(VI) remained stable during the extraction, and also that the tCr (also including Cr(III)) contained in the mixture did not influence the recovery of Cr(VI). Moreover, in order to further investigate the interconversion between Cr species, a tattoo ink sample (containing Cr(VI) at 0.30 mg/kg) was added to 10 mg/kg Cr(III) and subjected to the alkaline extraction procedure; the level of Cr(VI), after Cr(III) spiking, remained unchanged (0.27 mg/kg), showing that Cr(III) was not oxidized during the procedure.

Finally, another extraction method using phosphate buffer at pH 8.0 (ISO 17075 method for Cr(VI) determination in leather) was applied, in the framework of an intercomparison trial on cosmetics, to further confirm the validity of EPA 3060A extraction at pH 13. The 2 methods gave similar Cr(VI) levels on 2 independent cosmetic samples (±5% of difference). Table 2 also shows that the EPA 3060A method had a linear detection response in the interval range of 1 to 100 ng/mL Cr(VI) added to face powder and tattoo ink extraction solutions. The intraday repeatability as the coefficient of variation of 10 injections of the same extracted sample of face powder and tattoo

**FIGURE 1** (A), 10 µg/L Cr(III) and Cr(VI) in water. (B), 10 µg/L Cr(III) and Cr(VI) in alkaline extraction solution

ink was 8% to 9% (Table 2). The limits of detection (LoDs), calculated according to the 3 sigma criterion, were 0.03 mg/kg and 0.02 mg/kg Cr(VI) in face powder and tattoo ink, respectively (Table 2). All of these results showed that polyatomic interferences such as $^{40}\text{Ar}^{12}\text{C}^+$, $^{35}\text{Cl}^{16}\text{O}^{1}\text{H}^+$ and $^{35}\text{Cl}^{17}\text{O}^+$ that may disturb the measurement of ^{52}Cr were eliminated by using helium in the collision cell and KED conditions of the ICAP-Q ICP-MS, while a high detection sensitivity was maintained.

3.2 | Content and risk evaluation of tattoo ink samples

The level of tCr as obtained by complete digestion of samples, Cr(VI) levels after the alkaline extraction of this species, and the percentage of Cr(VI) relative to tCr, determined in 29 tattoo inks, are shown in Table 3. The samples analysed contained tCr in the range 0.22 to 4.72 mg/kg and Cr(VI) in the range 0.16 to 4.09 mg/kg; percentages of Cr(VI) relative to tCr ranged from 15% to 99%. The results for tCr and Cr(VI) levels separated for colour shades are shown in Figure 2 by means of bars representing mean $\pm$ SDs. The tCr and Cr(VI) levels found in US products as compared with those in EU inks are shown in Figure 3 by means of box-plots representing median and interquartile range. The tCr and Cr(VI) levels were not statistically different with respect to the origin of samples (Mann-Whitney test *P* values of .31 and .22, respectively). In Figure 4, Cr(VI) levels in tattoo inks are compared with the ResAP(2008)1 limit of 0.2 mg/kg and the dermal sensitization limit of 1 mg/kg. The Cr(VI) SED and MoS results calculated for each ink are shown in Table 4.

4 | DISCUSSION

4.1 | Exposure to Cr(VI) in tattoo inks

The safety of tattoo inks has increased in Europe since the implementation of ResAP(2008)1, which resulted in improved quality control of pigment raw materials, owing to the definition of impurity limits that manufacturers should refer to. However, inks used for tattooing were not developed or produced for applications involving a long-term

presence in the dermis or contact with bodily fluids. Therefore, some inks do not comply with the purity limits of ResAP(2008)1, even though it states that every distributed tattoo ink should not contain Cr(VI) above the limit.¹⁰ In addition, the limit for Cr(VI) requires that it is linked to a clearly defined analytical method, whereas present recommendations for Cr(VI) in tattoo inks do not mention any analytical method. Failing to provide an appropriate method may lead to invalid and questionable results, which often cause legal disputes that are ultimately resolved by a judge with regard to the method that should have been applied.²³ Moreover, the lack of standardized methods for Cr(VI) identification in tattoo inks leaves space to bypass ResAP(2008) 1 by simply reporting non-detectable values for this species.

As an example, in a report of the New Zealand Ministry of Health, 100% of 169 tattoo inks were below the LoD for Cr(VI) and were declared to be compliant with the ResAP(2008)1 limit.²⁴ In Australia, control activities undertaken in 2014 to 2015 showed that the majority of tattoo inks (40/49; 82%) contained tCr at a level of up to 23 mg/kg, but Cr(VI) was not detected in any of the tattoo inks.²⁵ It needs to be considered that these studies used the US EPA Method 218.7, which deals with IC and postcolumn derivatization and ultraviolet-visible Cr(VI) detection in drinking water samples, and that LoD values in ink matrix were not reported.

In the current study, direct determination by the online IC-ICP-MS coupling permitted complete separation of Cr(III) and Cr(VI) in <200 seconds (Figure 1), linearity between 1 and 100 ng/mL (Figure 2), a recovery rate of 99%, and, more importantly, an LoD value in the matrix (0.02 mg/kg) that was 10 times lower than the ResAP(2008)1 regulatory limit of 0.2 mg/kg (Table 2). All inks showed levels of Cr(VI) above the LoD value, the median level of Cr(VI) was 1.50 mg/kg, and the median level of tCr was 0.60 mg/kg. The data and their SDs showed great variation, which might reflect the different colours, brands, and conditions of pigment production (Table 3 and Figures 2 and 3). Analysis showed that Cr(VI) represented the 45% (as median percentage) of the tCr in inks. Relatively low Cr(VI) median levels were found in blue (0.32 mg/kg) and green (0.68 mg/kg) inks, and higher levels were found in red-orange (1.70 mg/kg) and violet (1.33 mg/kg) inks (Figure 2). Cr(VI) was previously reported as a contaminant of Cr(III)-based pigments, for example, the Cr_2O_3 and $\text{Cr}_2\text{O}(\text{OH})_4$ pigments used for green. Nevertheless, our data showed that green inks contained less Cr(VI) than inks of other colours (such as red and violet), so the presence of Cr(VI) can be only partly ascribed to contamination of Cr(III)-based pigments. Otherwise, the presence of Cr(VI) might be attributable to contamination of organic pigments or to uncontrolled release of Cr(VI) during pigment production because of work practices and environmental conditions.^{26,27} The results of patch testing of 90 cases with a selection of tattoo ink stock products showed only 9 individuals with positive reactions, mainly associated with red inks and violet inks; however, the responsible haptens for the allergenic reaction did not include metals, but possibly other compounds, such as products of photochemical cleavage of red azo pigment.²⁸ Another study on 493 tattoo complications linked the complications originating from tattoo inks more often to organic pigments.²⁹

The present study also showed that, even though the difference was not statistically significant, a lower median level of Cr(VI) in samples

TABLE 2 Method performances for Cr(VI) determination

Cosmetics	
Recovery (%) ^a	99.0
Linearity 1-100 µg/L (<i>R</i> ²)	0.9996
LoD (mg/kg)	0.03
Intraday repeatability (CV%)	9.2
Tattoo inks	
Recovery (%) ^b	99.1
Linearity 1-100 µg/L (<i>R</i> ²)	1.00
LoD (mg/kg)	0.02
Intraday repeatability (CV%)	8.1

Abbreviations: CV%, coefficient of variation; LoD, limit of detection.

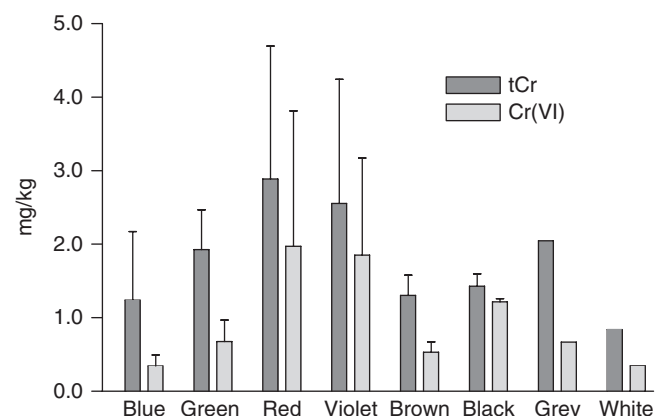
^a Mixture of NIST SRM 2701 and face powder containing 11.94 µg/g of Cr(VI).

^b Mixture of Cr(VI) standard solution and tattoo ink containing 10 µg/g of Cr(VI).

TABLE 3 Total chromium (tCr) and Cr(VI) levels in tattoo inks

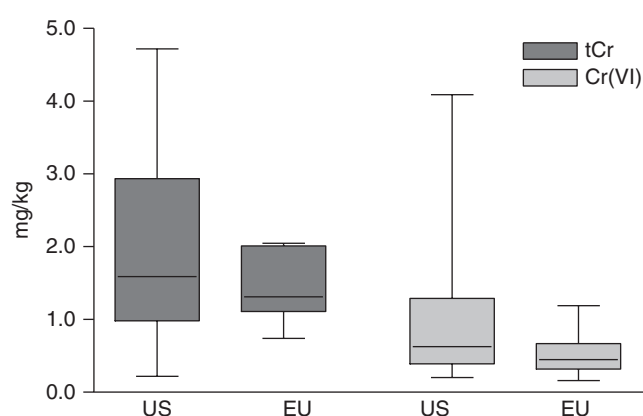
Product	Producer	Country	tCr (mg/kg)	Cr(VI) (mg/kg)	Cr(VI)/tCr (%)
Deep blue	Starbrite	USA	1.37	0.22	16
Country blue	Starbrite	USA	0.52	0.30	58
Brite Orange	Starbrite	USA	4.72	2.94	62
Red scarlet	Starbrite	USA	1.07	0.46	43
Deep violet	Starbrite	USA	4.05	2.38	59
Deep Tourquoise	Starbrite	USA	1.81	0.38	21
Lime green	Starbrite	USA	1.79	0.42	23
Green grasshopper	Intenze	USA	2.53	1.15	45
True magenta	Intenze	USA	4.67	3.91	84
Baby blue	Intenze	USA	0.64	0.60	94
Violet grape	Intenze	USA	1.46	1.33	91
Cherry bomb	Intenze	USA	4.14	4.09	99
True black	Intenze	USA	1.55	1.25	81
Acquamarine	Intenze	USA	0.22	0.20	91
Hunter green	Intenze	USA	2.22	0.68	31
Ice blue	Millenium	USA	3.34	0.52	16
Violent violet	Millenium	USA	1.71	0.98	57
Doo doo Brown	Millenium	USA	1.50	0.43	28
Mean green	Millenium	USA	1.09	0.68	63
Power white	Millenium	USA	0.84	0.35	41
Light purple	Eternal	USA	0.89	0.65	73
Crimson red	Eternal	USA	1.63	0.40	25
Blue turquoise	Diabolo	German	1.40	0.40	28
Grey Grey	Diabolo	German	2.05	0.67	33
Basic green	Diabolo	German	2.01	0.45	23
Ultramarine	Diabolo	German	1.11	0.16	15
Black	Zuppe	German	1.31	1.19	91
Blue	Dermagol	UK	0.74	0.32	43
Chocolate Brown	Micky Sharpz	UK	1.11	0.63	57
All inks					
Median			1.50	0.60	45
Minimum-maximum			0.22-4.72	0.16-4.09	15-99

produced in the EU (0.45 mg/kg) than in those produced in the United States (1.31 mg/kg) (Figure 3). This could possibly be linked to the absence of a specific regulation for the production of tattoo inks in the United States. There may be an issue with products from non-European countries where good manufacturing practices are not mandatory.⁹

**FIGURE 2** Total chromium (tCr) and Cr(VI) in different tattoo inks

Recently, an increase in the frequency of adverse reactions has led the US Food and Drug Administration to promote investigation into tattoo inks and their safety, and the development of regulations.³⁰

Regarding compliance with ResAP(2008)1, only 3 blue inks showed Cr(VI) levels of ≤ 0.2 mg/kg; all of the other inks (or 90%)

**FIGURE 3** Total chromium (tCr) and Cr(VI) in US and EU tattoo inks

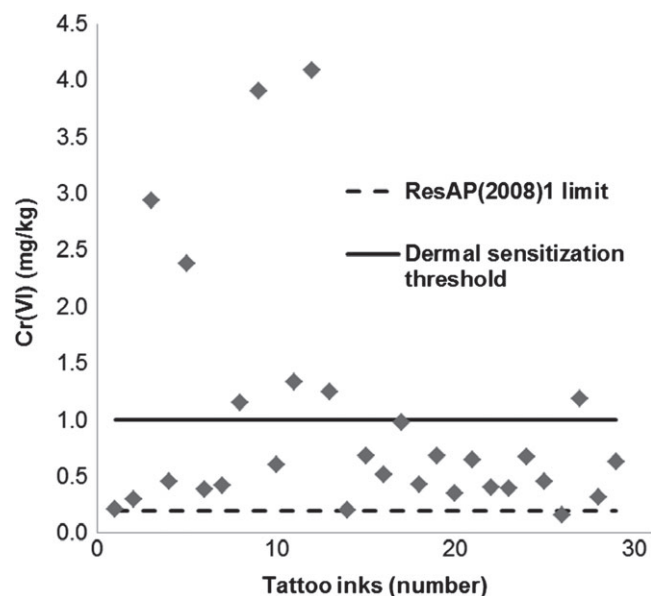


FIGURE 4 Cr(VI) in tattoo inks as compared with safe limits

showed Cr(VI) impurities ranging from 0.22 to 4.09 mg/kg, which is higher than the recommended maximum level (Figure 4). Another observation was that none of the ink bottles had labelling indicating the presence of traces of Cr(VI), as recommended by ResAP(2008)1. According to the literature, Cr(VI) is also found in other consumer goods. Of the 5 cosmetics tested and available for purchase on the Italian market, 2 eye shadows had Cr(VI) levels of 0.7 and 9.8 mg/kg.²¹ A survey of Cr(VI) in 22 eye shadows and 6 colouring agents showed a level of 97.6 mg/l in 1 of the colouring agents, and in a level of >5 mg/L 2 of the eye shadows.³¹ Recently, Cr(VI) levels of ~0.3 mg/kg were found in pearly pigment and pearly powder eye shadow samples.³²

4.2 | Dermal and systemic risk assessment

With a level of Cr(VI) above the threshold value of 0.2 mg/kg, the tattoo inks analysed are not recommended for tattooing (Figure 4). This is an alarming fact, considering that, in the EU, all Cr(VI)-based pigments are prohibited from use in cosmetics, even though these are only applied to the skin surface. In tattooing, hundreds of milligrams are injected into the skin, and health concerns would appear to be much greater. Even though the biotransformation of Cr(VI) to Cr(III) in the body reduces the toxicity after Cr(VI) oral and inhalation intake, dermal exposure to high quantities of Cr(VI) by tattooing could represent a problem. In fact, Cr(VI) has high skin permeability, and is transported into cells mainly as chromate through active anion channels; once it has entered the cells, it is easily converted to highly reactive species, including both unstable, namely, Cr(V) and Cr(IV), and stable, namely, Cr(III), species, which interact with critical intracellular components.³³ According to the Cosmetic Regulation,⁵ if metal impurities are present in cosmetics, it is mandatory to show that the cosmetic product containing these impurities does not pose a threat to consumer health. In addition, on the basis of the Commission Decision 2010/15, non-compliance with limit values does not automatically mean that the product presents a risk for health, so a risk assessment is required.³⁴

TABLE 4 Systemic exposure dosage (SED) and margin of safety (MoS) calculations based on Cr(VI) levels in tattoo inks

Product	SED (mg/kg BW/d)	MoS (NOAEL/SED)
Deep blue	1.67×10^{-7}	49 615
Country blue	2.32×10^{-7}	35 722
Brite Orange	2.28×10^{-6}	3645
Red scarlet	3.54×10^{-7}	23 450
Deep violet	1.85×10^{-6}	4497
Deep Tourquoise	2.96×10^{-7}	28 054
Lime green	3.25×10^{-7}	25 577
Green grasshopper	8.90×10^{-7}	9327
True magenta	3.03×10^{-6}	2742
Baby blue	4.65×10^{-7}	17 832
Violet grape	1.03×10^{-6}	8040
Cherry bomb	3.17×10^{-6}	2620
True black	9.68×10^{-7}	8573
Acquamarine	1.55×10^{-7}	53 584
Hunter green	5.27×10^{-7}	15 760
Ice blue	4.03×10^{-7}	20 609
Violent violet	7.61×10^{-7}	10 913
Doo doo Brown	3.31×10^{-7}	25 098
Mean green	5.27×10^{-7}	15 737
Power white	2.70×10^{-7}	30 795
Light purple	5.03×10^{-7}	16 487
Crimson red	3.10×10^{-7}	26 792
Blue turquoise	3.07×10^{-7}	27 062
Grey Grey	5.20×10^{-7}	15 948
Basic green	3.51×10^{-7}	23 657
Ultramarine	1.26×10^{-7}	65 747
Black	9.22×10^{-7}	9006
Blue	2.48×10^{-7}	33 490
Chocolate Brown	4.88×10^{-7}	17 011
All inks		
Median	4.65×10^{-7}	17 832
Minimum-maximum	1.26×10^{-7} to 3.17×10^{-6}	2620-65 747

Abbreviations: BW, body weight; d, day; NOAEL, no observed adverse effects level.

SED was calculated by the use of: ink use = 2.5 mg/cm²; area = 900 cm²; absorption = 100%; period = 42 days; BW = 70 kg.

To this end, a risk assessment (dermal and systemic) of tattoo inks with respect to their Cr(VI) levels was performed in this study. First, for the evaluation of dermal risks, data were compared with the acceptable dermal exposure regarding the induction and elicitation of allergic contact dermatitis. It was suggested that, for products that came into contact with the skin, such as liquid detergents and cosmetics, a Cr(VI) level of 1 mg/kg would pose essentially no sensitization risk.^{6,35} The results of this study showed that 21 of 29 tattoo inks (or 72.4%) contained Cr(VI) levels of ≤1 mg/kg; therefore, for the majority of samples, the likelihood of the induction of sensitization may be very low (Figure 4). However, for a number of inks (27.6%), the Cr(VI) level (in the range 1.19–4.09 mg/kg) (Figure 4) could represent a possible cause of skin reactions, especially if a consumer has already been sensitized to Cr(VI) from other sources.³⁶

Regarding Cr(III), the literature reports patients having positive reactions at ~6 mg/kg in 10% of cases and at ~89 mg/kg in 50% of cases; these higher levels of Cr(III) compounds needed to elicit an allergic reaction have been attributed to lower skin permeation of the trivalent form than of the hexavalent form.³⁷ In this study, even if we assume that the difference between tCr and Cr(VI) is attributable to Cr(III) only, the levels found in tattoo inks (median, 0.76 mg/kg; maximum, 2.82 mg/kg) were much lower than the limit (6 mg/kg) that is able to elicit a positive dermal reaction. On the other hand, the Cr(III) minimum elicitation thresholds were derived from studies on intact skin and for only 48 hours of patch test exposure. In comparison, in the case of tattoos, Cr(III), present originally or derived from Cr(VI), is already inside the skin, where it remains for a very long time. Cr(III) ions then bind to cellular proteins to form Cr-protein complexes, which are responsible for the allergic reactions. Hence, from a molecular perspective, Cr(III) could be as interesting as Cr(VI), if these species are already inside the skin, as in the case of injection of pigments.

In addition, in order to evaluate the systemic risks of tattoo inks, an exposure scenario must be developed and some assumptions need to be made regarding exposure, namely, the normal and foreseeable use of inks, the tattooed area, the period of exposure, and the normal and foreseeable absorption of inks. Values that are currently considered to be acceptable have been previously described: an average normal level of pigment used for tattooing of 2.53 mg/cm²; a tattooed surface area of 30 × 30 cm (900 cm²); absorbance of the substance over a period of 6 weeks (or 42 days); and a worst-case absorption rate of 100%.^{19,38} By the use of these parameters and a default human body weight (BW) of 70 kg,³⁹ the SED was calculated as the amount of tattoo ink/kg BW/d, with the following equation: SED = (concentration_{Cr(VI)} × ink use × area × absorption)/BW × period. Then, an MoS based on the no observed adverse effects level (NOAEL), which is the highest dose level without any adverse effects, was calculated to characterize the risk as the NOAEL/SED ratio.²⁰ The NOAEL for Cr(VI) systemic effects of 2.5 mg/kg BW/d in rats exposed to 2.5 mg of potassium chromate in drinking water for 1 year was used as a starting point, to which an uncertainty factor of 100 was applied for the intraspecies and interspecies variability to account for the difference in susceptibility between animals and humans; moreover, an additional factor of 3 was applied to compensate for the less-than-lifetime exposure duration in the animal study.⁴⁰ The Cr(VI) SED and MoS results calculated for each ink are shown in Table 4. The SED of Cr(VI) resulting from the use of the tattoo inks ranged from 1.26×10^{-7} to 3.17×10^{-6} mg/kg BW/d for a 100% absorption scenario, as the worst case. The MoS values for Cr(VI) occurring in all tattoo inks ranged from 2620 to 65 747. Because an MoS of 100 has been considered acceptable for the safe use of products,^{20,41} the values calculated, always above 100, are not expected to increase the risk to consumers. The data obtained were also compared with the acceptable daily intake (ADI) for Cr(VI) equal to 8.3×10^{-3} mg/kg BW, which is the dose that can be assumed to be consumed daily by an adult of 60 kg for the person's lifetime without any appreciable risk to health. The daily exposure to Cr(VI) from tattoo inks represented 0.002% to 0.038% of the ADI. This very low percentage and the great difference between SED values and the ADI value for Cr(VI) confirmed that the use of tattoo inks can be regarded as safe regarding systemic

risks, even if products exceeded the concentration limit of 0.2 mg/kg reported by ResAP(2008)1. Notwithstanding this, our data indicated that the ResAP(2008)1 threshold is a challenge, as tattoo ink manufacturers still did not achieve Cr(VI) levels lower than threshold in the final products.

5 | CONCLUSIONS

The sensitive and accurate analytical method developed in this study can be routinely applied to determine the level of Cr(VI) in tattoo inks; this is an important result, considering that ResAP(2008)1 did not specify the analytical method to be used. The present results showed that 90% of tattoo inks exceeded the ResAP(2008)1 maximum allowable level (0.2 mg/kg) and 27.6% exceeded the dermal sensitization threshold of 1 mg/kg recognized in the literature. These data suggest that the specific European regulatory provisions for Cr(VI) in tattoo inks are insufficient, particularly for this type of product, characterized by extended exposure of the dermis. An absence of any indication on the labels concerning Cr(VI) levels was also observed; complete labeling of tattoo inks would play an important role in informing the consumer that certain tattoo inks may contain Cr(VI) as an allergen; this information could contribute to reducing possible dermal complications. Moreover, although the present risk characterization showed MoS values higher than the safety threshold and a negligible risk of systemic adverse effects, considering the sensitization potential, manufacturers must implement measures to further reduce the amounts of Cr(VI) present as impurities in the final products.

Conflict of interest

The authors have no potential conflict of interest to disclose.

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