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Analytical survey of tattoo inks—A chemical and legal perspective with focus on sensitizing substances

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Abstract

Background: Tattoo inks have been reported to elicit allergic contact dermatitis.

Objectives: To investigate the labels and the contents of metals and pigments in tattoo inks, considering restrictions within the European Union.

Methods: Seventy-three tattoo inks currently available on the market, either bought or donated (already used), were investigated for trace metals and pigments by inductively coupled plasma mass spectrometry and by matrix-assisted laser desorption/ionization time of flight tandem mass spectrometry.

Results: Ninety-three percent of the bought tattoo inks violated European, legal requirements on labeling. Fifty percent of the tattoo inks declared at least one pigment ingredient incorrectly. Sixty-one percent of the inks contained pigments of concern, especially red inks. Iron, aluminium, titanium, and copper (most in green/blue inks) were the main metals detected in the inks. The level of metal impurities exceeded current restriction limits in only a few cases. Total chromium (0.35-139 μg/g) and nickel (0.1-41 μg/g) were found in almost all samples. The levels of iron, chromium, manganese, cobalt, nickel, zinc, lead, and arsenic were found to covary significantly.

Conclusions: To prevent contact allergy and toxic reactions among users it is important for tattoo ink manufacturers to follow the regulations and decrease nickel and chromium impurities.

KEYWORDS
allergic contact dermatitis, hazardous substances, metals, regulation, tattoo inks

1 | INTRODUCTION

Tattooing is done by injecting colored inks under/into the dermis layer of the skin to leave a permanent design. The inks consist of pigments and auxiliary compounds, such as solvents, binders, and pH regulators.[1-3] Tattoo art has been an increasing fashion phenomenon globally, and already involves 12% of Europeans and up to 30% of United States' citizens, in particular in young generations.[3-6] In parallel, tattoo removal is becoming more frequent. Tattoo inks might contain sensitizing/hazardous substances that may cause adverse health effects linked to the application and removal of tattoos, and a certain proportion of the ink could be transported within the body via the blood.[5,7] These effects include acute allergy directly after tattooing or delayed hypersensitivity after long-term exposure to the chemicals in the inks.[4,6-10] As an example, about 70% of 3411 tattooed individuals reported skin problems immediately or a few weeks after tattooing.[10] Skin cancer risks from tattooing have been neither proved nor excluded.[4,11] Sensitizing substances might induce allergic contact dermatitis (type IV hypersensitivity), an inflammatory skin reaction caused by direct contact with these substances.[12] A patch test is a clinical diagnostic standard method for type IV hypersensitivity, aiming to identify an allergen in an allergic patient by applying the diluted substance under occlusion on the skin under standardized conditions.[12] It can be used to detect specific allergies in a patient with an allergic reaction to a tattoo. A patch test study on 90 patients with a selection of tattoo ink stock products revealed only nine individuals with positive reactions, mainly associated with red inks.[13] This suggests that many culprit allergens in tattoo inks are neither not yet known nor included in baseline and specialized tattoo ink patch test series.[13]

The pigments used in tattoo inks are produced mainly for large-scale applications in construction or cosmetics industries, not specifically for use in injecting into the skin, and they generally show low purity (70%-90%).[3,4,14] Metals are often used in different substances as dyes or pigments, either in inorganic pigments, such as metal oxides, or in metal–organic complexes. Tattoo inks have been confirmed to contain harmful impurities that are known or suspected to cause adverse effects in humans, such as hexavalent chromium (Cr^{VI}) in Cr oxides; nickel (Ni), copper (Cu), and cobalt (Co) in iron (Fe) oxides; aromatic amines in azo-colorants; and polycyclic aromatic hydrocarbons in carbon black.[4,14-16]

Considering the increasing popularity of tattooing and the possible presence of harmful substances in the products used for tattoos, there is a need for rules to limit the risks posed by unsuitable tattoo inks. In 2003, the Council of Europe (CoE) published a resolution (revised in 2008, ResAP) on the requirements and criteria for the safety of tattoos and permanent makeup (PMU),[1,17] regarding the labeling of packages, prohibition of some harmful pigments, limits for the maximum concentration of certain impurities, and a safety assessment by the manufacturer. Followed by the CoE ResAPs (either of 2003 or 2008), seven Member States have developed their national legislation with rather minor deviations from the resolutions.[4] The Swedish Medical Product Agency has published a regulation on tattoo inks in 2012, covering product directory, labeling, product information, and importation and usage of tattoo inks.[18] A report of the Joint Research Center (JRC) of the European Commission (EC), compiled by experts from research and risk assessment, aimed to set a legislative framework to protect consumer safety.[4] Based on the evidence provided by the JRC of the presence of tattoo inks on the European market not complying with the limits set by the CoE, the European Chemicals Agency (ECHA) submitted in 2019 a restriction proposal on substances used in tattoo inks and PMU to the Committees for Risk Assessment (RAC) and Socio-economic Analysis (SEAC) for their evaluation.[14] Finally, a legal requirement for substances in tattoo inks or PMU at the EU-wide level was published on December 14, 2020, and will come into force on January 5, 2022 due to a transition period.[19]

Several relatively recent studies have reported the occurrence and potential risks posed by hazardous chemicals in tattoo inks. Bocca et al.[15] found that Cr^{VI} in tattoo inks could be a possible cause of dermal adverse reactions, and 90% of the investigated inks contained Cr^{VI} above the maximum allowed level (0.2 μg/g), but no information appeared on the label. An investigation on a set of tattoo inks with various shades[16] showed that the concentrations of Cr, Cu, and lead (Pb) were above (5- to 500-fold), the maximum allowed levels regulated in ResAP(2008)1. In another published market study in Italy, several toxic elements, such as cadmium (Cd), antimony (Sb), Pb, vanadium (V), and manganese (Mn), exceeded 1 μg/g in some cases.[20] In the same study, the sensitizing metals Cr, Ni, and Co were above the safe limit in 62.5%, 16.1%, and 1.8% of the studied 56 tattoo inks, respectively. The presence of the prohibited pigments and the prevailing pigments behind chronic allergic reactions (Pigments Red 22, Red 210, and Red 170) were revealed in several studies on tattoo inks, by different analytical techniques such as Raman spectroscopy and mass spectrometry.[9,16,21] According to a previous report compiled by the Swedish Chemicals Agency in 2010, only 5 of 31 analyzed tattoo inks in various shades were free of hazardous substances, and the others contained aromatic amines (classified as carcinogenic, mutagenic, and allergenic) and different metals at levels above the recommended limits. In a Swiss study (2009), 41% of the samples had nonpermitted chemical contents.[5]

This study aimed at assessing potential hazards with tattoo inks, and how those are related to concomitant content of substances/impurities, to labeling, to color, and to brand. This study increases knowledge about which substances are relevant to include in a patch test when testing a patient with an allergic reaction to a tattoo. In this study, a total of 73 tattoo inks known to be used in Sweden and many other countries, were either collected from a store and a tattoo studio in Sweden or ordered online. These samples were investigated on their contents of metals and pigments, and whether their labeling fulfilled legal requirements. Matrix-assisted laser desorption/ionization time of flight tandem mass spectrometry (MALDI-ToF-MS^n) was used for identification of organic pigments and inductively coupled plasma mass spectroscopy (ICPMS) for the quantification of metal present in the tattoo inks.

2 | EXPERIMENTAL

2.1 | Collection and preparation of tattoo inks

A total of 73 tattoo inks were supplied from different places: samples 1-29 from Killer Ink (online, https://www.killerinktattoo.se/), samples 30-36 from East Street AB (store in Sweden), samples 37-56 from Wish (online, www.wish.com), and samples 57-73 from a tattoo studio in Sweden. Details on shade (name of color or shade on bottle), colors (white, yellow, orange, red, pink, green, blue, purple, gray, black, as well as brown [only in sample 63], confirmed by four different persons), and brands are listed in Table S1, Appendix S1. These investigated tattoo inks were manufactured by a range of top brands, including World Famous Tattoo Ink (abbreviated as "WF"), Intenze Advanced Tattoo Ink ("In"), Radiant Colour ("RC"), Fusion Tattoo Ink ("Fu"), Eternal Ink ("Et"), Solid Ink ("So"), Dynamic ("Dy"), Tang Dragon Tattoo ("TD"), and Kuro Sumi Colours ("KS"). Samples 1-56 were bought between March 2019 and January 2020, and samples 57-73 were old or previously opened samples kindly provided by a tattoo studio. The latter samples were excluded from some evaluations, as their selling date might be prior to some legal requirements, and their previous opening could have caused evaporation, resulting in higher concentrations of substances. The label information on each tattoo ink bottle was inspected to investigate compliance with the requirements set in ResAP(2008)1[1] published by CoE. Correct label reading was confirmed by two persons.

2.2 | Chemicals

Acetonitrile (ACN, Sigma-Aldrich, St Louis, MS, USA), trifluoroacetic acid (TFA, Sigma-Aldrich), sinapinic acid (SA, Bruker Daltonik, Bremen, Germany), and ethanol (95%, Solveco, Rosersberg, Sweden) were the chemicals used for the MALDI-TOF-MS^n analysis. The calibration was based on a peptide calibration standard (covering mass range: 1000-3200 Da, Bruker Daltonik). Isopropanol (Sigma-Aldrich) and deionized water were used for cleaning the target plate.

For ICPMS analysis, nitric acid (HNO_3, ≥65%, Chem-Lab NV, Zedelgem, Belgium), hydrochloric acid (HCl, 25%, Merck, Darmstadt, Germany), and phosphate-buffered saline (PBS, 8.77 g/L NaCl, 1.28 g/L Na_2HPO_4, 1.36 g/L KH_2PO_4, of analytical grade and from VWR, Sweden, adjusted with 50% NaOH to pH 7.2-7.4) were used (standards for quantification described in Section 2.3.2).

The ultrapure water (Millipore) used in both the MALDI-ToF-MS^n and the ICPMS had a specific resistivity of 18.2 MΩcm at 25°C.

2.3 | Mass spectrometry analysis

2.3.1 | MALDI-ToF-MS^n

Seventy-three tattoo inks were analyzed by means of MALDI-ToF-MS^n to identify the pigments present in the samples. The samples were first vortexed to obtain a homogenous solution, and then 1 part sample was diluted with 9 parts of ethanol. Those samples that were found to contain polyethylene glycol (PEG), were first washed by adding water, vortexed, and their supernatant was removed after centrifugation (9500 g for 5 minutes, Heraeus Biofuge Pico, Hanau, Germany). The washing step was repeated three times, and the final sample was dissolved in ethanol. The dissolved samples were deposited directly onto the ground steel target plate (MTP 384, Bruker Daltonik) in four replicates, by adding 2 × 0.5 μL of sample at each target spot to minimize the spread. To ensure the ionization of all pigments, 0.5 μL of the samples was also deposited on a dried layer of 0.5 μL saturated SA matrix dissolved in two parts ACN and one part 0.1% TFA in water. The spots were dried at room temperature and ambient pressure.

For the MALDI-TOF analysis, an ultrafleXtreme MALDI TOF/TOF with a smartbeam-II laser operating at a wavelength of 355 nm (Bruker Daltonik), controlled by FlexControl software (Bruker Daltonik), was used. The samples were analyzed in positive mode in the mass to charge (*m/z*) range 20 to 3500 with no matrix suppression activated. The acceleration potential was set to +25 kV, with pulsed ion extraction at 130 ns. The method was calibrated using the monoisotopic masses in Bruker peptide calibration I, and the method was recalibrated every second sample spot. The MALDI-TOF spectra were the results of 500 laser shots collected to a total of 5000 laser shots, with partial sample random walk activated at 10 shots at raster spot. Analysis of the spectra was performed with FlexAnalysis software (Bruker Daltonik) and the spectra were processed using the centroid peak detection algorithm with a signal-to-noise (S/N) threshold of 2.

For tandem mass spectrometry (MS/MS or MS^2) analysis, the parent ions were chosen based on the intensity, S/N, and the *m/z* value, and the peaks with intensities >5000, S/N > 50 and *m/z* > 200 were selected. Argon (5.0 Lab line, Strandmøllen, Sweden) was used as collision gas at 3.5 bar with a detector gain boost of 150% and laser power boost of 90%. The spectra for the fragment ions were collected with 1000 laser shots collected to a total of 10 000 laser shots. The database searches were performed using MS Search v2.3 (NIST, Gaithersburg, MD, USA) with databases obtained from Dr. Ines Schreiver.[9] The database searches were performed with the same parameters for all samples.

To identify which pigments were present in each sample using MS, a corresponding peak for the pigment had to be found in the mass spectrum and the isotope pattern. For MS/MS identification, a probability score higher than 70% had to be obtained from the database search.

The target plate was washed with deionized water and liquid detergent and wiped gently with Kimwipes (Kimberly-Clark, Irving, TX, USA) until all visible pigments were removed, and washed extensively with deionized water to remove the detergent. The target plate was wiped with isopropanol before sonicating the plate in ultra-pure water for 15 minutes. Thereafter, isopropanol was used to wipe the target plate twice, and the plate was sonicated in isopropanol for 15 minutes. The target plate was then placed in an oven at 250°C for 3 hours.

2.3.2 | ICPMS

Quantitative analysis of both total (through microwave assisted digestion with concentrated HNO_3) and water-soluble (extracted in 0.9% NaCl, see below) trace metals in tattoo ink samples was conducted with ICPMS.

For total trace metals, the tattoo ink samples were digested using an Ultraclave IV microwave digestion system (MLS GmbH, Leutkirch, Germany). A total of 0.1 g of the tattoo ink was weighed into 10 mL quartz vessels, and 4.5 mL of sub-boiled concentrated HNO_3 was added into the vessel before closing it. The vessels were then placed in the autoclave with a pressure of 4×10^6 Pa of argon (grade 5.0, Messer, Austria). More details on the autoclave settings can be found in Table S2 in Appendix S1. After the samples were cooled down, the solutions were transferred into 50 mL tubes, and HCl and ultrapure water were added into the tubes to obtain a final concentration of 9% HNO_3 and 1% HCl in the solutions. The blank samples containing PBS were also diluted with ultrapure water (9% HNO_3 and 1% HCl) at a ratio of $1 + 9$. White precipitates were observed in many samples, suggesting nonsoluble (under these conditions) titanium dioxide.

For the extraction of the water-soluble metals from the tattoo inks, an aliquot of the tattoo inks (0.5 g) was mixed with 10 mL 0.9% NaCl and extracted in a shaking water bath at 37°C for 12 hours. After extraction and cooling down, the samples were centrifuged at 30 000 g. Afterwards, the supernatant was diluted 10 times with a solution of 1% HNO_3 and 0.1% HCl. A precipitate was observed after the addition of acids, so the samples were centrifuged again. Some samples had to be additionally filtered because the precipitates could not be removed.

The total and water-soluble metal concentrations were determined with an Agilent 7700x ICPMS (Agilent Technologies, Waldbronn, Germany). The instrument was equipped with a Micro Mist nebulizer (Glass Expansion, Melbourne, Australia), a Scott type double pass spray chamber, a 2.5 mm ID quartz torch, a sample cone made from Cu with a Ni tip and a Ni skimmer cone. A dilution gas was used to improve the measurements. An external calibration solution for V (V @ *m/z* 51), Cr (Cr @ *m/z* 52), Mn (Mn @ *m/z* 55), Co (Co @ *m/z* 59), Ni (Ni @ *m/z* 60), zinc (Zn @ *m/z* 66), gallium (Ga @ *m/z* 71), arsenic (As @ *m/z* 75), strontium (Sr @ *m/z* 88), molybdenum (Mo @ *m/z* 98), palladium (Pd @ *m/z* 105), silver (Ag @ *m/z* 107), cadmium (Cd @ *m/z* 114), tin (Sn @ *m/z* 118), antimony (Sb @ *m/z* 121), barium (Ba @ *m/z* 137), tungsten (W @ *m/z* 182), gold (Au @ *m/z* 197), mercury (Hg @ *m/z* 201), thallium (Tl @ *m/z* 205), lead (Pb @ *m/z* 208), bismuth (Bi @ *m/z* 209), thorium (Th @ *m/z* 232), and uranium (U @ *m/z* 238) was prepared, respectively, in the ranges of 0.01-100 μg/kg. For aluminum (Al), Fe, and Cu, the calibration solutions were prepared with a higher range, 0.1-10 mg/kg, due to higher sample concentrations. The calibration standards were prepared from single-element standards (1000 mg/kg) gravimetrically. Note that titanium (Ti) was not analyzed because it cannot be digested with the employed acid digestion method. All reported data were calculated based on the mean value of three different sample preparations sample with the respective blank sample concentration subtracted. Samples 57, 58, and 66 were not analyzed, since they were completely dried out.

2.4 | Statistical analysis

Jeffrey's Amazing Statistics Program (JASP, v. 0.14.1.0),[22] a multi-platform open-source statistics package, was used to determine if and how strongly different metal contents in tattoo inks are associated. Under JASP, classical correlation analysis was conducted with the inputted total and water-soluble metal raw data, respectively. The statistics relationship between two metals was expressed as Pearson's correlation coefficient ("r"), a value ranging from -1.0 (negative correlation) to $+1.0$ (positive correlation). The closer r is to 1, the more closely the two variables are related, where <0.1 is trivial, 0.1-0.3 a small effect, 0.3-0.5 a moderate effect, and >0.5 a large effect. In the cases of classical analyses, we used *P*-values as indicators for significance marked with asterisks (**P* < .05; ***P* < .01; ****P* < .001).

Other statistical analyses between two independent sets of samples were conducted with KaleidaGraph (v. 4.0) using an unpaired Student's *t* test with unequal variance and unpaired data.

Box plots can display the variation in samples of a statistical population, and in this study, they were used to show differences in soluble or total metal contents among different sample groups. In these graphs, each box represents 50% of the data, with the median value of the variable displayed as a line. The lines extending from the top and bottom of each box mark the minimum and maximum values within the data set that fall within the range R. Any values outside of this range are displayed as individual points. The range R is defined in Eqn. 1:

$$\mathrm{LQ} - 1.5 \times \mathrm{IQD} < \mathrm{R} < \mathrm{UQ} + 1.5 \times \mathrm{IQD} \tag{1}$$

where LQ is the lower quartile—the data value located halfway between the median and the smallest data value; IQD is the interquartile distance—the distance between the upper and lower quartiles (UQ – LQ); and UQ is the upper quartile—the data value located halfway between the median and the largest data value.

3 | RESULTS

3.1 | Inspection on label information

According to the instructions and requirements for labeling tattoos regulated in ResAP(2008)1 by the Council of Europe (CoE),[1] the name

TABLE 1 Marking on packaging labels in tattoo ink products, containing the listed six information groups according to regulations and requirements set in Resolution ResAP(2008)1,[1] with a summary of the percentage within each group

Sample ID	Name and address of the manufacturer	Date of minimum durability/period of maximum durability after opening	Guarantee of sterility	Batch number	Conditions of use and warnings	Correctly labeled ingredients
Sample 1-7 (WF)	Yes	Yes	Yes	No	Yes	3 of 7: Yes
Sample 8-17 (In)	No	Yes	Yes	Yes	Yes	9 of 10: Yes
Sample 18 (RC)	Yes	Yes	Yes	No	Yes	Yes
Sample 19-28 (Fu)	Yes	Yes	Yes	8 out of 10: Yes	Yes	3 of 10: Yes
Sample 29-33 (Et)	3 of 5: Yes	Yes	4 of 5: Yes	No	Yes	Yes
Sample 34,35 (So)	Yes	Yes	Yes	Yes	Yes	1 of 2: Yes
Sample 36 (Dy)	No	No	No	No	Yes	Yes
Sample 37-56 (TD)	No	No	No	No	Yes	5 of 20: Yes
Summary						
Percentage(meeting the regulation)	41% (23 of 56)	63% (35 of 56)	61% (34 of 56)	36% (20 of 56)	100% (56 of 56)	50% (28 of 56)

and address of the manufacturer, date of minimum durability, guarantee of sterility, batch number, conditions of use and warnings, and a list of ingredients need to be labeled on tattoo ink packages. Fifty-six tattoo ink samples (Samples 1-56 listed in Table S1, Appendix S1) were inspected, since the other samples might have been older than the regulation. The results are summarized in Table 1, with samples grouped based on the brands. A large majority (93%) of the investigated samples violated the requirements and criteria in the resolution, and only three samples from "Fu" and one from "So" were free of any violations. Among the tested samples, only 23 samples (41%) had the name and address of the manufacturer on the label. Samples 1-35 (63%) had a description about the maximum durability after opening. The "Et" samples had two different dates, 6 months and 365 days, which probably reflects the transition from older to newer requirements (older samples had more often 6 months duration on the label). Sixty-one percent of the samples marked the guarantee of sterility. Information on the batch number was found only in 36% inks. Although all investigated samples had a list of ingredients, only half of them had the correct labeling according to the detected ingredients in this work (see Sections 3.2 and 3.3). We could prove incorrect ingredients' labeling for 15 of 20 "TD" samples. For 5 of 20 "TD" samples, we could not disprove the correctness of the ingredients list; however, it would be impossible to make different colors with only a white and a black pigment, which were the only labeled pigments. All manufacturers had marks with conditions of use and warnings, even though they had a large variety of descriptions (shown in Table S3, Appendix S1). (See Discussion section.)

The degree of violation of labeling requirements varied among the brands (Table 1). None of the investigated manufacturers fully complied with the label requirements published by CoE. Samples 36-56 (from "Dy" and "TD") showed a larger deviation from the label requirements as compared with the other brands. The labels were exactly the same for all "TD" samples of different colors, including the list of ingredients. The labels stated that the product contained a "pure organic pigment," but the only pigments listed were two inorganic pigments (the white pigment TiO_2, CI77891 and the black pigment carbon, CI77266). Thus the labels on the bottles associated with samples 37-56 were considered completely unreliable.

3.2 | Identification of the pigments in tattoo inks

The pigments used in tattoo ink samples were analyzed both with and without MALDI matrix, since the matrix could result in interfering peaks in the lower *m/z* region. Detection without matrix was possible because the pigments were able to absorb laser energy. Table S4 (Appendix S1) shows a comparison between the labeled pigments in the ingredient list of the tattoo ink samples and the pigments detected with MALDI-TOF-MS and MS/MS. The mass spectra for all samples are also shown in Figure S1 (Appendix S1). For the 72 analyzed samples (sample 57 was completely dried out and not included in the analysis), 179 pigments were declared on their ingredient list. However, three of the pigments cannot be detected with MALDI-TOF-MS (marked as † in Table S4): Carbon Black (CI77266) due to the low mass and Pigment Red 101/102 (CI77491) and Pigment White 6 (CI77891) due to the lack of ionization sites. In addition, three of the declared pigments were not included in the library at the time of analysis (marked as ‡): Disperse Red 220 (CI12476), Pigment Red 269 (CI12466), and Reactive Orange 16 (CI17757). In total, 61 pigments were detected with both MS and MS/MS, whereas 23 (37.7%) of the pigments were declared. Other pigments were detected only with either MS or MS[2] and not included in the statistical analysis (below).

Polyethylene glycol (or PEG) was detected in 27 (37.5%) tattoo ink samples (Table S4), which none of the tattoo ink samples had declared. PEG is a common contamination in MALDI-TOF, and to confirm the presence of PEG in the tattoo ink samples, freshly prepared samples were analyzed. If the mass spectra contained PEG in both sets (with and without matrix), the sample was considered containing PEG.

Among the detected pigments, Pigment Red 22 (CI12315) (marked with "§" in Table S4, Appendix S1) is currently restricted (0.1% concentration limit) under an EU regulation for substances in tattoo inks or permanent make-up (published on December 14, 2020).[19] Pigment Red 170 (CI12475) is self-notified as skin sensitizing by companies (marked with "*"), although there is no harmonized classification in EU of this substance.[23] Both Pigment Red 22 and Pigment Red 170 were recently identified as the prevailing pigments behind chronic allergic reactions in tattoo inks.[9] Banning of another two pigments, Pigment Blue 15 (CI74160) and Pigment Green 7 (CI74260), is being discussed, but the ban is not in force because of the lack of safer and adequate alternatives for tattooing (marked with "#"). However, Pigment Blue 15 is banned for use in hair dyes, and Pigment Green 7 banned for use in hair dyes and eye products. Three detected pigments (Pigment Violet 23 – CI51319, Pigment Red 122 – CI73915, and Pigment Violet 19 – CI73900), marked with "+" in Table S4, are only allowed in rinse-off products by the European regulation (EC) No. 1223/2009 for cosmetic products,[24] but tattoo inks are no rinse-off products. For assessments of any violation of legal requirements, only samples 1-56 and pigments, which were detected by both MS and MS,[2] were considered. There were 34 tested inks (61%) containing pigments that may cause skin sensitization and other adverse effects. Unlabeled pigments were found in 28 samples (50%). Of the 10 different tattoo ink color groups, four colors (white, yellow, orange, and black) did not contain any of these potentially hazardous or nonsuitable pigments (Figure 1). The restricted Pigment Red 22, under current EU regulation for substances in tattoo inks or PMU, was only found in red inks.

3.3 | Quantification of metals in tattoo inks

The inorganic pigment CI77891 (TiO_2) was declared in many labels. Ti was not analyzed in this study; however, its presence was confirmed by the white precipitates observed after the digestion of the samples. Cu originates from the phthalocyanine pigment group (starting with CI74, Pigment Blue 15, and Pigments Green 7 and 36) and was found mainly in green, blue, purple, and gray inks. Copper ion is the central atom in the structures of these pigments and is firmly bonded to the base structure.[25] Fe originates mainly from the pigment CI77491 (Red Iron Oxide/Pigments Red 101/102).[9,26] Mo and W can be included in xanthene pigments (CI45170:2, Pigment Violet 1).[26] Of all the collected samples, one purple ink (sample 46) showed elevated levels of both Mo and W, and one blue ink (sample 32) showed only high Mo level. Other metals are unintended impurities. An EU-wide regulation published in 2020[19] has required a maximum concentration for many impurities in tattoos and PMU, as well as the resolution ResAP (2008)1.[1]

The total amount (μg/g) of selected detected metals (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Pd, Cd, Sn, Sb, Ba, W, Hg, Pb, and Bi) in the tattoo inks by means of ICPMS is summarized in Figure 2. Samples 57, 58, and 66 were excluded because they were partially dried out. Metals found in larger quantities (0.3 μg/g - 270 mg/g) were Fe, Al, and Cu. Fe showed the highest concentrations (4.39 μg/g - 270 mg/g) in some inks but its use or concentration is not restricted. Fe oxides have been approved as coloring agents in cosmetics[24] and food.[27] More hazardous metals (such as Cd, Pb, and Mn) and strongly sensitizing elements (such as Ni and Cr) were present in relatively lower amounts (shown in Table S5, Appendix S1). Hg, Sb, and Co were only above the detection limits in a few cases. Figure 3 shows the total or

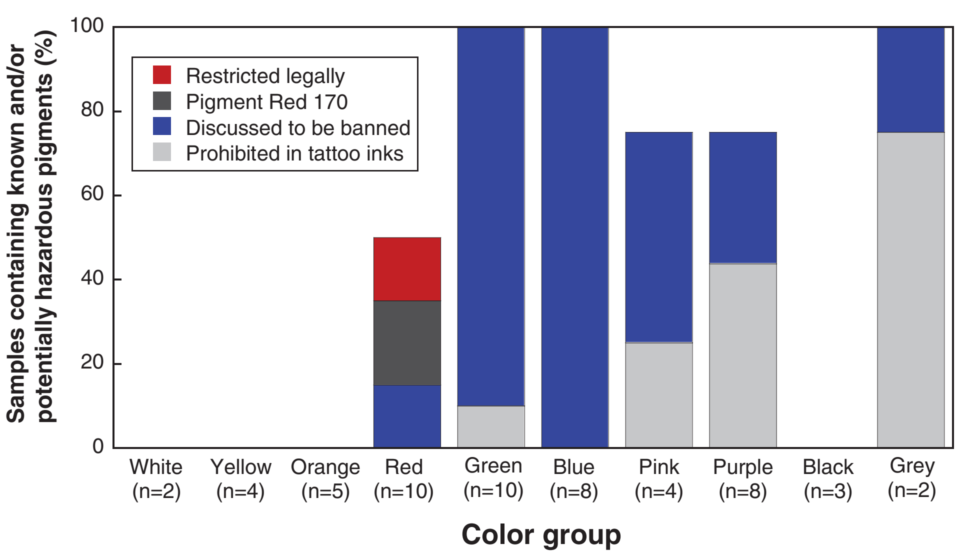

FIGURE 1 The proportion of samples containing restricted and/or potentially harmful pigments in different color groups: Pigment Red 22 (restricted, shown as red), Pigment Red 170 (self-notified sensitizing substance, shown as black), Pigment Blue 15 and Pigment Green 7 (discussed to be banned in tattoo inks, shown as blue), Pigment Violet 23, Pigment Red 122, and Pigment Violet 19 (only allowed in rinse-off products, shown as gray). Based on samples 1-56. n – the number of samples in each group

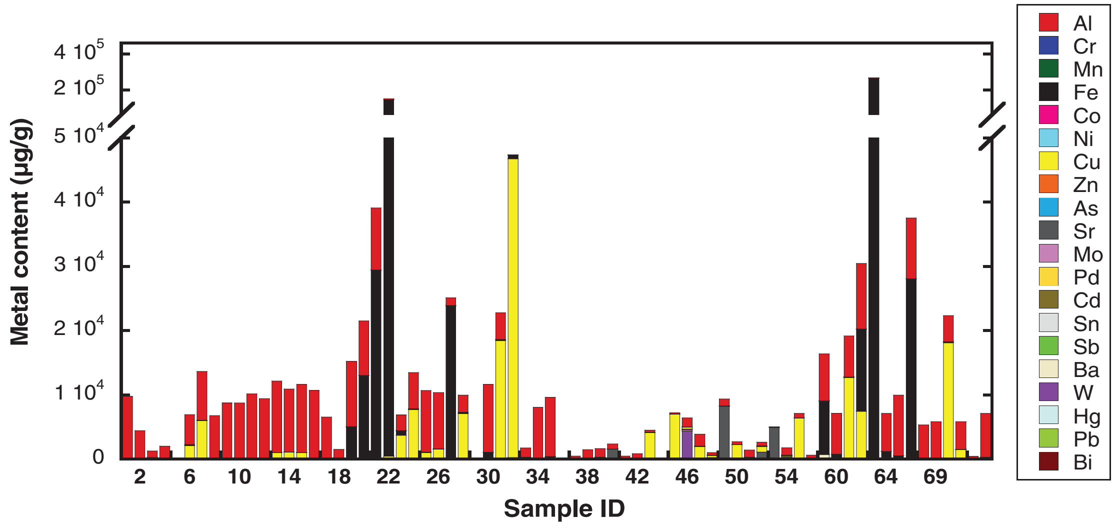

FIGURE 2 The total mean concentration (μg/g) of selected metals (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Pd, Cd, Sn, Sb, Ba, W, Hg, Pb, and Bi) in each sample by means of ICPMS. Three dried out samples (57, 58, and 66) were excluded

soluble (for Cu) concentrations (μg/g) of nine metals in 56 tattoo inks in comparison to the maximum allowable concentrations under the newly released EU regulation[19] and regulated in the resolution ResAP (2008)1.[1] Metals of Cd (0.0014-0.093 μg/g), Sb (0.00067-0.37 μg/g), and Zn (0.57-47.3 μg/g) were in all cases found below both restricted concentration limits. All samples also fulfilled the allowed limits for As (2 μg/g), Sn (50 μg/g), and Co (25 μg/g) regulated by ResAP(2008)1, but a few slightly exceeded the stricter limits under the EU regulation. The metals Hg (0.004-1.6 μg/g) and Pb (0.023-5.35 μg/g) were found at levels above both the EU regulated and CoE recommended limits in a few inks. All samples showed soluble Ba far below the limit (500 μg/g) regulated under EU, but total Ba (0.051-166 μg/g) was found above the CoE's limit (50 μg/g) in a few inks. Although the metals mentioned above are known as skin sensitizers and/or hazardous substances after short- or long-term human exposure,[28] very few samples exceeded the restricted amounts of these impurities. Cr (0.35-139 μg/g) was found in almost all samples. However, this study did not determine the Cr speciation. It is therefore not possible to judge whether the maximum allowed concentration level of 0.5 μg/g Cr^{VI} as defined by the EU regulation was exceeded. The restriction defined for Ni is 5 μg/g, and three inks were found to exceed it. However, the resolution ResAP(2008)1 recommended "as low as technically achievable" for Ni.[1] All tattoo inks contained quantifiable levels of Ni (0.1-41 μg/g). Although certain pigments containing no Ni could be found on the market, this is not true for all pigments, for example, inorganic Fe oxides pigments.[25] Both Cr and Ni are considered sensitizing elements, and to minimize potential health risk for sensitive individuals, it is recommended that its levels should not exceed 1 μg/g.[16,29,30] According to the newly released EU regulation, the presence of Cr^{VI} and Ni in tattoo products should be mentioned on the package together with a warning. Traces of Ni and Cr were mentioned on the labels for samples 31-35 (Ni) and 34-35 (Cr). These samples contained 0.3-8.0 μg/g Ni and 1.7-2.7 μg/g Cr. One of the investigated samples contained soluble Cu level higher than the maximum limit (200 μg/g), and three samples showed higher soluble Cu level (25 μg/g - 47 mg/g) than the CoE's recommended limit (25 μg/g).

The total metal content (μg/g) of Cu, Cr, Ni, Pb, and Ba is shown for the different brands investigated in this study in Figure 4. The highest median levels of Cu, Cr, and Ni were all observed in "Fu" inks (only statistically significant for Cu compared with "So" brand). Cr contents were statistically significantly greater in the "WF" brand as compared with "In" and "TD." The Ni contents in "In" were statistically significantly greater compared with the "TD" brand. Otherwise, there was no statistically significant difference in these metal contents among the brands. We also found a clear difference in Sr content, with higher levels in the "TD" (0.4 μg/g - 8.0 mg/g) and "KS" (1.8-275 μg/g) brands as compared to all other brands (0.2-12.5 μg/g), although this metal impurity is not regulated.

Figure 5 shows the total metal concentrations (μg/g) of Cu, Cr, Ni, Pb, and Ba in all investigated tattoo inks in different color groups. High concentrations of Cu were significantly ($P < .05$ or .01) more present in green (143 μg/g - 7.7 mg/g) and blue (214 μg/g - 47 mg/g) colors, compared to all other colors (except brown for one sample). White tattoo inks contained lower amounts of most metals (Ti not tested), which was statistically significant for Cu and Cr contents compared to blue and green colors. Gray colors contained higher amounts of Ni and Ba when compared to yellow and blue colors, respectively (Figure 5).

Correlation relationships between all different total metal contents (27 analyzed metals) were investigated in the 70 studied tattoo inks measured by ICPMS by means of statistical analysis. Most metals did not have any statistically significant correlation ($P < .05$) but those with significant correlation are summarized in Table 2. The amounts of Cr, Mn, Fe, Co, Ni, Cu, and Zn had moderate to strong positive correlations (r > 0.3) with other elements. Both Cr and Co were strongly related to the other metals ($r > 0.5$, $P < .01$), with the exception of a moderate correlation between Cr and Zn (r = 0.401). The correlation

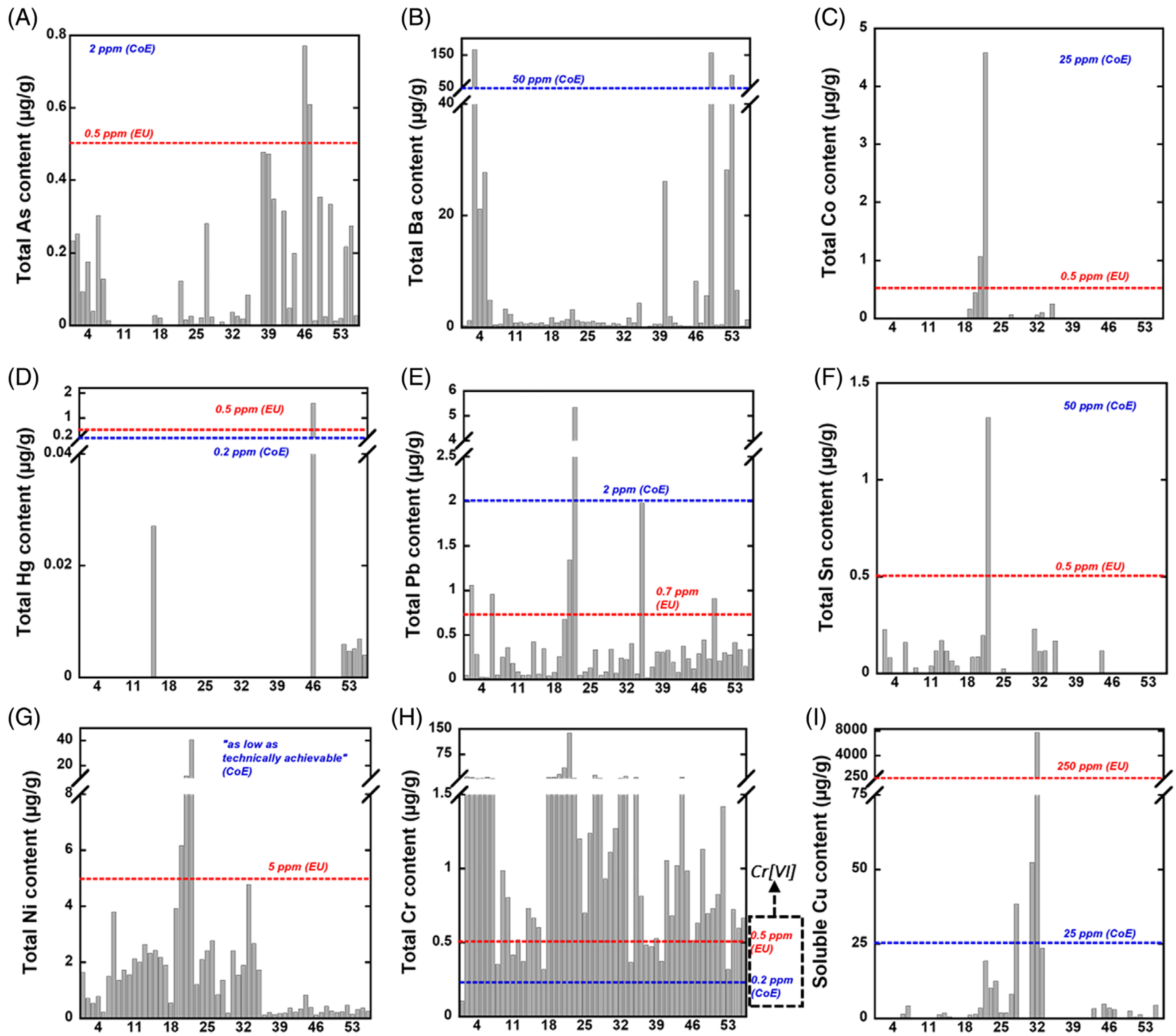

FIGURE 3 In samples 1-56, total metal concentrations (μg/g) of As (**A**), Ba (**B**), Co (**C**), Hg (**D**), Pb (**E**), Sn (**F**), Ni (**G**), and Cr (**H**), and water-soluble concentration (μg/g) of Cu (**I**), obtained by means of ICPMS. Corresponding concentration limits stipulated by the EU regulation[19] (as red dotted line) and in the ResAP(2008)1[1] (as blue dotted line). Mean value of triplicate measurements for each sample. Corresponding data in Table S5 (Appendix S1)

between Cr, Mn, Co, and Ni showed in all cases a large r value close to 1 (r > 0.9) with a highly significant correlation ($P < .001$). This means that if a tattoo ink contains Cr, it most likely also contains Mn, Co, and Ni. Fe, which is of special interest due to its high content in the tattoo inks (Figure 2) and common presence in pigments, had a positive, statistically significant correlation with Cr, Co, Ni, Mn, Zn, As, and Pb. Cu, the other common and pigment-included element, had only a positive, statistically significant, correlation with Mo. The impurities Mn, Co, Zn, As, and Pb were strongly correlated with several metals.

The CoE ResAP(2008)1,[1] recommends a maximum concentration of 25 μg/g soluble Cu in tattoo inks, but this concentration limit is increased to 250 μg/g by ECHA.[19] ECHA justifies its proposal in that soluble substances are not expected to accumulate in the organism but are excreted quickly (within a few weeks).[14] The new limit was exceeded by only one blue-colored sample from the brand "Et" (7760 μg/g) among samples 1-56 (Figure 3(I)). Two other samples (38 and 52 μg/g) exceeded the lower limit set by CoE. Similar to findings of total Cu concentrations in the tattoo inks (Figures 4 and 5), high water-soluble concentrations of Cu were mainly present in "Fu" and "Et" inks and in blue and green inks (Figure 6(A) and (B)). A correlation analysis performed using JASP confirmed a very clear positive correlation between total and water-soluble Cu in tattoo inks (r = 0.87, $P < .001$). The water-soluble Cu content was 2-2000 times lower than the total Cu content.

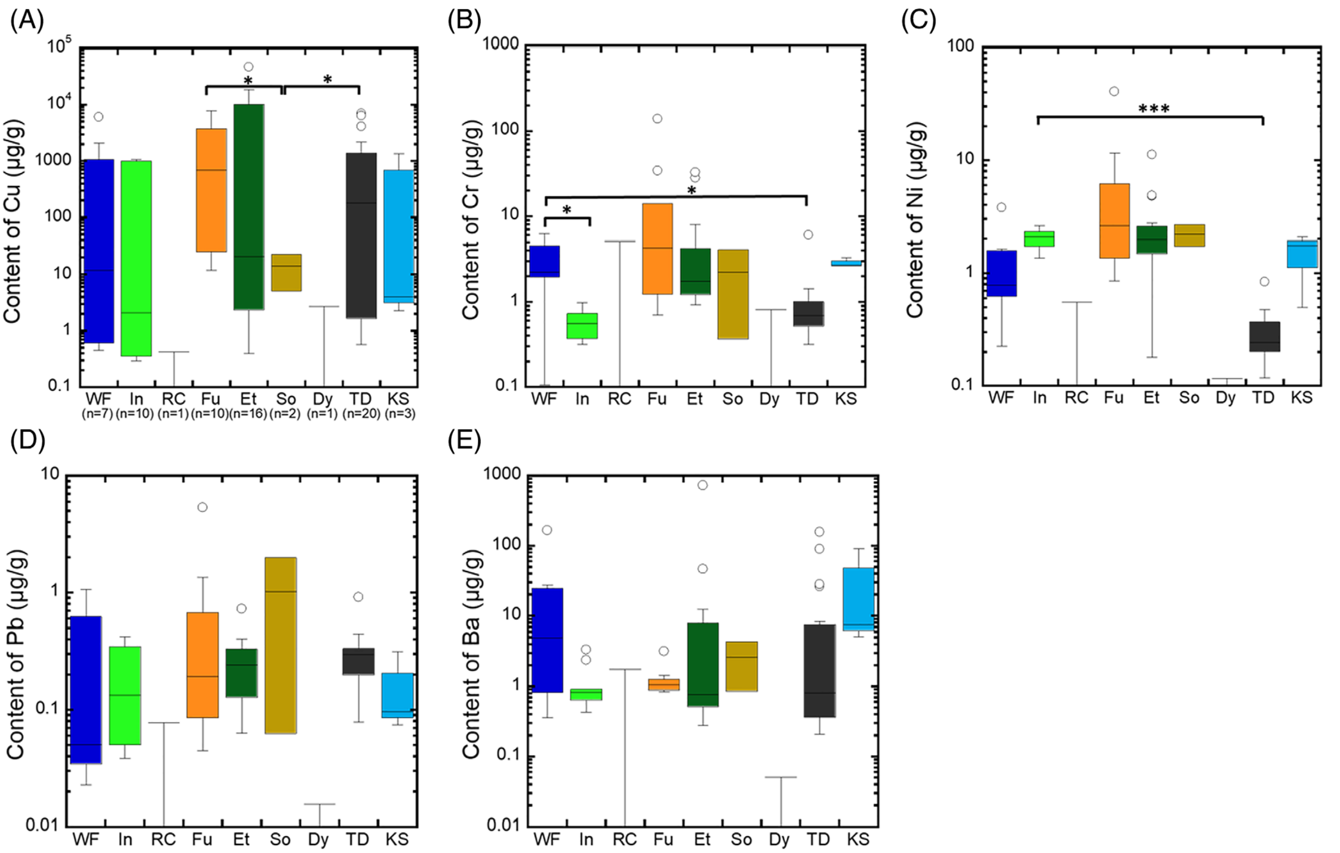

FIGURE 4 Box plots of total metal content (μg/g) of Cu (**A**), Cr (**B**), Ni (**C**), Pb (**D**), and Ba (**E**) as a function of brand for all investigated samples measured by means of ICPMS (70 samples). n – the number of samples in each group

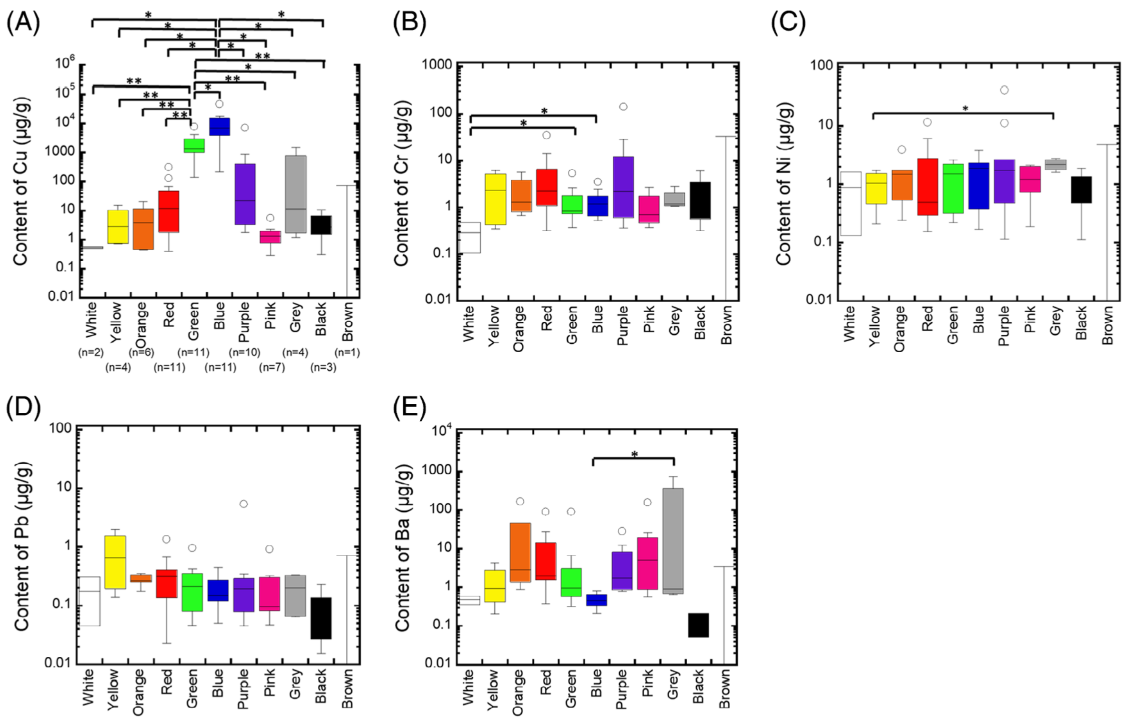

FIGURE 5 Box plots of total metal content (μg/g) of Cu (**A**), Cr (**B**), Ni (**C**), Pb (**D**), and Ba (**E**) as a function of color for all investigated samples measured by means of ICPMS (70 samples)

TABLE 2 Significant correlations between different metals (of 27) in tattoo inks investigated by JASP, expressed as Pearson's correlation coefficient ("r") with its *P* value (**P* < .05, ***P* < .01, ****P* < .001)

Correlation matrix	N	Pearson's r	*P*
Cr-Mn	70	0.97***	<.001
Cr-Fe	70	0.63***	<.001
Cr-Co	13	0.98***	<.001
Cr-Ni	70	0.97***	<.001
Cr-Zn	70	0.40***	<.001
Cr-Pb	70	0.89***	<.001
Mn-Fe	70	0.77***	<.001
Mn-Co	13	0.98***	<.001
Mn-Ni	70	0.91***	<.001
Mn-Zn	70	0.48***	<.001
Mn-As	51	0.35*	.011
Mn-Pb	70	0.86***	<.001
Fe-Co	13	0.57*	0.043
Fe-Ni	70	0.51***	<.001
Fe-Zn	70	0.53***	<.001
Fe-As	51	0.83***	<.001
Fe-Pb	70	0.49***	<.001
Co-Ni	13	0.96***	<.001
Co-Zn	13	0.69**	.009
Co-Pb	13	0.94***	<.001
Ni-Zn	70	0.32**	.006
Ni-Pb	70	0.86***	<.001
Cu-Mo	57	0.52***	<.001
Zn-As	51	0.61***	<.001
Zn-Pb	70	0.37**	.001

Note: n – sample size (only combinations with both content values above the detection limit were investigated). Corresponding scatter plots in Figure S2 (Appendix S1)

The restriction limit of 50 μg/g Ba in tattoo inks refers to total Ba content in the CoE ResAP(2008)1.[1] However, a soluble Ba limit of 500 μg/g has been regulated by ECHA.[19] As can be seen in Figure 6(C) and (D), soluble Ba (0.003-25 μg/g) was far below the restricted level of 500 μg/g in all investigated tattoo inks. Water-soluble Ba was also highly correlated with the total content ($r = 0.7$, $P < .001$) and 2-600 times lower than the total Ba content.

4 | DISCUSSION

This study revealed some alarming trends. From a consumer and medical perspective, the mislabeling of ingredients might be most problematic. There was some indication that mislabeling occurred intentionally, since confirmed (detected by both MS and MS^n) present pigments, not labeled on the ingredients list, were more likely to be among restricted, suspected nonsuitable, or discussed to be banned, pigments (29) as compared to other pigments (9) in this study. However, it cannot be ruled out that this trend is due to analytical limitations or sample selection in this study.

All samples from green, blue, and gray tattoo inks and 75% of the samples from pink and purple inks evaluated in this study contained the pigments that were either identified as not allowed to be used in cosmetics other than rinse-off products by the cosmetics regulation[24] (Pigments Violet 19, Violet 23, and Red 122) or were discussed to be banned but delayed due to the lack of alternatives for tattooing (Pigments Blue 15 and Green 7).[19] Pigment Blue 15 is banned for use in hair dyes, and Pigment Green 7 is banned for use in hair dyes and eye products. Cu-phthalocyanine colorants such as blue and green pigments are very common in cosmetics.[25] High Cu contents in blue and green tattoo inks were also reported in previous studies.[16,25,26] Up to 4310 μg/g soluble Cu in tattoo inks was also reported in an European market survey by EC.[4] The proposed ban or restriction of many Cu-containing pigments of the CI74 pigment group (Cu-phthalocyanine, such as Blue 15 and Green 7) is not necessarily the most urgent from a skin-sensitizing perspective. Cu contents were in this study correlated with only Mo. Both of these metals have a relative low skin-sensitization potential.[28,31] If this ban would result in more use of red colors, this would be detrimental.

Pigment Red 22 is the only detected (in this study) pigment restricted (0.1% concentration limit) under an EU regulation for substances in tattoo inks or PMU.[19] There is no harmonized classification within EU regarding the classification of Pigment Red 170, but many companies have submitted a notification on this substance to be sensitizing.[23] As an azo pigment (Pigment Red 22 and Red 170), the reductive cleavage of the azo could be a source of carcinogenic amines in the human body.[25,32] Pigment Red 22 and 170 are only found in red inks of this study (in 35% of red inks), and were recently identified as the prevailing pigments behind chronic allergic reactions in tattoo inks.[9] It was found previously by clinical investigation and a designed in vivo study in tattooed mice, that red tattoo inks are prone to cause allergic reactions[33] and increase skin cancer development,[34] compared to other colored tattoo inks. This study also found that Fe-containing pigment (another red pigment) might be a greater source of common sensitizers, such as Ni and Cr. It was reported that Fe oxide pigments contain minor amounts of Ni as impurities,[25] which was also identified in this study showing a large correlation ($r > 0.5$) between Fe and Ni with $P < .001$. Battistini et al.[26] found that a mixture of different kinds of metals were often observed simultaneously in tattoo inks, and that the mixture may alter the original toxicity of one metal.

Although this study did not quantify the amount of Ti, it confirmed its presence. Ti originates from the very common white pigment TiO_2 (CI77891, Pigment White 6). A large presence of Ti in tattoo inks was also found by Manso et al.[16] This pigment might be of comparably low concern; however, it is not totally harmless. TiO_2 as nanoparticles (like in pigments) is suggested to cause cancer and other adverse health outcomes.[35] Allergic contact dermatitis to Ti exists in

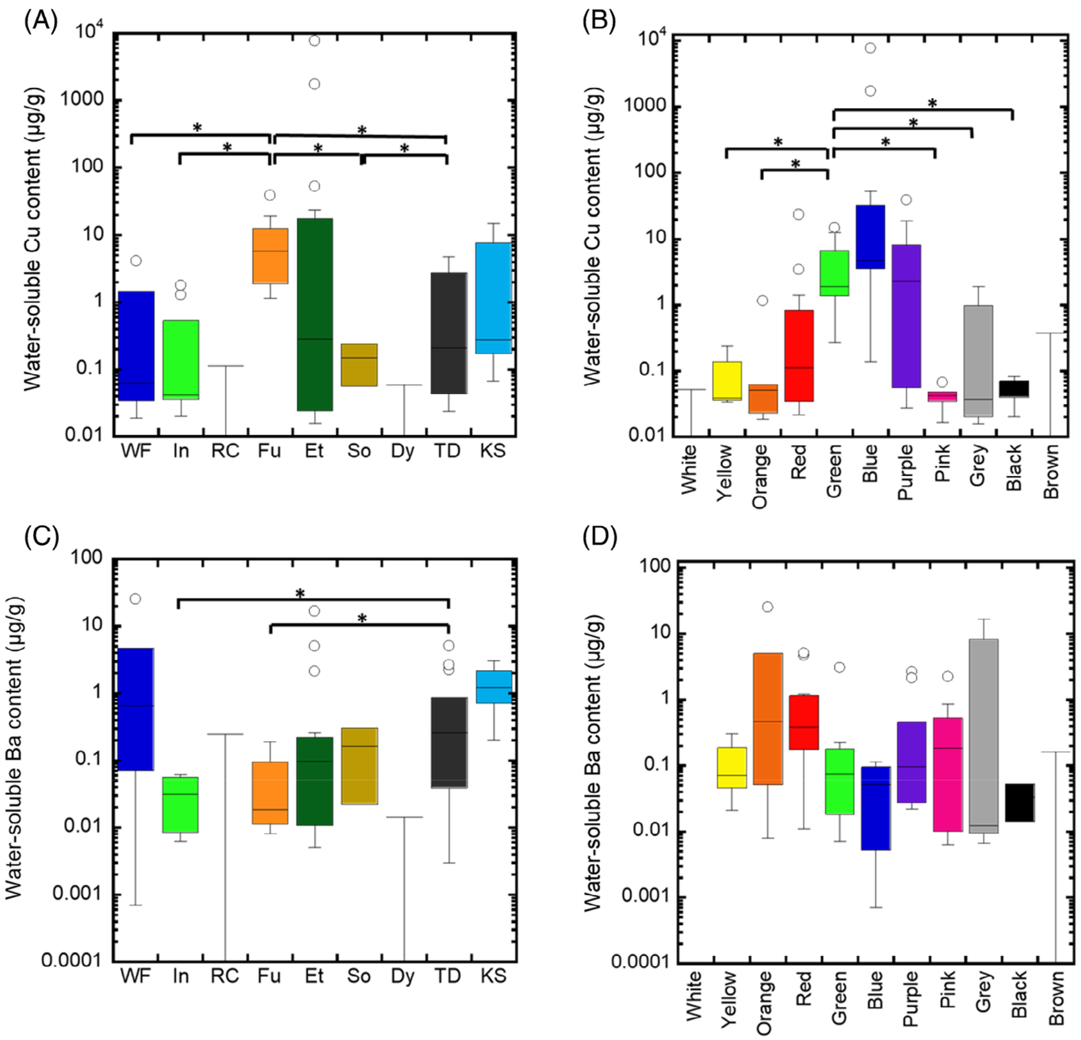

FIGURE 6 Box plots of water-soluble Cu and Ba content (μg/g) as a function of the brand (**A**, **C**) and the color (**B**, **D**) in 70 samples. Note that three dried-out samples (samples 57, 58, and 66) were not included, and data are the mean value of triplicate measurements for each sample. Corresponding data in Table S5 (Appendix S1)

rare cases.[36,37] Al is another element found in relatively high concentrations, possibly related to aluminum oxides and silicon oxides (Si was not analyzed), with similar and relatively low, but not absent, toxicity and sensitization potential.[38,39]

Ba in tattoo inks originates from $BaSO_4$, which is used to brighten darker shades and as a stabilizer.[40] $BaSO_4$ is of low concern, but soluble impurities can cause a number of adverse health effects,[41] for example, respiratory paralysis, cardiac arrest, or death.[42,43] This study did not reveal elevated soluble Ba contents in the investigated tattoo inks.

Although the overall amount of metallic impurities was relatively low in this study, several samples exceeded restriction limits or contained high amounts of Ni and (total) Cr. This study revealed that those impurities are more probable in samples containing other metals. Cr, Fe, Mn, Co, Ni, As, Pb, and Zn were highly interrelated.

The tattoo needles themselves can be a source of many nano- or micrometer-sized particles (rich in Ni and Cr), especially for inks that contain TiO_2, as described recently.[7,9] Hence, the mean concentrations of Ni and Cr in tattooed skin could be far higher than measured in the inks due to the tattoo needle wear. Both elements are common allergens, and their target levels in consumer products should be less than 1 μg/g.[16,29,30]

This study found polyethylene glycol (or PEG) in several tattoo inks. It is very common to find other substances in tattoo inks, in addition to the pigments, like binders, solvents, and additives, and a plausible source for PEG could be the use of polymeric binders, or surfactants Tween and Triton, which both have PEG as a sidechain.[44]

Ninety-three percent of the investigated tattoo inks violated at least one of the legal requirements for labeling by the CoE ResAP (2008)1.[1] In this study, the brands "In," "RC," "Fu," and "Dy" recommended an allergy or patch test before use, without instructions on how or where to conduct the test. A self-made patch test could be wrongly conducted or read, and even result in sensitization or a wrong belief of absent allergy. In addition, a negative patch test is never a guarantee that allergy is not developed in future (due to long-term exposure to the tattoo ink). Several manufacturers also declared a disclaimer that they would not be responsible for any allergic reaction.

This study is limited by its sample selection, its analytical method limitations, and sample size. However, the studied tattoo inks are sold and used globally. The analytical limitations mean that Ti and Si were not measured and that many possibly hazardous organic compounds were not investigated. This results in an underestimation of possibly hazardous substances in the tattoo inks of this study. Future studies could widen the pigment mass spectrometry library and improve the pigment analysis in tattoo inks in terms of detection limits, interferences, and quantification so that further pigments would be able to be detected. The sample size was primarily of concern for statistical comparisons among brands and colors, since some of the color and brand groups contained only a few samples.

This analytical survey provides color- and brand-resolved information on common pigments in typical tattoo inks and can therefore be used to select patch test substances to find culprit allergens in patients with a contact allergy to certain tattoo inks.

5 | CONCLUSIONS

The following main conclusions were drawn:

1. A large majority (93%) of 56 bought tattoo inks violated the label requirements and criteria in the European resolution ResAP (2008)1, regarding the name and address of the manufacturer, date of minimum durability, guarantee of sterility, batch number, and a list of ingredients. All manufacturers declared the conditions of use and warnings, but had various descriptions, some with misleading or dangerous information on skin allergy and patch tests. Only three "WF" and one "So" inks were free of any violations, and the inks from "TD" did not fulfill most of the requirements on tattoo ink labeling. Half of the tattoo inks declared at least one ingredient incorrectly on the label, with a higher probability to not declare a pigment listed as nonsuitable, sensitizing, or discussed to be banned within the EU. Among the detected pigments, only 37.7% were declared on the labels.
2. MALDI-ToF-MS^n analysis revealed the presence of several nonsuitable/harmful/sensitizing pigments in 61% of the 56 tattoo inks: Pigment Red 22, Pigment Red 170, Pigment Blue 15, Pigment Green 7, Pigment Violet 23, Pigment Red 122, and Pigment Violet 19. Pigment Red 22, restricted legally in the EU, was present in only red inks. The green, blue, pink, purple, and gray inks contained more often, or always, pigments with potential future restriction in the framework of the REACH regulation. Nondeclared PEG was found in 37.5% tattoo inks.
3. For 27 investigated metals in the tattoo inks, Fe, Al, and Cu were the highest concentrated metals (0.3 μg/g - 270 mg/g). A high amount of Ti was also confirmed due to white precipitates. The levels of most metals in tattoo inks were found below or slightly exceeding (in a few cases) the restriction limits of EU regulation and the resolution ResAP (2008)1. However, total Cr (0.35-139 μg/g) and Ni (0.1-41 μg/g) were found in almost all samples. Cu (0.29 μg/g - 47 mg/g) was clearly more present in green and blue colors, regardless of the brand. Most samples contained water-soluble Cu at levels below the restricted concentrations. High concentrations of metals were found mainly in "Fu" inks. Fe, Cr, Mn, Co, Ni, Zn, Pb, and As were found to significantly correlate with each other. This is of concern, as Fe pigments are common and present in high concentrations. Cu correlated with Mo content. Total and soluble Cu or Ba contents correlated as well, and soluble amounts were 2-2000 and 2-600 times lower than total amounts for Cu and Ba, respectively.
4. Our study suggests that regulatory measures should focus on correct labeling and on red tattoo inks and pigments, including impurities in Fe-containing pigment (CI77491). There is a great potential for contact allergy prevention.

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AUTHOR CONTRIBUTIONS

Xuying Wang: Formal analysis; visualization; writing-original draft. **Leila Josefsson:** Formal analysis; investigation; methodology; supervision; visualization; writing-review & editing. **Silvia Meschnark:** Investigation; methodology. **Marie-Louise Lind:** Conceptualization; supervision; writing-review & editing. **Åsa Emmer:** Resources; writing-review & editing. **Walter Goessler:** Resources; supervision; writing-review & editing. **Yolanda Hedberg:** Conceptualization; funding acquisition; investigation; project administration; supervision; visualization; writing-review & editing.

CONFLICT OF INTERESTS

The authors declare they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article. The data that support the findings of this study are also available from the corresponding author upon reasonable request.

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REFERENCES

1. European Commission. Resolution ResAP (2008) 1 on requirements and criteria for the safety of tattoos and permanent make-up (superseding Resolution ResAP (2003) 2 on tattoos and permanent make-up). https://rm.coe.int/16805d3dc4. Accessed May 27, 2021.
2. Krutak L. The cultural heritage of tattooing: a brief history. In: Serup J, Kluger N, Bäumler W, eds. *Tattooed Skin and Health, Current Problems in Dermatology*. Vol 48. Basel, Switzerland: Karger; 2015:1-5.
3. Giulbudagian M, Schreiver I, Singh AV, Laux P, Luch A. Safety of tattoos and permanent make-up: a regulatory view. *Arch Toxicol*. 2020; 94(2):357-369.
4. Piccinini P, Pakalin S, Contor L, Bianchi I, Senaldi C. JRC science for policy report: safety of tattoos and permanent make-up final. European commision technical report 2016. https://core.ac.uk/download/pdf/45616482.pdf. Accessed May 27, 2021.
5. Swedish Chemicals Agency. Farliga ämnen i tatueringsfärger - Utredning av tillsynsansvar samt behov av ytterligare reglering. https://www.kemi.se/download/18.6df1d3df171c243fb23a990f/1591454113466/rapport-3-10-tatueringsfarg.pdf. Accessed May 27, 2021.
6. Renzoni A, Pirrea A, Novello F, et al. The tattooed population in Italy: a national survey on demography, characteristics and perception of health risks. *Ann Ist Super Sanita*. 2018;54(2):126-136.
7. Schreiver I, Hesse B, Seim C, et al. Distribution of nickel and chromium containing particles from tattoo needle wear in humans and its possible impact on allergic reactions. *Part Fibre Toxicol*. 2019;16(1):1-10.
8. de Cuyper C, Lodewick E, Schreiver I, et al. Are metals involved in tattoo-related hypersensitivity reactions? A case report. *Contact Dermatitis*. 2017;77(6):397-405.
9. Serup J, Hutton Carlsen K, Dommershausen N, et al. Identification of pigments related to allergic tattoo reactions in 104 human skin biopsies. *Contact Dermatitis*. 2020;82(2):73-82.
10. Klügl I, Hiller K-A, Landthaler M, Bäumler W. Incidence of health problems associated with tattooed skin: a nation-wide survey in German-speaking countries. *Dermatology*. 2010;221(1):43-50.
11. Kluger N, Koljonen V. Tattoos, inks, and cancer. *Lancet Oncol*. 2012; 13(4):e161-e168.
12. Johansen JD, Aalto-Korte K, Agner T, et al. European Society of Contact Dermatitis guideline for diagnostic patch testing–recommendations on best practice. *Contact Dermatitis*. 2015;73(4):195-221.
13. Serup J, Hutton CK. Patch test study of 90 patients with tattoo reactions: negative outcome of allergy patch test to baseline batteries and culprit inks suggests allergen (s) are generated in the skin through haptenization. *Contact Dermatitis*. 2014;71(5):255-263.
14. European Chemicals Agency (ECHA). Committee for Risk Assessment (RAC) Committee for Socio-economic Analysis (SEAC) - Opinion on an Annex XV dossier proposing restrictions on substances used in tattoo inks and permanent make-up. https://echa.europa.eu/documents/10162/dc3d6ea4-df3f-f53d-eff0-540ff3a5b1a0. Accessed May 27, 2021.
15. Bocca B, Senofonte O, Petrucci F. Hexavalent chromium in tattoo inks: dermal exposure and systemic risk. *Contact Dermatitis*. 2018;79 (4):218-225.
16. Manso M, Pessanha S, Guerra M, et al. Assessment of toxic metals and hazardous substances in tattoo inks using Sy-XRF, AAS, and Raman spectroscopy. *Biol Trace Elem Res*. 2019;187(2):596-601.
17. European Commission. Resolution ResAP (2003) 2 on tattoos and permanent make-up. https://search.coe.int/cm/Pages/result_details.aspx?ObjectID=09000016805df8d3. Accessed May 27, 2021.
18. Laux P, Luch A. The European landscape of national regulations of tattoo inks and businesses. In: Serup J, Kluger N, Bäumler W, eds. *Tattooed Skin and Health, Current Problems in Dermatology*. Vol 48. Basel, Switzerland: Karger; 2015:196-200.
19. European Commission. Commission regulation (EU) 2020/2081 of 14 December 2020 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards substances in tattoo inks or permanent make-up. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32020R2081&qid=1609761602759. Accessed May 27, 2021.
20. Forte G, Petrucci F, Cristaudo A, Bocca B. Market survey on toxic metals contained in tattoo inks. *Sci Total Environ*. 2009;407(23):5997-6002.
21. Niederer M, Hauri U, Kroll L, Hohl C. Identification of organic pigments in tattoo inks and permanent make-ups using MALDI-TOF mass spectrometry. *F1000Res*. 2018;6:2034.
22. Goss-Sampson M. Statistical analysis in JASP: A guide for students. https://static.jasp-stats.org/Statistical%20Analysis%20in%20JASP%20-%20A%20Students%20Guide%20v2.pdf. Accessed May 27, 2021.
23. European Chemicals Agency (ECHA). Substance Information. https://echa.europa.eu/substance-information/-/substanceinfo/100.018.645. Accessed May 27, 2021.
24. European Commission. Regulation (EC) no. 1223/2009 of the European parliament and of the council of 30 november 2009 on cosmetic products. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02009R1223-20160812&from=EN. Accessed May 27, 2021.
25. Prior G. Tattoo inks: legislation, pigments, metals and chemical analysis. Serup J, Kluger N, Bäumler W. *Tattooed Skin and Health, Current Problems in Dermatology*. Basel, Switzerland: Karger; 2015;48: 152-157.
26. Battistini B, Petrucci F, De Angelis I, Failla CM, Bocca B. Quantitative analysis of metals and metal-based nano- and submicron-particles in tattoo inks. *Chemosphere*. 2020;245(Apr):125667.
27. European Commission. Regulation (EC) no. 1333/2008 of the European parliament and of the council of 16 december 2008 on food additives. https://www.fsai.ie/uploadedFiles/Consol_Reg1333_2008.pdf. Accessed May 27, 2021.
28. Bircher AJ. Metal allergy: other metals. In: Allergy M, Chen JK, Thyssen JP, eds. Springer; 2018:467-479.
29. Basketter DA, Angelini G, Ingber A, Kern PS, Menné T. Nickel, chromium and cobalt in consumer products: revisiting safe levels in the new millennium. *Contact Dermatitis*. 2003;49(1):1-7.
30. Bocca B, Pino A, Alimonti A, Forte G. Toxic metals contained in cosmetics: a status report. *Regul Toxicol Pharmacol*. 2014;68(3):447-467.
31. Bocca B, Forte G. The epidemiology of contact allergy to metals in the general population: prevalence and new evidences. *Open Chem Biomed Methods J*. 2009;2:26-34.
32. Berrie BH, Lomax SQ. Azo pigments: their history, synthesis, properties, and use in artists' materials. *Stud Hist Art*. 1997;57(2):8-33.
33. Serup J, Sepehri M, Carlsen KH. Classification of tattoo complications in a hospital material of 493 adverse events. *Dermatology*. 2016;232 (6):668-678.
34. Lerche CM, Heerfordt IM, Serup J, Poulsen T, Wulf HC. Red tattoos, ultraviolet radiation and skin cancer in mice. *Exp Dermatol*. 2017;26 (11):1091-1096.
35. Shakeel M, Jabeen F, Shabbir S, Asghar MS, Khan MS, Chaudhry AS. Toxicity of nano-titanium dioxide (TiO 2-NP) through various routes of exposure: a review. *Biol Trace Elem Res*. 2016;172(1):1-36.
36. Chan E, Cadosch D, Gautschi OP, Sprengel K, Filgueira L. Influence of metal ions on human lymphocytes and the generation of titanium-specific T-lymphocytes. *J Appl Biomater Biomed*. 2011;9(2):137-143.
37. Hamann C. Metal allergy: titanium. In: Chen JK, Thyssen JP, eds. *Metal Allergy*. Springer; 2018:443-466.

38. Hindsén M. Metal allergy: aluminium. In: Chen JK, Thyssen JP, eds. *Metal Allergy*. Springer; 2018:333-336.
39. Willhite CC, Karyakina NA, Yokel RA, et al. Systematic review of potential health risks posed by pharmaceutical, occupational and consumer exposures to metallic and nanoscale aluminum, aluminum oxides, aluminum hydroxide and its soluble salts. *Crit Rev Toxicol*. 2014;44(sup4):1-80.
40. Schmitz I, Prymak O, Epple M, Ernert C, Tannapfel A. Squamous cell carcinoma in association with a red tattoo. *J Dtsch Dermatol Ges*. 2016;14(6):604-609.
41. Oskarsson A. Barium. In: Nordberg GF, Fowler BA, Nordberg M, eds. *Handbook on the Toxicology of Metals*. Elsevier; 2015:625-634.
42. Ghose A, Sayeed AA, Hossain A, Rahman R, Faiz A, Haque G. Mass barium carbonate poisoning with fatal outcome, lessons learned: a case series. *Cases J*. 2009;2(1):1-4.
43. Johnson CH, VanTassell VJ. Acute barium poisoning with respiratory failure and rhabdomyolysis. *Ann Emerg Med*. 1991;20(10):1138-1142.
44. Dirks M. Making innovative tattoo ink products with improved safety: possible and impossible ingredients in practical usage. In: Serup J, Kluger N, Bäumler W, eds. *Tattooed Skin and Health, Current Problems in Dermatology*. Vol 48. Basel, Switzerland: Karger; 2015: 118-127.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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