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What's in My Ink: An Analysis of Commercial Tattoo Ink on the US Market

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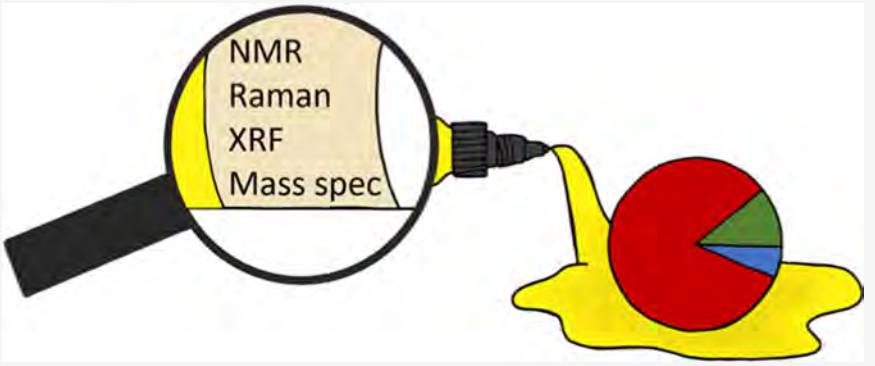

ABSTRACT: As tattoos continue to rise in popularity, the demand for tattoo ink has surged. Historically, tattoo inks have been underregulated in the US market. This study analyzes inks from nine different brands that are common in the United States, ranging from major to small manufacturers. Out of 54 inks, 45 contained unlisted additives and/or pigments. Major, unlisted adulterants include poly(ethylene glycol), propylene glycol, and higher alkanes. Many of the adulterants pose possible allergic or other health risks. Taken together, the results from this study highlight the potential for a significant issue around inaccurate tattoo ink labeling in the United States.

INTRODUCTION

Tattooing is a practice that has held cultural significance for thousands of years. The earliest record of tattoos can be found on Egyptian mummies from 2000 BCE and on "the Ice Man," which has been carbon-dated to be about 5300 years old.[1] In the present day, tattoos are increasingly common as a form of self-expression, with more than 20% of the United States population having at least one tattoo.[2] Beyond cosmetic purposes, interest in functional tattoos for medical diagnostics, electronics, and sensor applications is also growing at a remarkable pace.[3−7]

The process of tattooing involves rapid penetration of a needle into the dermal layer of the skin and insertion of tattoo ink into the dermis, approximately 1.5−2.0 mm below the skin's surface.[8] Tattoo ink is composed of molecular and/or solid pigments suspended in an aqueous/alcohol carrier solution. Most of the pigment inserted into the skin stays in place, creating images that last a lifetime, while the carrier package is assumed to leave the skin. However, the specific configuration of the tattoo within the dermis remains an unresolved question. Using mouse models, Malissen and colleagues proposed that the introduction of tattoo inks into the dermis prompts the entrapment of pigment particles by dermal cells, including fibroblasts and a specialized class of macrophages referred to as melanophages.[9] While tattoo pigments are often assumed to be immobilized in the dermal layer, previous work demonstrates small amounts of pigment can be distributed throughout the body, most commonly in the lymph nodes.[10] The presence of pigment in lymph nodes can complicate their removal for the treatment of breast cancer.[11,12] In addition, allergic reactions months or years post-tattooing are a common occurrence, particularly with red inks.[13] Recent research focused on individuals experiencing chronic allergic reactions due to red tattoos suggests that there is a connection between the onset of allergies and the photodegradation of tattoo inks, as observed in skin biopsies.[14] There are additional concerns regarding how pigments are broken down by light and if these byproducts pose health risks.[15,16] It has also been observed that exposure to sunlight often worsens an allergic response in patients.[17]

For more than a decade, the European Chemicals Agency has sought to monitor and regulate substances found in tattoo

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inks that could potentially cause harm through the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) regulations.[18,19] These substances include materials that can cause irritation, cancer, gene mutations, or those that can be harmful to reproductive health. However, although the FDA monitors the tattoo ink market, they are typically unable to regulate compositions or issue recalls due to the classification of tattoo inks as "cosmetic products."[20] This classification also means that the FDA does not need to approve new tattoo inks entering the market.[21] States are able to regulate some portion of the tattoo industry, but this is generally limited to maintaining safe and hygienic conditions to minimize the risk of infection. The FDA does require that manufacturers label their inks with the ingredients and any warnings, as indicated by the Fair Packaging and Label Act.[22] However, this information is typically not shared with tattoo clients, leaving those clients at risk of known and unknown adverse effects such as infections, allergic reactions, MRI complications, and granulomas.[23] As of 2022, the FDA has expanded its authority to regulate cosmetics, including tattoo inks, through the Modernization of Cosmetics Regulation Act (MoCRA). The passage of this act specifically expands upon the FDA's ability to regulate cosmetic products including allowing for recalls of products, required reporting for adverse events, and required product ingredients labeling that must be updated yearly.[24]

Despite their increasing popularity, little has been done to determine the composition of tattoo inks in the United States market. Work focused on the European tattoo market consistently highlights concerns about the purity of tattoo inks. A case in point is the work by Forte et al. in 2009, which scrutinized 56 commercially available tattoo inks within Europe and disclosed that 36% of the inks surpassed a 1 ppm threshold for at least one toxic metal (such as Cd, Mn, Pb, Hg, and V). Additionally, 62% and 16% of these inks exceeded established safe limits for chromium and nickel, respectively.[25] Recent investigations into a smaller sample of inks identified substantial concentrations of copper, nickel, chromium, and lead in six out of seven inks examined.[26] These metals frequently manifest in the form of oxide or metallic nanoparticles within tattoo inks, displaying sizes that range from tens to hundreds of nanometers.[27,28] One study was able to determine pigment composition of tattoo inks in situ using Raman spectroscopy, demonstrating this technique as a viable way to identify pigments found in tattoo inks.[29] Additionally, full analyses of tattoo inks have been conducted on European inks due to their stricter regulations and bans on specific components tattoo and permanent makeup (PMUs) inks. One study looked at 190 different inks designed for tattoos or PMUs sourced from various shops. Their results found that 37% of tattoo inks contained banned material and 53% were objectionable, meaning that they had high nitrosamine content, contained undeclared material, or declared material that was not present.[29] A study by Wang et al. found that a large majority of European tattoo inks violated regulations in terms of nonsuitable pigments, high metal concentrations, and misleading or dangerous labeling information.[30] It should be noted that historically, for both the European and the United States markets, tattoo inks were generally manufactured in the United States and identical for both markets. More recently, with the advent of the REACH regulations, EU-specific formulations have appeared on the market. These REACH compliant inks are still typically produced by manufacturers based in the United States.

Due to MoCRA, the question of tattoo ink labeling accuracy in the United States has suddenly become critical. Unfortunately, there is no published work analyzing the composition of a variety of commercial tattoo inks on the American market. In this study, we assess six common colors of tattoo ink (red, yellow, green, blue, white, and black) from nine major tattoo ink manufacturers to develop a representative snapshot of tattoo ink labeling accuracy in the United States. Using a combination of NMR spectroscopy, Raman spectroscopy, X-ray fluorescence (XRF) spectroscopy, and mass spectrometry (MS), this study also provides a simultaneous survey of both pigments and carrier package additives used in tattoo inks.

EXPERIMENTAL PROCEDURE

Materials. The following brands of inks were analyzed: Intenze, Dynamic, One Tattoo World, Solong, Starbrite, Raw Inks, World Famous, Solid inks, and Mom's Inks by Millennium Colors Inc. All inks besides One Tattoo World and Starbrite inks were sourced directly from the manufacturer and used as received. One Tattoo World and Starbrite tattoo inks were purchased through their official Amazon storefronts and used as received. Six different colored inks were analyzed for each brand: red, yellow, green, blue, white, and black. Deuterated acetonitrile and acetone were used as received from Cambridge Isotope Laboratories, Inc.

Characterization of Carrier Solution. Inks were dispensed into 2 mL centrifuge tubes and centrifuged at 8000 rpm using a Chemglass Life Sciences Mini Centrifuge for at least 6 h. Times varied depending on each ink's tendency to break suspension. After centrifuging, the inks were decanted into a round-bottom flask and distilled. A silicone oil bath was heated at 230 °C and the ink stirred to prevent bumping. All distillate was collected below 100 °C and the remaining material was considered pot residue. Both distillate and pot residue were then analyzed through NMR spectroscopy using a Bruker Avance III HD 400 MHz instrument and either deuterated acetonitrile (CD_3CN) or deuterated acetone ($(CD_3)_2CO$). ^1H, $^1H-^1H$ COSY, and $^1H-^{13}C$ HSQC analyses were performed on all the ink samples.

Pot residues of inks were further analyzed through gas chromatography−mass spectrometry (GC−MS). To prepare GC−MS samples, pot residues of concentrated pigments dissolved in water were analyzed and separated via high-performance liquid chromatography with diode array detection (HPLC-DAD) using a binary gradient (water/methanol) to identify peaks of interest for fraction collection. To do so, an Agilent 1100 series HPLC equipped with an Agilent 1100 series UV−Vis DAD and a Zorbax Eclipse Plus C18 column (4.6 × 100 mm, 3.5 μm) was used. For the binary gradient, the mobile phase started at 5% methanol with 0.1% (v/v) formic acid (pH 2.7) and linearly increased to 95% methanol at a flow rate of 1 mL/min over 4 min after a 2 min hold.

HPLC fraction collection was done either using a binary or isocratic gradient to collect analytes in a window that included the time frame of the peaks of interest ±0.1 min. Because the sample coming out of the HPLC was in such small quantities, the collection had to be done over 10 injections of 25 μL each. A 20 mL scintillation vial was used to recover the binary solution containing the analyte.

After fraction collection, methanol was evaporated from the sample using a rotovap at 39 Torr and 25 °C. Following this,

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an extraction using ca. 5 mL dichloromethane was performed, washing the aqueous layer four times, and collecting each organic wash in a clean 20 mL scintillation vial. This organic layer was then dried using magnesium sulfate to remove residual water and dried down even further using a rotovap at 171 Torr and 25 °C. Chloroform-d was then used to dissolve the residual analyte, which was subjected to HPLC-DAD, GC−MS, and NMR analysis. GC−MS was performed using a Shimadzu GCMS-QP2020 gas chromatograph mass spectrometer with an AOC020i auto-injector and SH-Rxi-5Sil MS column.

Characterization of Pigments. To analyze the pigments used in tattoo inks, an EZRaman-I Series High-Performance Portable Raman Analyzer with a 785 nm laser was used. Tattoo inks were cast on a glass microscope slide and dried in an oven at 120 °C for at least an hour before Raman analysis. Collected data were then compared to the SOPRANO Raman spectral database website, which contains Raman spectra of over 300 different pigments.[31] The various parameters used to obtain each reference spectrum can also be found on the SOPRANO website.

Additionally, XRF spectroscopy was used to confirm the presence of inorganic pigments in the dried pigment samples. A Thermo Electron MicronX instrument with Xpert Analysis software was used. The same samples used for Raman spectroscopy were also used for XRF. A beam width of 1.4 mm and a measurement time of 30 s were used.

RESULTS

Analysis of the Carrier Solution. A total of 54 inks were analyzed for their carrier solution and pigment composition. Each ink was distilled and the distillate below 100 °C was collected. Most of the tattoo ink distillates contained water and a variety of low-boiling alcohols, while the pot residue typically contained higher boiling alcohols and dispersants that enabled the pigments to remain in suspension (Table S1). All carrier solutions contained water and either ethanol and/or isopropyl alcohol. Of the 36 inks listing isopropyl alcohol as one of their ingredients, 17 contained unlisted ethanol. In addition, other alcohols were also observed. One Tattoo World Light Green and Mario's Blue contained 1-propanol, while Starbrite Scarlet Red and Solid Ink White both contained 1-butanol. A total of 15 inks contained benzyl alcohol, including all the World Famous and Solong Inks, as well as Mom's Millennium Ectoplasmic Green and Power White and Raw Ink's Light Yellow. We note that 40 out of 54 inks contained isopropyl alcohol, which is a restricted substance in EU REACH regulations.[18]

The most common listed additive in the inks was glycerol, with 36 out of 54 reporting the additive. However, by NMR we only observed glycerol in 29 out of 54 inks and failed to observe signals for glycerol in any of the One Tattoo World Inks nor in the Solid Ink Lining Black. We also observed characteristic NMR peaks for propylene glycol in 15 of 54 inks, though none of the 54 inks surveyed listed it as a component.

Out of the 54 inks characterized, 28 contained a ^{1}H NMR singlet around δ 3.56−3.60 ppm, which consistently coupled with a ^{13}C NMR peak around δ 70 ppm. No other ^{1}H or ^{13}C NMR signals could be observed that were related to this species. The NMR data are most consistent with a high molecular weight poly(ethylene glycol) (PEG), where we are only able to observe the backbone—CH_2—units due to the low concentration of the chain ends. The reported ^{1}H NMR stretches for the backbone—CH_2—units in PEG are between δ 3.42 and δ 3.82 ppm, with the major peak at δ 3.5−3.6 ppm.[32,33] As the molecular weight of PEG increases, the NMR resolution for peaks outside of the major peak decreases. The primary ^{13}C NMR peak for the backbone—CH_2—units in PEG is found at approximately δ 70 ppm.

Seven inks (Intenze Snow White Opaque, World Famous Great Wall Yellow, Starbrite Country Blue, Starbrite White, Solong Bright Red, Solong Lemon Yellow, and Raw White) exhibited coupled ^{1}H NMR peaks at δ 0.83−0.89 ppm and δ 1.28−1.29 ppm. ^{13}C NMR and ^{1}H−^{13}C HSQC demonstrated that these ^{1}H NMR were related to ^{13}C NMR peaks at around δ 14, δ 22, δ 29, and δ 32 ppm. Taken together, these data are consistent with higher alkanes (e.g., nonane and dodecane).[34−36] The peaks at δ 0.83−0.89 ppm are assigned to end methyl groups, while the peaks at δ 1.28−1.29 ppm are assigned to—CH_2—units. Integration and comparison of the peaks at δ 0.83−0.89 ppm and δ 1.28−1.29 ppm did not produce a consistent ratio, which may be due to either differences in the specific alkane between brands or poor resolution due to low concentration. Extraction of the Solong Bright Red pot residue with diethyl ether produced a ^{1}H NMR spectrum consistent with dodecane (Figure S354).

The pot residue for Solong Light Green showed ^{1}H NMR signals at δ 6.9450, δ 6.9635, and δ 7.3099 ppm, which were initially assigned to phenol (Figure S239). However, a sample of Solong Light Green pot residue was prepared in distilled water and compared against phenol and benzyl alcohol standards. The resulting Solong Light Green pot residue PDA spectrum showed similar absorbances to the phenol and benzyl alcohol standards, but the retention time of the sample differed from both (Figure 1). GC−MS demonstrated that the identity of the component was 2-phenoxyethanol and not phenol. This was confirmed by ^{1}H NMR of the component and isolated using HPLC (Figure S241). The ^{1}H NMR spectrum for each Solong tattoo ink pot residue appears to show 2-phenoxyethanol.

GC−MS of the Starbrite Jet Black Outline pot residue suggested the presence of hexamethylenetetramine. Hexamethylenetetramine would produce a singlet in ^{1}H NMR spectroscopy, which is consistent with the peak δ 4.61 ppm in the ^{1}H NMR spectrum (Figure S166). Furthermore, in hexamethylenetetramine, coupling can be seen between the ^{1}H singlet at δ 4.61 and a ^{13}C peak at δ 73 ppm, matching closely with the literature value of δ 74.84 ppm in $CDCl_3$.[22] Finally, ^{1}H−^{15}N HMBC NMR was performed to determine if nitrogen was present and appears to show coupling between the singlet at δ 4.61 ppm and a nitrogen atom at δ 44.18 ppm. Taken together, this appears to confirm the presence of hexamethylenetetramine.

GC−MS of the Starbrite Canary Yellow identified a component with a mass of 205 that could correspond to *N*-methyl-2-pyrrolidone, butylated hydroxytoluene (BHT), and 2,2′-methylenebis(6-*tert*-butyl-4-methylphenol) (Figure S157). No 2,2′-methylenebis(6-*tert*-butyl-4-methylphenol) was observed in the NMR data; however, it still may be present in concentrations too low to be observed using this technique. ^{1}H−^{13}C HSQC NMR also did not show evidence of *N*-methyl-2-pyrrolidone. ^{1}H NMR and ^{1}H−^{13}C HSQC were used to confirm that the GC−MS data corresponded to BHT in this sample (Figure S158).

A total of 15 of out 54 inks contained one or more components that were visible by ^{1}H and/or ^{13}C NMR but

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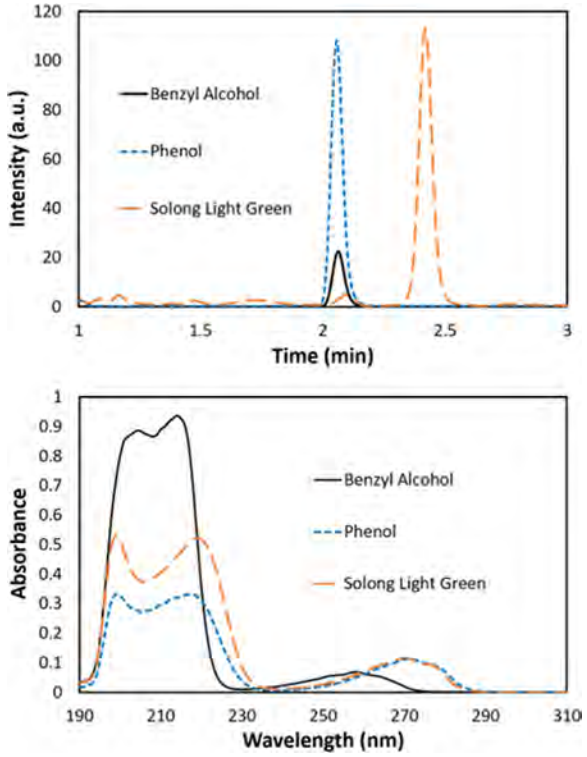

Figure 1. (Top) HP-LC chromatogram of Solong Light Green pot residue, 50 ppm benzyl alcohol, and 20 ppm phenol and (bottom) PDA spectra of Solong Light Green pot residue, 50 ppm benzyl alcohol, and 20 ppm phenol standard.

could not be satisfactorily identified. These inks include Intenze Bright Red, Solid Nice Blue, Solid White, World Famous Great Wall Yellow, World Famous Pitch Black, Starbrite Scarlet Red, Starbrite Country Blue, Starbrite Brite White, Millennium Ectoplasmic Green, Millennium Power White, Solong Bright Red, Solong Lemon Yellow, Solong True Black, Raw Green, and Raw White. Generally, these species were found in the pot residue; however, in the case of Solong Lemon Yellow, Solid Nice White, and Solid Nice Blue unidentified species could also be found in the distillate, suggesting a molecule with a boiling point less than 100 °C.

Analysis of the Pigments. Molecular pigments were identified using Raman spectroscopy (Supporting Information) and the resulting spectra were compared to spectra from the SOPRANO spectrum database for identification.[14,37] The intensity of the signals from the SOPRANO spectrum was decreased to allow for easier comparison. Additionally, XRF spectroscopy was performed to further analyze solid pigment composition among the tattoo inks. This was particularly helpful for inks with Pigment White 6 (TiO_2, PW6), which could not be easily identified with Raman. Table S2 summarizes all pigments identified using Raman spectroscopy and XRF.

Out of the 54 inks analyzed, 29 inks were found to contain only the listed pigments. Specifically, there were no discrepancies between the listed and observed pigments for the Dynamic, Starbrite, and Mom's Millennium inks. Two of the Intenze inks, Lemon Yellow and Snow White Opaque, did not show evidence of containing barium sulfate, although it is listed on its packaging; however, only the remaining listed pigments were observed. All pigments identified in Solid Ink products were listed on the safety data sheets but not assigned to specific inks.[38]

In several cases, the presence of TiO_2 (PW6) is inferred by the presence of titanium in the XRF data. For example, Starbrite Country Blue lists Pigment Blue 15 and PW6 on its label. The Raman spectrum only showed evidence of Pigment Blue 15, with characteristic peaks at 1530, 1452, 1342, 1308, 1144, 954, 748, and 680 cm^{-1} (Figure 2). Unfortunately, the

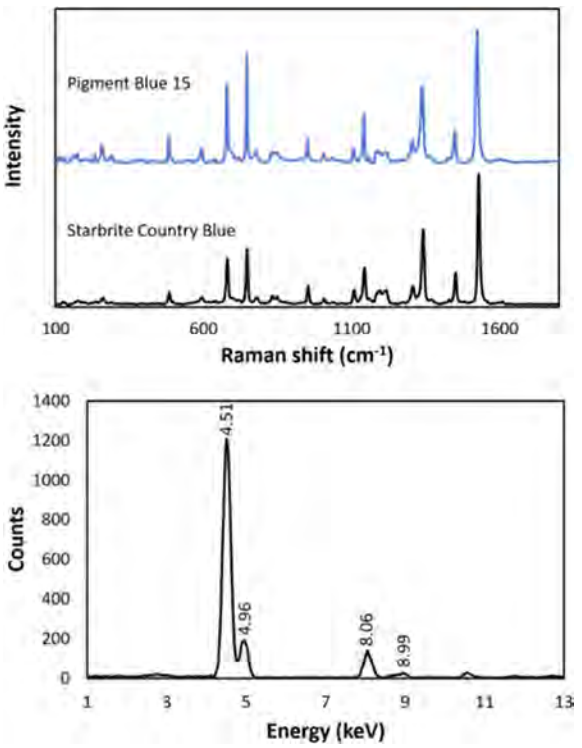

Figure 2. (Top) Raman spectrum of Starbrite Country Blue with parameters of 785 nm, 175 mW, 5 s integration, 1 average, 0 boxcar, autobaseline on and Pigment Blue 15 standard spectrum[31] and (bottom) XRF spectrum of Starbrite Country Blue.

characteristic peaks of PW6, located at 610, 448, and 232 cm^{-1} (Figure S53), were not obvious in the Raman spectrum; however, the XRF spectrum did show peaks at 4.52 and 4.96 keV, and 8.06 and 8.95 keV which correspond to the $K\alpha_1$ and $K_{\beta 1}$ lines of titanium and copper, respectively (Figure 2).[39] The presence of PW6 (TiO_2) was inferred from XRF for 22 inks (Table S2).

There were 11 total inks that showed complete inaccuracy in terms of listed pigment compositions, including Intenze Light Green. The data for Intenze Light Green ink were not consistent with the pigments listed for the ink, Pigment Yellow 14 (PW14) and Pigment Blue 15 (PB15), or the presence of barium sulfate, which is used as a filler. Instead, the observed pigments were consistent with containing Pigment Green 7 (PG7) and TiO_2. All the Solong inks listed the same three pigments, Pigment Red 210 (PR210), Pigment Orange 13 (PO13), and Pigment Yellow 65 (PY65) but none were found to contain any of these pigments. One Tattoo World did not list pigment information on their bottles or safety data sheets.

Pigment assignments could not be completed for two inks. One Tattoo World Lemon Yellow ink showed characteristic $K\alpha_1$ and $K\beta_1$ lines for zinc. The corresponding pigment could not be identified but zinc was historically used in paints in the

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form of zinc chromate or zinc oxide,[40−42] though we could not make a conclusive assignment due to lack of other data. The Raman spectrum for Solong Light Green is consistent with PB15, while the XRF for that ink shows both copper and titanium. Both PB15 and PG7 are copper phthalocyanine, though we do not observe evidence for PG7 in the Raman (Figure S266). To achieve a green ink with PB15, a third, yellow pigment would be expected.

DISCUSSION

We classified each of the 54 inks according to three classifications: minor label discrepancies, major label discrepancies, and correct labels. Inks with minor label discrepancies include those that have the unlisted type of low-boiling alcohol (ethanol versus isopropyl alcohol) or are missing a listed pigment or carrier solution component. Inks with major label discrepancies were those with unlisted pigments or additives. Figure 3 summarizes the classifications for all inks in this study.

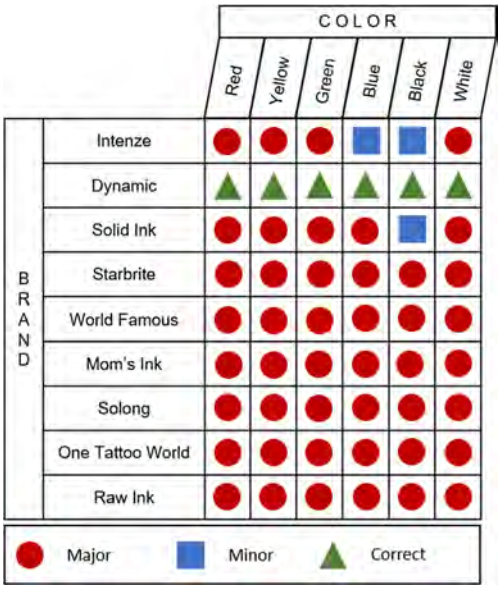

Figure 3. Summary of label discrepancies categorized by type of discrepancy: major, minor, or no discrepancy (correct). Major discrepancy inks contain unlisted pigments or additives and minor discrepancy inks are missing components or include an unlisted low-boiling alcohol.

Of all the inks analyzed, only the inks from Dynamic showed complete agreement between the listed and observed compositions. Three inks were found to have minor discrepancies: inclusion or exclusion of unlisted low-boiling alcohols (ethanol versus isopropanol) or had missing components. The only discrepancy between Intenze Mario's Blue and True Black was the presence of ethanol; however, the Hamamelis Virginiana extract may be the source of the ethanol in these inks. All the Intenze Inks and Solid Ink Black are reported to contain Hamamelis Virginiana extract and showed unlisted ethanol. However, we note that all the Solid inks are also reported to contain Hamamelis Virginiana extract but only the black ink contained ethanol.

The remaining 45 inks in this study were found to have major discrepancies, specifically unlisted additives and pigments. PEG was the most common unlisted additive and was found in 28 inks. Unlisted PEG is a known issue in tattoo inks. Hedberg and co-workers identified it in 37.5% of inks they evaluated,[30] which is consistent with our results. PEG is generally added to inks to increase viscosity and decrease surface tension and may also decrease pigment aggregate size.[43] The inclusion of PEG in tattoo inks likely plays a similar role in modifying the viscosity and performance of the ink. PEG may also play a role in improving pigment fixation within the skin after tattooing. However, Schreiver and co-workers highlight safety concerns with PEG and classify it a Category 2, Specific Target Organ Toxicant (repeated exposure).[44] Metabolization of PEG into low molecular monomers can result in acidosis, which may ultimately lead to kidney or heart failure. The lack of signal from the PEG end groups and observation of only a singlet in the ^{1}H and ^{13}C suggests a high molecular weight PEG that may be unlikely to leave the dermal layer with the rest of the carrier package. This could heighten concerns around long-term PEG degradation and kidney exposure.

Outside of pigments, water, and isopropyl alcohol, the most commonly listed additive is glycerol. Glycerol is generally added to tattoo inks to increase the viscosity and is generally considered to have low toxicity.[45] Though listed as a component in 36 inks, we only observed it in 29 inks. In the seven inks that are missing glycerol it is possible that the concentration of glycerol was too low to be detected. In 15 inks, we found strong evidence for the presence of unlisted propylene glycol. Propylene glycol has a very similar structure to glycerol but comes with a high potential for allergic reactions. In 2018, propylene glycol was named the American Contact Dermatitis Society's Allergen of the Year, as it has been known to elicit allergic reactions such as redness, swelling, itching, and fluid-filled blisters.[46,47] Propylene glycol is also known to increase viscosity and may benefit the ink performance in that way. Additionally, propylene glycol also exhibits antibacterial properties[48] and thus may be in the ink to prevent microorganism growth. All told, 41 inks contained either glycerol, propylene glycol, and/or PEG. Of the remaining 13 inks, seven are reported to contain glycerol, which may have been at a concentration too low for observation by NMR.

The observation of higher alkanes in seven inks is somewhat surprising as these compounds are not commonly known to be in tattoo inks and to the best of our knowledge have not been observed in the scientific literature around tattoo inks. Nonpolar extraction of Solong Bright Red suggests the presence of dodecane (Figure S354). Dodecane is a known emollient in cosmetics and lotions that is generally considered to have low toxicity.[49] Dodecane is also known to promote emulsion formation and may also be serving to stabilize the pigment suspension in water.[50] Either of these applications may help to explain why higher alkanes are present in tattoo inks.

We were also able to identify a handful of other, unlisted additives including 1-butanol, 2-phenoxyethanol, BHT, and hexamethylenetetramine. Hexamethylenetetramine is an antibiotic most commonly used to treat urinary tract infections[51] and BHT is used as a preservative,[52] with both likely included in ink to prevent the growth of microorganisms. Both hexamethylenetetramine and BHT are generally considered to be safe when used in an approved manner, though we note that neither additive has been considered for use in tattoos. 2-Phenoxyethanol is an antimicrobial agent approved by the European Union for use up to 1% in cosmetics.[53] Though rare,

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cases of contact dermatitis and hives with 2-phenoxyethanol are documented.[54,55] In addition, the US FDA has warned against the nervous system and diarrhea problems with nursing infants exposed to 2-phenoxyethanol by their mothers.[56]

Only ten unique pigments were identified in this study: PR254, PR170, PR210, PR112, PY74, PY14, PG7, PB15, PW6, and Carbon Black. It is possible that other molecular pigments are present in the inks but have their Raman signals masked; however, we do not observe evidence in the XRF samples for other solid pigments (e.g., iron oxide). In general, the agreement between the listed and observed pigments is better than what we observed with carrier package additives. In total, 29 inks showed complete agreement between the listed and observed pigments, while two additional inks contained only listed pigments but lacked barium sulfate. The labels for Solid Ink and One Tattoo World do not report specific pigments in their inks and therefore should not be considered accurate. Of the remaining 11 inks that contained discrepancies between the listed and observed pigments, six of those inks were made by Solong. In that case, all of the inks list PR210, PY65, and PO13, none of which we observe in the inks. Of the five inks with unlisted pigments not made by Solong, three are green inks that contain unlisted PG7. The final two, Raw Ink Light Red and Raw Ink Yellow, rely on different red and yellow pigments than what is listed.

The choice of pigments in this study is generally common in tattooing. Niederer and co-workers characterized the organic pigment in 396 tattoo inks in the Swiss market and Pigment Blue 15 and Pigment Green 7 were the most commonly used molecular pigments in tattooing,[57] which is consistent with our results. Additionally, they also observed the use of PR254, PR170, PR210, PY74, and PY14 in 3% or more of the 396 tattoo ink samples. Of the ten pigments that we observe in tattoo inks, six are legally restricted for use by EU REACH regulations (PY74, PY14, PR112, PR210, PB15, and PG7).[20]

Multiple people have observed PB15, PR170, and PR210 (PR210 is a combination of PR170 and PR266) as being prevalent in 17−40% of skin biopsies with adverse reactions to tattoos.[58,59] Other pigments identified in this study (PY74, PY14, PG7, and PR112) were also observed in biopsies of skin with adverse reactions, albeit less frequently. In general, the majority of adverse reactions in these skin samples were chronic allergic reactions. Five of the pigments identified in this study contain azo functional group (PR170, PR210, PR112, PY74, and PY14), leading to potential concerns over the release of primary aromatic amines (PAA).[54] Many PAA, such as *o*-toluidine, are known or probable carcinogens in humans.[60] A recent review from Lachenmeier and co-workers demonstrated that red and yellow tattoo pigments released high concentrations of PAA.[61] For example, EU REACH has established a maximum concentration of 0.5 mg/kg for class 1A or 1B carcinogens; however, red and yellow inks included in the review by Lachenmeier were observed to have a variety of PAA with concentrations in excess of 100 mg/kg, with some in excess of 1000 mg/kg.

There are likewise concerns around the use of TiO_2 (PW6) and carbon black in tattoo inks, both of which are used by all nine ink manufacturers in this study, with TiO_2 found in 21 of the 54 inks. Both pigments are classified as possible carcinogens in humans,[62] possibly via the formation of reactive oxygen species that may lead to lung cancer.[60] Particle sizes less than 100 nm are a concern with both TiO_2 and carbon black, due to the potential to cross blood tissue barriers. Finally, polycyclic aromatic hydrocarbons (PAH) are a known contaminant in carbon black, with concentrations as high as 201 mg/kg.[61] PAH are generally considered to be carcinogens.[62]

Finally, we note some limitations of this study. At least 14 components that we are currently unable to identify, appear in the inks included in this study. In general, this is because peaks could be observed in the NMR spectra but there was insufficient data to make a reasonable assignment. Partly, this is a function of lower-concentrations and partly this arises from the challenge of achieving adequate separation of all the ink components. Inks are suspensions of water-insoluble pigments in a complex carrier solution, which can make separating the components challenging. In addition, as demonstrated above, the pigments may be a mix of molecular and solid pigments, further complicating separation. Typical pot residues contain materials that boil near 300 °C, the upper limit of our GC−MS capabilities, and many different components have similar molecular structures, making separation and use of a technique like LC−MS difficult. From a characterization standpoint, the typical limit of detection for ^{1}H NMR spectroscopy is 2000 ppm, meaning that any components below this threshold may not be observed with this technique. Finally, as noted above, some pigments may also be masked or not observable with Raman spectroscopy. We assume that titanium in our XRF data corresponds to TiO_2 and likewise assume that any copper is related to Pigment Blue 15 or Pigment Green 7.

CONCLUSIONS

In the US market, there is little oversight regarding the regulation of tattoo ink composition. Although the US FDA does require accurate labeling for tattoo inks, only six of the 54 inks analyzed in this study matched their reported composition with complete accuracy. Three inks had relatively minor discrepancies with the reported composition. However, 45 inks from eight different manufacturers had unlisted pigments and/or additives. By far, the most common adulterant was PEG, followed by propylene glycol. Both additives primarily function to modify the viscosity and surface tension of the inks, likely explaining their inclusion in tattoo ink. Evidence for unlisted higher alkane(s), likely dodecane, was found in seven inks. Other unlisted additives included 2-phenoxyethanol, BHT, and hexamethylenetetramine. Only 29 inks correctly listed the pigments found in the ink. The other 25 inks either had a discrepancy between the listed and observed pigments or neglected to report the pigment in the ink.

The high percentage of inks exhibiting unlisted additives or pigments should raise concerns for both manufacturers and consumers. It may be that manufacturers are unaware of the purity of the bulk components they purchase and need to develop better protocols for purchasing and purity control. Alternatively, if additives are being intentionally added to inks, manufacturers have a legal and ethical obligation to report this in the product documentation. Many of these additives come with potential health concerns that tattoo artists and their clients may wish to consider in their choice of ink. In addition, while most of the emphasis around the safety of tattooing has focused on the potential carcinogenicity of the pigments, the presence of additives like PEG and 2-phenoxyethanol in the inks suggest that a wider range of potential health issues (e.g., kidney and nerve disorders) should also be considered in discussions around the safety of tattooing.

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It should also be emphasized that the unlisted additives we observed in tattoo inks are at concentrations on the order of thousands of ppm. Other components may be present at concentrations too low to observe by NMR but that are otherwise significant (e.g., hundreds of ppm). Out of an abundance of caution, we did not list any components that were suggested by mass spectrometry but were not observable by NMR. This difficulty in accurately characterizing lower concentration components further emphasizes the need for careful manufacturing controls and characterization of raw materials before they are combined into a complex ink mixture.

Finally, there are many manufacturers not considered in this study and even among the manufacturers that are considered, the typical portfolio of inks is much larger than the six we analyzed. However, the nine manufacturers included in this study run the gamut from extremely large manufacturers to small producers. At all levels, our results indicate notable deficiencies around accurate reporting of ink composition. This suggests that issues with accurate labeling are likely to be widespread. In addition, it is not uncommon for multiple ink manufacturers to have the same owner. For example, in addition to World Famous Inks, which were included in this study, the Body Art Alliance owns two additional tattoo ink brands (Black Buddha and Kuro Sumi) as well as two PMU brands (Evenflo and Perma Blend). Likewise, our data do not show any manufacturers where some inks are correctly labeled and some are not. Thus, while we are only considering six inks per manufacturer, there is reasonable cause for concern that labeling issues are likely to extend to other inks not considered in this study. Taken together, the results of this study suggest labeling inaccuracies are likely to be common across tattoo inks on the US market and that stronger efforts to ensure accurate labeling must be undertaken.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.3c05687.

^{1}H NMR spectra, ^{13}C NMR spectra, Raman spectra, and XRF data for all 54 inks; ^{1}H−^{1}H COSY NMR and ^{1}H−^{13}C HSQC NMR spectra for selected inks; mass spectra for selected inks; reference Raman spectra for barium sulfate, carbon black, and titanium dioxide; ^{1}H NMR of diethyl ether extraction of Solong Bright Red Ink (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Lineberry, C. *The Worldwide History of Tattoos*. Smithsonian Magazine, 2023. https://www.smithsonianmag.com/history/tattoos-144038580/.

(2) Niederer, M.; Hauri, U.; Kroll, L.; Hohl, C. *F1000Research* **2017**, *6*, 2034.

(3) Hosu, O.; Mirel, S.; Sandulescu, R.; Cristea, C. *Anal. Lett.* **2019**, *52*, 78−92.

(4) Promphet, N.; Ummartyotin, S.; Ngeontae, W.; Puthongkham, P.; Rodthongkum, N. *Anal. Chim. Acta* **2021**, *1179*, 338643.

(5) Bandodkar, A. J.; Jia, W.; Yardimci, C.; Wang, X.; Ramirez, J.; Wang, J. *Anal. Chem.* **2015**, *87*, 394−398.

(6) Gogurla, N.; Kim, Y.; Cho, S.; Kim, J.; Kim, S. *Adv. Mater.* **2021**, *33*, 2008308.

(7) Butterfield, J. L.; Penoncello, G. P.; Dikshit, K.; Bruns, C. J. *ACS Appl. Nano Mater.* **2022**, *5*, 13840−13844.

(8) Grant, C. A.; Twigg, P. C.; Baker, R. P.; Tobin, D. J. *Beilstein J. Nanotechnol.* **2015**, *6*, 1183−1191.

(9) Baranska, A.; Shawket, A.; Jouve, M.; Baratin, M.; Malosse, C.; Voluzan, O.; Vu Manh, T.-P.; Fiore, F.; Bajenoff, M.; Benaroch, P.; Dalod, M.; Malissen, M.; Henri, S.; Malissen, B. *J. Exp. Med.* **2018**, *215*, 1115−1133.

(10) Friedman, T.; Westreich, M.; Mozes, S. N.; Dorenbaum, A.; Herman, O. *Plast. Reconstr. Surg.* **2003**, *111* (6), 2120−2122.

(11) Soran, A.; Menekse, E.; Kanbour-Shakir, A.; Tane, K.; Diego, E.; Bonaventura, M.; Johnson, R. *Breast Dis.* **2017**, *37*, 73−76.

(12) Aguillar, V. L. N.; Tucunduva, T. C. d. M.; Carvalho, F. M.; Mazucato, M. B.; Viana, M. P.; Torres, U. S.; Mello, G. G. N. *Breast J.* **2020**, *26*, 764−766.

(13) Serup, J.; Sepehri, M.; Hutton Carlsen, K. *Dermatology* **2016**, *232*, 668−678.

(14) Serup, J.; Hutton Carlsen, K.; Dommershausen, N.; Sepehri, M.; Hesse, B.; Seim, C.; Luch, A.; Schreiver, I. *Contact Dermatitis* **2020**, *82*, 73−82.

(15) Engel, E.; Vasold, R.; Santarelli, F.; Maisch, T.; Gopee, N. V.; Howard, P. C.; Landthaler, M.; Bäumler, W. *Exp. Dermatol.* **2010**, *19* (1), 54−60.

(16) Hauri, U.; Hohl, C. *Curr. Probl. Dermatol.* **2015**, *48*, 164−169.

(17) van der Bent, S. A. S.; de Winter, R. W.; Wolkerstorfer, A.; Rustemeyer, T. *J. Eur. Acad. Dermatol. Venereol.* **2019**, *33*, e384−e386.

(18) European Union. 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:32020R2081#document1 (accessed Nov 15, 2023).

(19) ECHA. Tattoo inks and permanent make-up. https://echa.europa.eu/hot-topics/tattoo-inks#:~:text=To%20protect%20European%20citizens%2C%20thousands%20of%20hazardous%20chemicals,reproduction%20as%20well%20as%20skin%20sensitisers%20and%20irritants (accessed Nov 16, 2023).

(20) U.S. Food and Drug Administration, ePublications. Tattoos and Permanent Makeup, 2015. https://www.fda.gov/media/93379/download#page=2 (accessed Nov 15, 2023).

(21) U.S. Food And Drug Administration. FDA Authority over cosmetics: How cosmetics are not FDA-Approved, but are FDA-Regulated. https://www.fda.gov/cosmetics/cosmetics-laws-regulations/fda-authority-over-cosmetics-how-cosmetics-are-not-fda-approved-are-fda-regulated (accessed Nov 25, 2023).
(22) US Code Title 15, Chapter 39 (2018): Fair Packing and Labeling Program. https://law.justia.com/codes/us/2018/title-15/chapter-39/(accessed Nov 15, 2023).
(23) U.S. Food And Drug Administration. Tattoos & Permanent Makeup: Fact sheet. https://www.fda.gov/cosmetics/cosmetic-products/tattoos-permanent-makeup-fact-sheet.(accessed Nov 16, 2023).
(24) U.S. Food And Drug Administration. *Modernization of Cosmetics Regulation Act of 2022 (MOCRA)*. U.S. Food And Drug Administration, 2023.
(25) Forte, G.; Petrucci, F.; Cristaudo, A.; Bocca, B. *Sci. Total Environ.* **2009**, *407*, 5997−6002.
(26) Manso, M.; Pessanha, S.; Guerra, M.; Reinholz, U.; Afonso, C.; Radtke, M.; Lourenço, H.; Carvalho, M. L.; Buzanich, A. G. *Biol. Trace Elem. Res.* **2019**, *187*, 596−601.
(27) Hauri, U. *Inks for Tattoos and PMU (Permanent Make-up)/ Organic Pigments, Preservatives and Impurities Such as Primary Aromatic Amines and Nitrosamines, Report for the State Laboratory of the Canton Basel City*; State Laboratory of the Canton Basel City, 2011.
(28) Bocca, B.; Sabbioni, E.; Mičetić, I.; Alimonti, A.; Petrucci, F. *J. Anal. At. Spectrom.* **2017**, *32*, 616−628.
(29) Poon, K. W. C.; Dadour, I. R.; McKinley, A. J. *J. Raman Spectrosc.* **2008**, *39* (9), 1227−1237.
(30) Wang, X.; Josefsson, L.; Meschnark, S.; Lind, M.-L.; Emmer, A.; Goessler, W.; Hedberg, Y. S. *Contact Dermatitis* **2021**, *85*, 340−353.
(31) SOPRANO. SOP Spectral Library. https://soprano.kikirpa.be/index.php?lib=sop.
(32) Pasek-Allen, J. L.; Wilharm, R. K.; Bischof, J. C.; Pierre, V. C. *ACS Omega* **2023**, *8*, 4331−4336.
(33) Dust, J. M.; Fang, Z.-h.; Harris, J. M. *Macromolecules* **1990**, *23*, 3742−3746.
(34) Kreis, M.; Palmelund, A.; Bunch, L.; Madsen, R. *Adv. Synth. Catal.* **2006**, *348*, 2148−2154.
(35) Eisch, J. J.; Dutta, S. *Organometallics* **2005**, *24*, 3355−3358.
(36) Black, P. J.; Edwards, M. G.; Williams, J. M. J. *Eur. J. Org Chem.* **2006**, *2006*, 4367−4378.
(37) Fremout, W.; Saverwyns, S. *J. Raman Spectrosc.* **2012**, *43* (11), 1536−1544.
(38) Solid Tattoo Inks; *CAS RN: 56-81-5; CAS RN: 84696-19-5; CAS RN: 67-63-0; CAS RN: 7732-18-5*; Solid Ink: Miami, FL, 2020. https://thesolidink.com/pages/msds (accessed Nov 16, 2023).
(39) Periodic Table and X-Ray Emission Line Lookup Chart − AMPTEK − X-Ray Detectors and Electronics. https://www.amptek.com/resources/periodic-table-and-x-ray-emission-line-lookup-chart.
(40) Zanella, L.; Casadio, F.; Gray, K. A.; Warta, R.; Ma, Q.; Gaillard, J. F. *J. Anal. At. Spectrom.* **2011**, *26* (5), 1090.
(41) Otero, V.; Carlyle, L.; Vilarigues, M.; Melo, M. J. *RSC Adv.* **2012**, *2* (5), 1798−1805.
(42) Osmond, G. *AICCM Bull.* **2012**, *33* (1), 20−29.
(43) Tang, Z.; Fang, K.; Song, Y.; Sun, F. *Polymers* **2019**, *11*, 739.
(44) Schreiver, I.; Hutzler, C.; Andree, S.; Laux, P.; Luch, A. *Arch. Toxicol.* **2016**, *90*, 1639−1650.
(45) Vedova, R. D.; Wassermann, A.; Cadel, S. *Arch. Sci. Med.* **1976**, *133*, 71−84.
(46) Contact Dermatitis Institute. Propylene Glycol, 2014 https://www.contactdermatitisinstitute.com/pdfs/allergens/Propylene%20glycol.pdf (accessed Nov 16, 2023).
(47) McGowan, M. A.; Scheman, A.; Jacob, S. E. *Dermatitis* **2018**, *29* (1), 6−12.
(48) Nalawade, T. M.; Bhat, K.; Sogi, S. H. P. *J. Int. Soc. Prev. Community Dent.* **2015**, *5*, 114−119.
(49) Environmental Working Group. Dodecane, n.d. https://www.ewg.org/skindeep/ingredients/727547-DODECANE/(accessed Dec 12, 2023).
(50) Li, Q.; Xu, M.; Yang, Y.; Guo, J.; Wan, Z.; Yang, X. *Food Res. Int.* **2021**, *150*, 110733.
(51) National Center for Biotechnology Information. Methenamine. PubChem. Compound Database, n.d. CID = 4101, https://pubchem.ncbi.nlm.nih.gov/compound/4101 (accessed Dec 12, 2023).
(52) National Center for Biotechnology Information. Butylated Hydroxytoluene. PubChem. Compound Database, n.d. CID = 31404, https://pubchem.ncbi.nlm.nih.gov/compound/31404 (accessed Dec 12, 2023).
(53) National Center for Biotechnology Information. Phenoxyethanol. PubChem. Compound Database, n.d. CID = 31236, https://pubchem.ncbi.nlm.nih.gov/compound/31236 (accessed Dec 12, 2023).
(54) Chasset, F.; Soria, A.; Moguelet, P.; Mathian, A.; Auger, Y.; Frances, C.; Barete, S. *J. Dermatol.* **2016**, *43*, 318−320.
(55) Bohn, S.; Bircher, A. J. *Allergy* **2001**, *56*, 922−923.
(56) Akgündüz, M. Ç.; Çavuşoğlu, K.; Yalçın, E. *Sci. Rep.* **2020**, *10*, 1209.
(57) Niederer, M.; Hauri, U.; Kroll, L.; Hohl, C. *F1000Research* **2018**, *6*, 2034.
(58) Serup, J.; Hutton Carlsen, K.; Dommershausen, N.; Sepehri, M.; Hesse, B.; Seim, C.; Luch, A.; Schreiver, I. *Contact Dermatitis* **2020**, *82*, 73−82.
(59) Brungs, C.; Schmid, R.; Wolf, C.; Berg, T.; Korf, A.; Heuckeroth, S.; Hayen, H.; van der Bent, S.; Maijer, K.; Rustemeyer, T.; Karst, U. *Anal. Chem.* **2022**, *94*, 3581−3589.
(60) Foerster, M.; Schreiver, I.; Luch, A.; Schüz, J. *Cancer Epidemiol.* **2020**, *65*, 101655.
(61) Fels, P.; Lachenmeier, D. W.; Hindelang, P.; Walch, S. G.; Gutsche, B. *Cosmetics* **2023**, *10*, 141.
(62) Baan, R.; Straif, K.; Grosse, Y.; Secretan, B.; El Ghissassi, F.; Cogliano, V. *Lancet Oncology* **2006**, *7*, 295−296.

https://doi.org/10.1021/acs.analchem.3c05687
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