Abstract
Black is the most common tattoo color, but only a few studies have shed light on the multitude of functional and contaminating chemicals present in black inks. These studies have generally shown that black inks are a diverse group, containing anything from 5 to 50+ organic components. Little is known about the possible effects on humans of internalizing these chemicals. Analysis has shown that the production of the main component, carbon black, can lead to the formation of pigments with polycyclic aromatic hydrocarbon (PAH) contents that range from very high to almost completely absent. Similar variations in PAH concentrations are observed in black inks. PAHs are known carcinogens and thus, low recommended levels have been suggested by the Council of Europe. Reactive oxygen species (ROS) have recently been a topic in scientific literature related to tattoo ink. Again, it has been shown that some inks produce deleterious ROS (e.g. singlet oxygen or peroxyl radicals), presumably via either adhered organic compounds or particle surface defects. It has been shown that black tattoo inks may contain a multitude of chemicals, including carcinogens and allergens, and some have unknown toxicologies. However, it has additionally been demonstrated that some black inks already on the market do not produce ROS and also contain PAHs at levels that are below those recommended by the Council of Europe and very few additional contaminants.
content of black ink was thoroughly characterized have reached the literature. The main substance in black inks is carbon black (CB). One project has shown that the average CB content in 4 inks is 0.3 g/g [1], whereas another has reported ultra-centrifuged dry weight percentages of between 31 and 67% for 11 black inks [4]. CB is one of the major chemicals used in the world with an annual global production of 5–10 million tons per year. By far, the majority (70%) is used for the reinforcement of rubber in the production of automobile tires, and 20% is used for the production of black plastic [5, 6]. Nine percent is used for inks and paints, which includes tattoo inks. CB is very diverse, being produced by many different manufacturers from different types of highly controlled incomplete combustion of hydrocarbons (gases or liquids). Thus, there is a wide variety of particle sizes, surface areas, structures, purities and adhered chemicals, such as polycyclic aromatic hydrocarbons (PAHs). An illustration of the latter has been demonstrated by a characterization of 5 commercial CB products, in which a 3,000-fold difference (0.123–330 µg/g) in PAH content was detected between the highest and lowest extractable PAH contents. For a strong carcinogen and the most frequently examined PAH (benzo[a]pyrene), this difference was 0 versus 6.8 µg/g [1]. The latter of these two products contained even higher amounts of PAHs than those found in standard diesel exhaust particles from a forklift engine (NIST SRM 2975).

A wide variety of chemicals with different functions may be added to tattoo ink, including dispersed pigment particles, dispersants (polymers and surfactants), solvents (water and/or organic solvents), polymeric resins for binding, antifoaming agents (e.g. fatty oils), wetting agents (surfactants), rheological modifiers to control viscosity, pH modifiers, and biocides to prevent microbiological growth. In addition to the intentionally added chemicals, inks contain contaminants that are associated with the added chemicals, e.g. PAHs in CB. However, it must also be mentioned that some inks used in tattoo shops are commonly used for paper printing and are thus not manufactured for the purpose of tattooing and being injected into the human body.

We have previously shown in a study of 11 black tattoo inks that they contain a wide variety of at least 50 different organic compounds in amounts (excluding low boiling solvents) of about 1% (w/w) or below [4]. All of the investigated tattoo inks contained CB and 2–3 other main components in addition to surfactants and contaminants. The main components constituted 65–100% of the organic compounds and were all oxygenated compounds, such as butanediol, glycerol and phenol, but in some cases, more exotic compounds were observed, such as Texanol, which is usually used in waterborne paints. In 6 of the 11 inks, surfactants were found, including nonylphenol ethoxylates (Surfonic N-X), octylphenol ethoxylates (Triton X), heptylphenol propoxylates, alkenyl ethoxylates, a mixture of polyethylene glycol, isosorbide oligomers and sorbitan ethoxylates (Tween), alcohol ethoxylates and 2,4,7,9-tetramethyl-5-decyne-4,7-diol ethoxylate (Surfynol 4XX). However, their contents were not quantified. Most of the tentatively identified minor organic compounds were derivatives of PAHs, with a few exceptions, such as trichlorobenzene, and were all considered contaminants. The inks were screened for dimethyl phthalate, diethyl phthalate, butyl benzyl phthalate and di-octyl phthalate, but only dibutyl phthalate in concentrations of up to 5 µg/g and di-(2-ethylhexyl) phthalate in concentrations of up to 19 µg/g ink were found. One of the interesting findings of this study was that the total content of PAHs varied by up to a factor of 63 among the 11 black inks (0.46–29 µg/g), showing that there are commercial inks with PAH contents that are below the Council of Europe recommendation of <0.5 µg/g. This finding was also supported by Regensburger et al. [7], who showed that 19 commercial black tattoo inks contained total PAHs at concentrations ranging from 0.14 to 201 µg/g ink. A third study showed
Similar results, in which 8 black inks were found to contain from 0.8 to 118 µg/g, and two inks were shown to contain no PAHs (i.e. under the detection limit) [1]. All of these results clearly show that it is possible to produce black tattoo inks with very low levels of carcinogenic PAHs and that such products already exist on the market. PAHs represent a large group of organic chemicals with more than two fused aromatic rings, which are often formed as a result of incomplete combustion. Within inks, these chemicals have received special attention due to their possible toxicities. Although only one PAH has been classified by the International Agency for Research on Cancer (IARC) as a group 1 human carcinogen (benzo[a]pyrene), numerous others have been classified as group 2b (probably) and group 2a (possibly) human carcinogens [8]. Several metabolized PAH intermediates (via the cytochrome P-450 subfamily) are able to interact with DNA, forming bulky adducts with known carcinogenic effects. Skin cells are able to metabolize and activate PAHs, although this occurs at a much lower rate compared with other cell types e.g. liver and lung cells [9]. Human skin cells may be less sensitive to the genotoxicity of PAHs. However, it should be considered that the dermis is not the only and final destination of inks. Local lymph nodes are also recipients [10], and it is likely that systemic exposure occurs as well. Circulating particles above the size of glomerular filtration of <10 nm (a small subset of 2% of renal pores are 15–20 nm) [11] will likely be largely taken up by the liver. However, removing PAHs from particle surfaces requires harsh chemical treatments. Borm et al. [12] did not find any leakage of PAHs when CB particles were shaken in a water bath (24 h at 37°C in the dark) in a range of saline/dipalmitylphosphatidylcholine concentrations. Thus, the bioavailability of particle-adhered PAHs in inks can be questioned. Because metabolized PAHs are excreted in the feces or urine, and such excretion has been demonstrated, for example, for PAHs taken up through the skin [13, 14], it should be possible to determine whether PAHs in deposited ink are systemically bioavailable after tattooing. Although mutations and cancers are the main events in PAH carcinogenesis, some in vitro studies have indicated that nongenotoxic mechanisms may influence this process. For example, PAHs have been linked to promoter hypermethylation and increased cell proliferation [15, 16]. In addition to carcinogenesis, PAHs have been linked to several other toxicological end points, such as skin sensitization, skin photosensitivity, and immunotoxicity (see discussion in [17]).

The production of chemically highly reactive oxygen species (ROS) by tattoo inks has so far only been shown and discussed in two publications. It has previously been shown that PAHs are able to generate singlet oxygen (O2·) under ultraviolet A (UVA) radiation [18]. Importantly, UVA is able to penetrate the skin to a depth of 1.5 mm. This includes the entire dermis with tattooed particles and PAHs. Regensburger et al. [7] tested the production of singlet oxygen by all PAHs present in a wide selection of black inks. Then, they exposed primary normal human dermal keratinocytes to PAH extracts of inks and UVA light. A good correlation between the generation of singlet oxygen by PAHs (the PAH composition of the extract) and decreased mitochondrial activity in the keratinocytes was observed [7]. Høgsberg et al. [4] used a broad indicator of ROS when they analyzed the production of 11 black inks. Surprisingly, they found huge differences in the ability to produce ROS, identifying 2 inks that produced very high amounts and 9 that produced relatively low amounts. Experiments with different specific ROS scavengers were performed to specify which ROS were produced by the two inks. For the first ink, it was shown that the peroxyl radical accounted for 44–69% of the ROS, whereas hydrogen peroxide and hydroxyl radicals accounted for less than 10%, depending on the tested concentration. For the second ink, it showed relatively similar results, with the peroxyl radical accounting for
45–72% of the ROS, and hydrogen peroxide and hydroxyl radicals accounting for less than 16%. Additionally, these results were somewhat similar to those detected for a highly pure (>99.9% C; 75 ng PAH/g [19]), high ROS-producing CB product (Printex 90), with peroxyl radicals, hydrogen peroxide and hydroxyl radicals accounting for 26–57, 1–20 and 0–10%, respectively [4].

The production of ROS by inks is an important finding that indicates the possibility of new mechanisms that may lead to mild or even more severe toxicological symptoms in tattooed individuals. Possible mechanisms underlying ROS production by inks are illustrated in figure 1. Toxicological symptoms could be anything from membrane destabilization, over reduced skin integrity to genotoxicity and cancer; proximal or even distal to the site of deposition. In this regard, it should be mentioned that Printex 90 has previously been shown to be able to both oxidize DNA and induce DNA strand breaks in a pulmonary cell line and in vivo in rodent lungs following pulmonary exposure [21–23]. Prolonged in vitro exposure caused mutations to form in the damaged DNA [21]. Mutation spectrum analysis has revealed that the mutations possess the same genetic fingerprint as that of high ROS production [24]. It is therefore important that both of the abovementioned studies have shown that there are several commercial black inks without or with very limited ROS production. Based on these findings, one could expect that long-term exposure to some black inks may cause genotoxicity, mutations and cancer. On the other hand, a recent literature survey has indicated that cancer in tattoos is rare and coincidental [25]. However, these cases have not been linked to specific ink products, and as mentioned above, it appears that...
only some samples produce high amounts of ROS. Additionally, no one has examined whether a correlation exists for other possible target sites, such as the lymph nodes or liver.

Regensburger et al. [7] also measured phenol and found concentrations of up to 385 μg/g. Phenol is thus far unclassifiable as a human carcinogen (IARC group 3). However, in addition to causing cutaneous burns, it has been shown in mice that the external skin application of 3 mg of phenol twice a week for up to 20 weeks promotes papilloma formation with the risk of malignant conversion [26]. However, the implication of the detected concentrations in inks is unknown.

Lehner et al. [27] investigated 14 black tattoo inks and found that they all contained dibutyl phthalate in concentrations ranging from 0.12 to 691.2 μg/g. Some of the inks also contained the contaminants hexachloro-1,3-butadiene (0.08–4.52 μg/g), methenamine (0.08–21.64 μg/g), dibenzofuran (0.02–1.62 μg/g), benzophenone (0.26–556.66 μg/g), and 9-fluorenone (0.04–3.04 μg/g) [27]. Some of these compounds have been suspected of causing irritant skin reactions or allergies.

The Danish Environmental Protection Agency measured metals and other elements, primary aromatic amines and phenylene diamine in 61 tattoo inks, including 11 black inks. The only problematic metals/elements found in the black inks were (in μg/g ink) the following: Cr, 8.9 and Ni, 2.5 [1]. The council of Europe recommends that hexavalent Cr should be present at <0.2 μg/g, whereas the level of Ni should be as low as possible due to the possibility of allergies. Additionally, these elements have been linked to genotoxicity via direct DNA-Cr adducts or the inhibition of DNA repair enzymes [28]. One black ink was screened for 23 different primary aromatic amines, and only o-anisidine was detected, at a concentration of 4.9 μg/g. O-anisidine is possibly carcinogenic to humans (IARC group 2b) based on sufficient evidence of carcinogenicity in animals [29].

Some black tattoo inks have been shown to contain a multitude of chemicals, including known allergens and carcinogens. This might well be connected to the fact that a large fraction of the tattooed population appears to suffer from persistent skin reactions, including sunlight sensitivity [30, 31]. It is therefore important to mention that all of the abovementioned studies of ink composition have shown that there are products that are presently on the market that contain very low amounts or the complete absence of PAHs, ROS production and other associated chemicals.

References


27 Lehner K, Santarelli F, Vasold R, König B, Landthaler M, Bäumler W: Black tattoo inks are a source of problematic substances such as dibutyl phthalate. Contact Dermatitis 2011;65:231–238.


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Carbon Black and Other Constituents of Ink and Potential Harm in Tattooed Humans