Long perceived as a form of exotic self-expression in some social fringe groups, tattoos have left their maverick image behind and become mainstream, particularly for young people. Historically, tattoo-related health and safety regulations have focused on rules of hygiene and prevention of infections. Meanwhile, the increasing popularity of tattooing has led to the development of many new colours, allowing tattoos to be more spectacular than ever before. However, little is known about the toxicological risks of the ingredients used. For risk assessment, safe intradermal application of these pigments needs data for toxicity and biokinetics and increased knowledge about the removal of tattoos. Other concerns are the potential for phototoxicity, substance migration, and the possible metabolic conversion of tattoo ink ingredients into toxic substances. Similar considerations apply to cleavage products that are formed during laser-assisted tattoo removal. In this Review, we summarise the issues of concern, putting them into context, and provide perspectives for the assessment of the acute and chronic health effects associated with tattooing.

Introduction

Figurative art is an inherent expression of human culture, and the body art of tattooing has been present in mankind at least since the late Neolithic period, with specimens found as early as 3300–3200 BCE. In Europe and the USA, tattooing was previously a fringe fashion, but this once rebellious tribal sign for sailors, soldiers, and mavericks has turned into a popular mainstream accessory, similar to piercings or jewellery (appendix). Today many individuals receive their first tattoo at age 16–20 years and, with up to 36% of people younger than 40 years having at least one tattoo, reassessment is needed of tattooing from a toxicological perspective.

The main risks associated with tattooing were previously poor standard of hygiene and associated risks of infection. The introduction of rigorous health and safety standards have made this mostly a threat of the past. In conjunction with an increasing public display of tattoos by role models, widespread perception is that tattooing is fairly safe. However, apart from a risk for severe dermatological complications, the inks used today have little in common with classic colourants and none have been toxicologically assessed for their use in tattoos (ie, intradermal application). Additionally, up to 50% of tattooed individuals regret their decision to get a tattoo at some point. Although modern laser tattoo removal is not as unpleasant as the scorching described by Mark Twain, it still harbours the risk of post-treatment scarring. Moreover, we know little about the physiological or toxicological fate of tattoo pigments after laser-induced photolysis.

In an effort to close some of these knowledge gaps, the German Federal Institute for Risk Assessment (BfR) organised a conference about tattoo safety, which was held in Berlin, Germany, on June 6–7, 2013. This Review is a result of this workshop and here we address the composition and application of tattoo inks, toxicological aspects, tattoo removal, pigment fate, and regulatory aspects.
(eg, crystallisation aids, dispersing agents). From 2008 to 2013 Swiss health authorities analysed 416 samples of 73 different brands of tattoo inks. They identified 39 organic colourants, none of which were ever tested for the use in contact with the human body, and found that their use had increased from 39% to 56% between 2009 and 2011.

Although modern tattoo inks mostly contain organic pigments, heavy metals still feature prominently, be it as chromophores (appendix), shading additives, or as contaminants. Analysis of commercial inks shows that titanium, barium, aluminium, and copper are predominantly used as colourants, whereas antimony, arsenic, cadmium, chromium, cobalt, lead, and nickel tend to be contaminants. Respective particle sizes lie within the submicrometer range or can be true nanoparticles (particles smaller than 100 nm). Some metal oxides (eg, aluminium oxide, titanium oxide) are intentionally added as nanoparticles to create special effects, their fate and effects in the human body remaining uncertain. However, most particles form aggregates visible by the naked eye in biopsy samples. Analyses have shown a trend in the reduction and replacement of notorious culprits of the past (eg, mercury and cadmium salts or oxides of chromium and cobalt), although these substances are still detectable in concentrations ranging from μg/kg to mg/kg of ink.

Meanwhile metals such as titanium, copper, and aluminium are found in ink in concentrations as high as 180–9 g/kg, 31·3 g/kg, and 5·9 g/kg, respectively (Bocca B, unpublished), and a survey from Denmark reported high concentrations for toxic metals such as chromium (31 mg/kg), nickel (18 mg/kg), and lead (10 mg/kg).

Because of their high concentrations in tattoo inks, colourants dominate the analytical focus, and preservatives and impurities seem to be regarded as less of a problem. However, in Switzerland preservatives banned for the use in cosmetics were found in up to 14% of some 416 samples tested (Hauri U, unpublished). Among these banned substances were 1,2-benzisothiazolin-3[2H]-one (benzisothiazolinone; 56 samples, 0·4–245 mg/kg), 2-octyl-4-isothiazolin-3-one (octhilinone; 15 samples, 40–450 mg/kg), phenol (12 samples, 40–4300 mg/kg), formaldehyde (55 samples, 0·004–0·23%), and the known strong sensitisers methylisothiazolinone/methylchloroisothiazolinone (21 samples, 0·5–82 mg/kg). Other substances included N-nitrosamines such as N-nitrosodiethanolamine (56 samples, up to 24 mg/kg), N-nitrosomorpholine (nine samples, up to 625 μg/kg), N-nitrosodibutylamine (two samples, up to 93 μg/kg), and N-nitrosodimethylamine (one sample, 17 μg/kg). The samples also contained other undisclosed ingredients, for example β-naphthol ethoxylate (15 samples), nonylphenol ethoxylate (seven samples), or octylphenol ethoxylate (eight samples). Similarly, anecdotal evidence from the USA suggests that preservative issues and impurities cause some of the adverse tattoo issues that are reported.

Tattoo-associated complications

From a medical perspective, tattooing involves overcoming the skin barrier and thus carries some risk of infection because the skin surface is not sterile. About 1–5% of tattooed people have tattoo-related bacterial infections after receiving a tattoo. These infections can be superficial local skin infections or more severe systemic cases, with pathogens encompassing specific bacterial strains as well as multibacterial communities, fungi, or blood-borne viruses such as hepatitis C or B or HIV. Although difficult to treat, infections with fungi and viruses are rare. Bacterial infections are far more prominent and culprits consist of, among others, group A Streptococcus spp (eg, *Staphylococcus aureus*), *Streptococcus pyogenes*, mycobacteria (nontuberculous and tuberculous) and pseudomonads.

The risk of infection depends mainly on the conditions under which the tattooing is done, and unhygienic practices such as moistening of the needle with saliva have traditionally been a major source of pathogens. Because of an increased awareness of hygiene, infections are now caused mainly by opportunistic pathogens and commensal skin microorganisms. In exceptional cases, the results can be as severe as pyogenic infection with abscess formation, erysipelas, cellulitis, fasciitis, and gangrene and can include systemic and life-threatening infection, especially sepsis and endocarditis.

Additionally, inks have been underestimated as a source of bacterial contamination. Investigators have reported that up to 20% of sampled inks are contaminated, with bacterial counts as high as 10⁸ colony forming units per mL, including inks labelled as sterile. Contaminations can either originate from poor manufacturing practice or are the result of the use of tap water as an unsterile diluent. Of particular concern is *Mycobacterium chelonae*, an environmental pathogen, which has been identified as the causative agent for many tattoo-related infections.

In a 2010 German survey, about 68% of tattooed people reported complications as a result of procedure, with coloured tattoos reported as being of most concern. Of the problems reported, 7% were systemic and 6% were persistent. However, usually only individuals with severe cases will seek medical attention. Experiences in Denmark show that chronic adverse events are dominated by reactions of an allergic nature, with red remaining the most problematic colour (Surup J, unpublished). Reactions can appear months or years after the tattoo was done. This is a remarkably long period of sensitisation induction and, although the exact reasons have not yet been elucidated, this delayed complication is an example of the much wider problem—intradermal deposit of tattoo pigments results in lifelong exposure. Most reactions are inflammatory and can
range from ulceration in severe allergy to excessive epidermal hyperplasia or even conjunctival uveitis.1,2,9,10,11 The underlying inflammatory histological hallmarks are interphase dermatitis and T-cell lymphocyte infiltration.10–11 This series of events has also been shown for an allergic reaction against a red tattoo ink.12

Meanwhile patch testing seems unsuitable to identify the corresponding tattoo dyes as allergens. The tattoo clinic of the Bispebjerg University Hospital (Copenhagen, Denmark) tested a set of 43 standard allergens, 32 textile dyes, and a battery of eight tattoo inks in a study of 90 patients who had previously been tested positive for allergic tattoo reactions.13 Most positive results related to nickel as the primary allergen (n=16), and only two patients reacted to textile dyes and four to tattoo inks. Concomitantly, individual patch tests against specific culprit dyes were mostly negative. The study concluded that allergic reactions against tattoos develop slowly and are unlikely to be caused by an allergen directly present in the tattoo ink. In accordance with the general clinical impression, reactions in red tattoos were predominant.

Apart from nickel as a contaminant, red is among the few colours that frequently tested positive,19,38,39 even though modern inks try to avoid the use of mercury sulphide (cinnabar), which historical case reports identified as a photosensitising potential of cadmium has been suggested. In Europe REACH legislation (Registration, Evaluation, Authorisation and Restriction of Chemicals) requires testing for all chemicals marketed in the European Union. The extent of testing depends on the registered volume and will cover acute toxicity, genotoxicity, reproductive toxicity, carcinogenicity, and repeated dose studies.4 Data for dermal toxicity will cover local irritation, corrosiveness, and the potential for sensitisation, but not the application of intradermal skin deposit (figure). Therefore manufacturers of the respective raw materials cannot support the use of their pigments for tattooing. Moreover, assessments under REACH refer to the pigments alone. They are neither applicable to the ready-to-use formulations, nor do they include data on any impurities.

Additionally, pigments can be susceptible to cleavage, as is the case for the acid-catalysed dissociation of pigment red 57:1.44 Furthermore, many colourants contain inherently problematic substances such as PAAs, nitrosamines, metal pigments, or various PAHs (including benzo[a]pyrene), and the formulations used for tattooing might additionally contain phenols, formaldehyde, or phthalates.45 Some inks, such as red biolip 27, have proved to have strong cytotoxic potential.5

The aromatic chromophores in tattoos are subject to photodecomposition.19,32 Apart from the purely cosmetic effect of colour fading, some of the decomposition products are suspected or known carcinogens in man. Examples include the cleavage of pigment yellow 74 to various primary amines or the photodecomposition of pigment orange 13 to substances such as 3,3'-dichloro-4-aminoazobiphenyl or 3,3'-dichlorobenzidine (Howard PC, unpublished).46 Similarly, studies have shown pigment red 22 to be photocleavable.47–49 Generally, azo or bisazo dyes have a tendency to decompose at the azo group,
Tattoos and dyes and pigments are used to colourise foods and consumer products. Toxicological testing for the latter will routinely address oral toxicity as well as extradermal toxicity and sensitisation, but will not consider prospective consequences of a lifelong intradermal deposit. REACH legislation—Registration, Evaluation, Authorisation and Restriction of Chemicals (in Europe). —www.thelancet.com Published online July 24, 2015 http://dx.doi.org/10.1016/S0140-6736(15)60215-X

**Metabolism and carcinogenicity**

After their intradermal deposition, tattoo pigments can react with the surrounding tissue and be subject to intracellular uptake. However, the issue of metabolism is disputed. Some have argued that the low solubility renders the respective pigments to be biologically unavailable; making them basically inert. Indeed, the persistence of tattoo colouring indicates that any metabolic processes are slow. Yet, low solubility is not a feature of all colourants and ink components and, with a lifelong deposit, even slow metabolism is relevant.

Furthermore, some pigments contain nanoparticles, which have different biokinetics (ie, distribution, metabolism and excretion). Pigment yellow 74 is oxidised by isolated cytochrome P450-dependent mono-oxygenases (CYPs), leading to a hydroxylisation at the 4′-position, followed by O-demethylation. The implicated CYPs (eg, CYP1A1, 1A2, 1B1, and 3A4) are key molecules in eukaryotic phase one metabolism. Likewise, the nitro group of pigment yellow 74 is reduced by nitroreductases, resulting in the formation of aryl nitroso and hydroxylamine derivatives, which are reactive to DNA and other nucleophiles. Additionally, tattoos are part of normal skin clearance. In the SKH-1-tattooed mouse, up to 32% of injected pigment red 22 is cleared after its application, and an initial study in people estimated pigment clearance to be as high as 87–99%. Because of low solubility and absence of phase two clearance, respective pigments remain potentially systemically available. Together, evidence indicates that tattoo colourants are subject to phase one metabolism. To what extent and which organs besides skin might be affected is unknown.

Likewise the potential local and systemic carcinogenic effects of tattoos and tattoo inks are unclear (figure). The finding that commercial inks can contain potentially carcinogenic PAHs in concentrations of 0.14–201 μg/g has already led to repeated calls of caution. Although the respective PAHs will stay partly at the site of injection, concentrations of up to 11.8 μg/g of tissue have been reported in local lymph nodes. In addition, the respective inks can contain potential carcinogens such as the azo dyes, some aromatic amines, phenol, hexachloro-1,3-butadiene, methylene amine, dibenzofuran, benzophenone, and 9-fluorenone. However, without reliable in-vivo studies, the issue remains fiercely debated. The issue is further complicated by photolysis and species-specific metabolism. Other factors to consider are the initial trauma of tattooing on skin as well as the subsequent inflammatory reaction that can occur over a lifetime. The available epidemiology data are scarce and have many confounding social and environmental factors. Another limitation is the fact that the long latency of cancer would require a sufficiently big cohort to detect development. An extensive review of the scientific literature reported a seemingly low number of about 50 cases of skin cancer that were possibly related to tattoos. Therefore, so far, any association has to be regarded as coincidental.

**Tattoo removal and technical prospects**

Practitioners surveys indicate that up to 50% of tattoo recipients have, at some point, had second thoughts about their decision. However, only a few pursue tattoo removal because of high cost and the risk of scarring.
Historical techniques listed in published work include abrasion of the skin with salts, chemical cauterisation, or the use of wire brushes to remove the skin. Other techniques try to induce a cutaneous inflammatory response (eg, with trichloroacetic acid or lactic acid). However, there are hardly any controlled studies of removal effectiveness. Moreover, such procedures bear the risk of scarring and dyspigmentation. In the case of allergic reactions, surgical excision or dermabration shaving seems a preferable option since no residue of pigment or degradation products is left in skin.

Laser removal is less extreme and routinely preferred in absence of allergic reactions. The corresponding wavelength-induced thermophotolysis allows selective targeting of colourants without destroying the skin. The preferred three types of lasers are Q-switched versions of the ruby laser (λ=694 nm; effective against black, blue, and green), the Nd:YAG laser (λ=1064 nm and 532 nm; effective against black and dark blue or red orange and some yellows), and the alexandrite laser (λ=755 nm; effective against black, blue, and green). From four to more than ten treatment sessions are needed to rid an individual of their unwanted tattoo. In some cases, complete removal is never achieved (eg, particularly for multicoloured tattoos), which is frequently caused by inorganic pigments such as iron, zinc, and titanium oxides. Furthermore, the degradation products of laser-cleaved dye can lead to unforeseen immune reactions. A recent case report describes regional lymphadenopathy in two individuals after laser treatment of black and blue–green tattoos. Another risk is a local allergic reaction to the original tattoo after laser treatment. Ablative fractional resurfacing laser treatment has been discussed as a method for complete tissue removal in individuals who have had an allergic reaction, although a case of a systemic reaction after such a treatment has been reported.

Pulses of lasers in the picosecond range could enhance removal effectiveness and seem beneficial when it comes to targeting very small pigment particles. Other advances include the use of index-matching compounds and the application of several passes of Q-switched lasers. Topical application of perfluorodecalin reduces the effect of laser-induced whitening, allowing for faster sequences of laser passes per session. Although this diversity of new laser methods is good news for patients considering tattoo removal, the toxicological safety of these methods remains to be established.

Microencapsulation is discussed as one option to provide dyes and pigments with defined toxicological properties and predictable biokinetics. In the case of tattoos, encapsulated soluble dyes would lead to dye stability as well as an unproblematic clearance after laser treatment. Bruce Klitzman’s group at Duke University (NC, USA) developed encapsulated tattoo inks with a capsule that could be disrupted through application of a specific exogenous energy pulse. The motivation originated from the occasional need to correct the position of medical nipple tattoos during the course of breast reconstruction. Proving the principle, the group showed that 80% of the respective colourant could be removed after one laser treatment of tattooed hairless rats and guineapigs, compared with 20% of colourant in conventional ink in the control group.

**Regulatory aspects**

The number of people with one or several tattoos is constantly increasing, and traditionally used dyes and pigments are being replaced by colourants that have never been used before. This development coincides with an increase in reports of adverse reactions and thus poses a challenge for the regulation and risk assessment of tattoo inks worldwide. In the USA, tattoo inks are regulated as cosmetics under the Federal Food, Drug and Cosmetic Act sections 601 and 602 and the pigments used in the inks are regulated as colour additives, which fall under a different section of the act, section 721. The practice of tattooing might or might not be regulated by state or local jurisdictions—each jurisdiction has different requirements in legislation, jurisdictional authorities, and professional certifications. Because they are treated as cosmetics, the act does not require pre-market review or approval of tattoo inks. However, to be marketed, they must not be adulterated, nor misbranded, and must be compliant with the 1970 Poison Prevention Packaging Act. By contrast, colour additives, with the exception of coal tar hair dyes, require the submission of a petition to establish safety. When a colour additive is intended for injection, as is the case for tattoo inks and permanent make-up, a colour additive must specifically provide for such use (Code of Federal Regulations 21: 70·5(b); safe defined as “that there is convincing evidence that establishes with reasonable certainty that no harm will result from the intended use of the colour additive”). However, no colour additives have yet been listed for use in injection; consequently, tattoo inks (including permanent make-up) are unapproved colour additives. So far the US Food and Drug Administration (FDA) has not exercised its regulatory authority for colour additives over tattoo inks or tattoo pigments because of their historical use and the low numbers of reported adverse events. Because of the recent rise in reports of adverse events, the implication of manufactured sealed inks as the source of contamination, and other information received about reactions related to tattoo inks, the FDA is reconsidering its approach. Meanwhile, the FDA is investigating reports of adverse events, taking appropriate actions when problems are identified, and is educating consumers, industry, and health professionals about the sources and problems associated with tattoo inks and permanent make-up.

Similarly, European tattoo regulation is still work in progress. Instead of a common regulation there are
several national regulations, which are mostly based on resolution ResAP (2008). Commonly these regulations provide general instructions regarding composition and labelling as well as negative lists of hazardous chemicals that should not be present in tattoo inks. Even with tattoo regulations in place, market surveillance is a great challenge. Switzerland has had legal restrictions for inks used for tattooing and permanent make-up since 2006. Yet, a 2011 nationwide survey showed that of 37% of 167 inks and 9% of 23 permanent make-up products did not comply with current regulations.

Attempts for balanced regulation are further complicated in countries where tattooing has a strong traditional background such as in New Zealand. Traditional tattoos (moko, pe’a) are a taonga (treasure), protected by the Treaty of Waitangi. They signify connections to family, culture, and life achievements. However, the issues with tattoo inks in New Zealand are remarkably similar to those in other countries, and concern has been raised against inks with uncertain ingredients and sterility, many of which are imported via the internet without restrictions. Infections remain one of the greatest problems with traditional tattooing techniques. Because it is a special situation, authorities in New Zealand favour an approach of soft regulation, and no specific regulation of tattooing is present in the primary legislation. Instead, the Ministry of Health, the Environmental Protection Authority and local government authorities have implemented national guidelines, standards, and bylaws to manage tattoo-associated risks. These measures include practical advice on sterility, and on management of injuries and bleeding, biological waste management, and information and documentation issues. Additionally, the New Zealand Environmental Protection Authority has introduced standards for the chemical safety of tattoo inks (Tattoo and Permanent Makeup Substances Group Standard 2011), which are partly similar to the Council of Europe resolution ResAP (2008).

Conclusions

International measures for consumer protection are needed urgently. In Europe, 100 million people have one or more tattoos. Although industry needs to comply with the existing regulations and to take a more proactive stance on tattoo safety, regulators and scientists have a responsibility to address potential risks of tattoos. Harmonised scenarios for risk assessment of tattoo inks are a pressing need. Ideally these assessments should result in approval of substances that are safe for intradermal application up to a defined dose. However, because of uncertainties about topics such as pigment biokinetics and metabolism, positive lists will probably not be possible in the short term. The seemingly more feasible regulatory approach of negative lists will also be subject to limitations as exemplified by the case of allergies against red tattoo inks. Without the mechanism and nature of causal agents being known, banning certain ingredients will be of little effect. What is therefore urgently needed is the establishment of a legal framework that considers tattooing as a unique application scenario.

For the period until an adequate risk assessment is achieved, step-by-step exclusion or limitation of substances for which evidence exists of adverse health effects could greatly enhance the level of consumer safety. Information about existing body art should be included as routine in questionnaires on health status to aid the identification of consequences of tattooing. Epidemiological prospective studies into the potential effect of tattoo inks on health would also help this aim. Alongside regulation, standardisation is an important element for implementation of high quality requirements for tattoo inks and tattooing. Normalising approaches to standardisation nationally and internationally might therefore help to implement quality and public safety faster than would regulation alone. Last, awareness should be raised in tattoo artists and people getting tattooed that a tattoo, besides being an aspect of art, involves a lifetime internal exposure to a mixture of ingredients that have not been characterised with regard to possible adverse health effects.

Contributors

All authors contributed equally to the generation of this manuscript. All authors also contributed equally to the 1st International Conference on Tattoo Safety held at the German Federal Institute for Risk Assessment (BfR) in 2013. The presentations and discussions at this meeting established the scientific grounds for the initiation and writing of this comprehensive Review.

Declaration of interests

WB received grants from Deutsche Forschungsgemeinschaft (DFG) during the generation. EB is Head of Medical Advisory Board and a consultant for Candela. HC is an employee of New Zealand Ministry of Health. BK is the inventor listed on US Patent number 6,013,122. NK is an honorary member of the French tattooists union (syndicat national des artistes tatoueurs, SNAT). All other authors declare no competing interests.

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