Requirements for Tattoo Inks

BfR opinion No. 013/2013, 28 August 2012

For tattoo inks and substances used in permanent make-up, the regulations of the Food and Feed Code (LFGB) apply. According to the LFGB, products must be safe for consumers and must not have any detrimental effects on human health. The manufacturer is responsible for the safety of such products. However, so far, no comprehensive assessments of individual substances are available regarding their use in tattoo products. This means that it is often not known what effect substances entering the body through tattoo inks have on the organism. Tattoo products can, apart from colourants, also contain other substances such as solvents, thickeners, preservatives as well as various impurities. The Federal Institute for Risk Assessment (BfR) has recommended that the Tattoo Ink Regulation which has been in effect in Germany since 2009 is supplemented with a positive list of colourants[1]. This list should name all colourants which may be used in tattoo inks without any adverse effects on human health. Evidence of their safety is to be supplied by the manufacturers. In addition to the positive list for colourants, lists for other components are currently being discussed. The BfR has compiled criteria which are to be applied when assessing the safety of tattoo inks and which can serve as a basis for making decisions regarding the inclusion of individual substances in positive lists.

The testing criteria presented by the BfR correspond to the current international standards and methods for the toxicological assessment of substances. Despite the limited data available, an exposure assessment should additionally be conducted.

The BfR is of the opinion that there is a need for research particularly on the distribution, metabolisation and deposition / excretion of colourants and all other components and potential breakdown products of tattoo inks in the body. It is to be assumed that the soluble components of the carrier liquid are systemically available and are metabolised immediately. In contrast, pigments are usually insoluble. They are initially deposited in the skin. However, some studies show that they do not remain in the tattoo completely but migrate to lymph nodes to some extent.

To ensure that colourants can be included in the relevant positive lists, they should, in the view of the BfR, be tested for the following criteria among others: solubility, purity / contamination with heavy metals, additives used as well as stability against UV and laser radiation and skin bacteria. They must not give rise to any breakdown products such as carcinogenic aromatic amines, which could be formed in the body as a result of metabolic processes or through UV and laser radiation. In addition, toxicological data are required on whether the colourant has genotoxic or carcinogenic properties, is detrimental to human fertility, is irritating skin or mucous membranes and whether it can trigger allergies.

This opinion describes the general criteria according to which safety assessments of tattoo inks should be conducted. However, some points, notably the biokinetics of pigments, require further investigation.

1 Introduction

[1] Opinion No. 012/2009 of the BfR
http://www.bfr.bund.de/cm/343/anforderungen_fuer_eine_sicherheitsbewertung_von_taetowiermitteln.pdf

Provided they are used as intended and even in case of reasonably foreseeable misuse, tattoo inks should not pose a threat to the health and safety of humans. To ensure that, the manufacturer or the person responsible for placing the product on the market should conduct a safety assessment. This assessment should be based on toxicological data and findings. The assessment should be summarised in a documentation which can be viewed by the competent authorities at any time.

In what follows below, the Federal Institute for Risk Assessment (BfR) has developed criteria providing a basis for the safety assessment of tattoo inks. It must be ensured that the safety assessments do not refer to pigments only but to all ingredients. The assessment of substances was discussed in a task force of the Council of Europe. The following suggestions of the BfR on relevant test methods are based on the results of these discussions. Manufacturer dossiers for the assessment of tattoo inks should use the Specifications for Cosmetics Products as guidance (SCCS Notes of Guidance 2010).

2 Necessary Specification of the Ingredients of Tattoo Inks

The following specifications are relevant for the ingredients of tattoo inks:

(1) *Chemical identity*
Precise specification of chemical properties and structural formula of all active ingredients and, if possible even the breakdown products, CAS number, CI number (where applicable) and EC number. If a substance cannot be unambiguously identified on the basis of its structural formula, sufficient information should be provided on the method of production and on the base material so that the likely structure and activity of the substance can be concluded.

(2) *Physical form*
Description of the physical form (powder, liquid, gel etc.).

(3) *Molecular weight*
in Dalton; in case of mixtures to be given for each individual substance.

(4) *Characterisation and purity of the chemical*
Specification of the experimental conditions of the techniques used for characterising the substance (UV, IR, NMR, MS, elemental analysis, etc.) and the resulting spectra, chromatograms etc, specification of the degree of purity, demonstration of the validity of the method used. The substance used for testing must correspond to that used in the commercial product.

(5) *Characterisation of impurities and / or accompanying contaminants*
Identification of significant contaminants that may be present and their concentration. The results of safety studies on a specific substance are only relevant if they refer to the relevant substance including its specific purity and contamination patterns. The scientific validity of studies of different batches of the substance with different contaminants is questionable. The manufacturer should ensure that different batches of the commercial end product do not contain different contaminants and / or different concentrations of a contaminant.

(6) *Solubility*
Specification of solubility (EC A.6) of the substance in water and / or other relevant organic solvents (in g/l at °C). Some substances (e.g. pigments) are not readily soluble in aqueous solution.

(7) *Partition coefficient (Log P_{ow})*
Specification of the n-octanol / water partition coefficient [EC A.8] with specification of pH and temperature. Where the coefficient is calculated, specification of the method.

- *Further relevant physical and chemical specifications*
  A typical chemical and physical data record consists of:
  - Physical state (solid, liquid, gas)
  - Organoleptic properties (colour, smell, taste (where relevant))
  - Solubility properties in water and organic solvents (at ..°C)
  - Partition coefficient [EC A.8] (Log P_{ow}, at ..°C)
  - Flash point [EC A.9]
  - Physical properties depending on state of aggregation:
    (for liquids: boiling point [EC A.2], relative density [EC A.3] (at ..°C), pKa (at ..°C), viscosity (at ..°C), steam pressure [EC A.4] (at ..°C), for solid substances: general appearance (crystal form, amorphous, ...), melting temperature [EC A.1], pKa (at ..°C), for UV-absorbing active ingredients: UV absorption spectrum)

(9) *Homogenity and stability*
The homogeneity of test solutions with regard to the dispersion of the test substances should be ensured. The stability of the test substance under different experimental conditions should be indicated as should the stability of under storage conditions and in the typical end product. The stability against UV and laser light should be specified. Breakdown products should be declared. Where necessary, a safety assessment of the breakdown product(s) should be conducted.

(10) *Function and uses*
Specification of concentration, function and effects of the substances to be assessed in the end product to be marketed. Specification of other types of use and concentrations (e.g. consumer products, industrial products).

3 Necessary Toxicological Endpoints for the Safety Assessment of Tattoo Inks

A toxicological data set for the safety assessment of tattoo inks should cover at least the endpoints listed below:

- skin corrosion / skin irritation
- Eye irritation
- Photo irritation
- Sensitisation
- Photosensitisation
- Genotoxicity
- Carcinogenicity
- Reproductive toxicity
- Systemic toxicity
- Toxicokinetics
- In addition, data should be available on toxic degradation products.

The test methods used to generate toxicological data should, if possible, be in agreement with existing guidelines (e.g. OECD, EU). Table 1 gives an overview of suitable test methods for the different toxicological endpoints. The following methods are considered reasonable based on the current state of knowledge:

Table 1: Toxicological test methods, applicable to the safety assessment of tattoo ingredients

Toxicological endpoint	Method	Result	Assessment
Skin irritation[1]	Intracutaneous reactivity test (ISO/FDIS 2009)	negative	+
		positive	-
	Data from other validated methods[7]	negative	o
		positive	-
Mucous membrane irritation	OECD 405: acute eye irritation / chemical burn	negative	+
		positive	-
	Data from other validated methods[7]	negative	o
		positive	-
Phototoxicity[2]	OECD 432: in vitro 3T3 NRU phototoxicity test	negative	+
		positive	-
Sensitisation	OECD 406: Guinea Pig Maximisation Test (GPMT)	negative	+
		positive	-
	Data from other validated methods[7]	negative	o
		positive	-
Mutagenicity / genotoxicity[3]	Test group: OECD 471, OECD 476, OECD 478	negative	+
		positive	-
Carcinogenicity[4]	OECD 451, OECD 453	negative	+
		positive	-
	Data from other validated methods[7]	negative	o
		positive	-
Reproductive toxicity	OECD 414, OECD 416	negative	+
		positive	-
	Data from other validated methods[7]	negative	o
		positive	-
Acute toxicity[5]	OECD 420, OECD 423, OECD 425	LC50	
Repeated dose toxicity[6]	OECD 407		
	OECD 408, OECD 409	NOAEL	

+: No misgivings relating to the use of tattoo inks with regard to the tested endpoint
-: Substance not recommended for use in tattoo inks
o: Testing with the recommended method required for further evaluation

[1]: Substances with a pH <5 or >9 not recommended for tattoo inks
[2]: For substances absorbing light of a wavelength 290-700 nm
[3]: The safety assessment should include tests for gene mutation, clastogenicity and aneugenicity. In case of ambivalent or inconclusive results making a decision regarding genotoxicity impossible, further tests should be conducted (e.g. OECD 473 or *in-vivo* micronucleus test, in order to carlify positive *in vitro* results)
[4]: In certain cases, carcinogenicity tests may be required, especially for non-genotoxic carcinogens
[5]: Substances which according to GHS are classified as lethal, toxic or harmful should not be allowed in tattoo inks.
[6]: At this point in time there are no harmonised models for estimating an MOS in tattoo inks.
[7]: Even test methods which have not been accepted as an alternative yet may be suitable for collecting supporting data (see Text / Notes of Guidance). In addition, findings gained from studies of humans, epidemiological data or information from the GHS classification may be used.

Skin corrosion / skin irritation

Generally, substances which act as irritants when used in topical applications are not suitable for tattoo inks. For this reason, any substances identified as irritating or corrosive are ruled out for this purpose. Since tattoo inks are applied under the skin, suitable substances must meet additional requirements.

To test tattoo inks for their potential to cause skin corrosion and / or skin irritation the “Intracutaneous Reactivity Test” (International ISO/FDIS Standard - ISO/FDIS 2009) is recommended, because the substance is applied intradermally as part of this test. For all other validated test methods, a topical application is used which does not simulate the tattoo procedure.

Becase very acidic or alkaline substances can cause chemical burns / skin irriations, the pH of tattoo inks should be between 5 and 9. As a matter of principle, tattoo ink with a pH < 5 or >9 should never be used.

Eye irritation
For this toxicological endpoint, it is possible to use the “Notes of Guidance” by the Scientific Committee on Consumer Safety (SCCS) of the European Commission. According to this document, a test should be conducted in accordance with OECD 405 (Acute Eye Irritation / Corrosion).

Photo irritation
Only substances which absorb light of a wavelength between 290-700 nm need to be tested for photo irritation. For cosmetic products, the *in vitro* “3T3 Neutral Red Uptake Phototoxicity Test” (3T3 NRU PT; EC B.41, OECD 432) is recommended. This test can also be used for tattoo inks. In addition, other relevant data should be taken into account.

Sensitisation
For cosmetic products, three sensitisation tests are available according to SCCS guidelines: Local Lymph Node Assay (LLNA; EC B.42, OECD Guideline 429), the Magnusson Kligman Guinea Pig Maximisation Test (GPMT; EC B.6, OECD 406) and the Buehler test (EC B.6, OECD 406).

For tattoo inks, only the GPMT test is relevant, because by means of Freund's Adjuvant it simulates a stimulation of the immune system of the same type that can be triggered when the pigment is applied through the tattoo needle, and because in this test, the simulation is linked to intradermal injection. In contrast, the two other methods are not suitable for establishing the safety of a tattoo ink. Negative test results from these methods are not sufficient proof that substances are safe.

Photosensitisation
Only if a substance can absorb light of a wavelength of 290-700 nm and is positive in 3T3 NRU PT it should be tested further for photosensitisation. For tattoo inks, an *in vivo* method involving guinea pigs can be used (Ichikawa et al. 1981). As part of this test, an intradermal application of Freund's Adjuvant is combined with subsequent topical testing of the substance to be investigated.

Genotoxicity
Genotoxic substances should not be contained in tattoo inks (see also Item 8 of this report). The SCCS lays down standards for testing cosmetic products for genotoxicity in its “Notes of Guidance” (Paragraph 3-4.6 “Mutagenicity / Genotoxicity”). These standards can be used for testing tattoo inks and include tests in accordance with OECD 471 (Bacterial Reverse Mutation Test), OECD 476 (*In Vitro* Mammalian Cell Gene Mutation Test) and OECD 487 (*In Vitro* Micronucleus Test). In addition, the breakdown products of the base materials should be tested for genotoxicity.

Carcinogenicity
In exceptional cases, OECD Tests 451 (Carcinogenicity Test) and 453 (Combined Chronic Toxicity / Carcinogenicity Test) can be used for tattoo inks.

Reproductive toxicity
OECD Tests 414 (Teratogenicity Test - Rodent and Non-rodent) and 416 (Two-generation Reproduction Toxicity Test) should be used for tattoo inks.

Systemic toxicity
According to the SCCS “Notes of Guidance” for cosmetic products, the following test should be used for testing repeated dose toxicity: OECD 407 (Repeated Dose (28 days) Toxicity (oral)), OECD 408 (Sub-chronic Oral Toxicity Test: Repeated Dose 90-day Oral Toxicity Study in Rodents), OECD 409 (Sub-chronic Oral Toxicity Test: Repeated Dose 90-day Oral Toxicity Study in Non-rodents). This also applies to tattoo inks.

Toxicokinetics
Tattoo inks are essentially composed of pigments (this term is used as the designation for the colour substance contained in the tattoo product and comprises inorganic and organic substances) and a carrier liquid which contains numerous additional ingredients, for example preservatives.

It is to be assumed that the soluble components of the carrier liquid become systemically available and are metabolised and excreted. For assessing substances serving as carrier fluids, existing toxicokinetic data may therefore be used, provided that there are no data on / indications of different toxicokinetic behaviour of the substance as a component of tattoo inks.

The situation for pigments is different. These are usually insoluble and are deposited in the skin where some of them form aggregates. One important aspect is migration, i.e. pigments moving away from the tattoo. This migration can occur immediately after tattooing but also for long periods thereafter. This also applies to potential metabolisation of the substances. According to a study by Engel et al. (2010) 32 % of the applied pigment disappeared from the tattoo in the course of 42 days. These processes are probably substance-specific and can lead to systemic toxicity, if the substances enter the bloodstream. Currently, the data on migration and metabolic degradation are insufficient. For this reason, several scenarios have to be considered for an exposure assessment.

Exposure estimation and Margin of Safety (MOS)
Due to a lack of data, exposure assessment for insoluble components such as pigments cannot reflect reality accurately at present. Additional research is needed on kinetics (distribution – metabolism – deposition / excretion). Minipigs provide a suitable animal model for these studies, because their skin properties are very similar to those of human beings (Sullivan et al. 2001). For first exposure assessments, the data of Engel et al. (2008; 2010) can be used. The BfR used this procedure for an assessment of polycyclic aromatic hydrocarbons (PAH). Several scenarios were considered within this assessment (BfR Opinion No. 044/2011[2]).

For soluble components, the Margin of Safety (MOS) can be determined according to the "Notes of Guidance of the SCCS". For this purpose, the following parameters must be known:

(1) Exposure level
This value can be roughly estimated on the basis of the studies conducted by Engel et al. (2008; 2010). According to this study, the average amount of tattoo ink suspension applied is 0.025 ml/cm^2 of skin. Since the tattoo ink does not have to overcome the skin barrier, 100 % systemic availability is to be assumed for soluble substances.

(2) NOAEL
Both, an intravenous and an oral NOAEL (No Observed Adverse Effect Level) can be used. However, in oral studies, the oral resorption rate and a possible First-Pass-Effect in the liver must be taken into account.

Toxic degradation products
Toxic breakdown products, for example aromatic amines from azo pigments, can be formed spontaneously through exposure to UV light or as part of photolytic cleavage by laser light, for example when a tattoo is removed (Engel et al. 2010; Vasold et al. 2004). Possible breakdown products should be tested for genotoxicity; genotoxic substances and their precursors should not be contained in tattoo inks.

In individual cases, it should be ascertained whether testing of specific endpoints can be dispensed with. For that purpose, procedures in accordance with REACH or the TTC concept could be used. Where applicable, additional data or tests may be relevant in conformity with the authorities.

Hardly any data are currently available on the long-term effects of tattoos. It would be desirable to systematically collect empirical data based on studies on humans.

The BfR recommends drawing up, in the medium term, a positive list of substances that are permitted in tattoo products. For this purpose, manufacturers must make available the necessary data. An evaluation of the colourants should have priority.

Microbiological aspects of the safety assessment of tattoo inks
Tattoo inks should be produced under sterile conditions. It is technologically possible to sterilise tattoo inks and / or to manufacture them in a sterile environment and to ensure that this sterility is preserved when the tattoo ink is taken out of its packaging. Sterility can be preserved, for example, by using disposable packaging, through sterile removal of the tattoo ink from the packaging, and through physical methods. For example, bioactive glass ware

[2] http://www.bfr.bund.de/cm/343/taetowiermittel-koennen-krebserregende-pak-enthalten.pdf

has been described with biocidal properties against bacteria, bacterial endospores and fungi (Charnock 2006).

As a matter of principle, no germs should be detectable in tattoo inks, especially no pathogenic microorganism which can cause wound infections (for example *Proteus vulgaris, Pseudomonas aeruginosa,* haemolysing streptococci, *Clostridium spp*).

4 Exposure estimation on the basis of the current state of knowledge and data published so far

The size and number of tattoos vary greatly from person to person. There appear to be two main groups: the group of those who have a tattoo of limited size (approximately 35 % of respondents; Klügl et al. 2010), often in a location that can be covered with clothing; and a second group with more than 6 tattoos (approximately 14 per cent of respondents). The size of tattoos varies greatly too: between 25 cm^2 (approximately 8 % of respondents) and exceeding 900 cm^2 (approximately 16 % of respondents; Klügl et al. 2010). An additional element of uncertainty in exposure estimation is that there are significant differences in the quantity of tattoo ink that is applied, depending on the experience of the tattooist (between 0.63 and 2.49 mg/cm² for 10 % test solutions or between 1.42 and 9.42 mg/cm^2 for 25 % pigment solution (median 3.5 mg/cm^2); Engel et al. 2008).

This means that exposure assessments are unable to give a clear indication of the real situation. Instead, several scenarios have to be considered. An improvement of the data situation regarding the kinetics of tattoo inks and / or their active ingredients would, though urgently required, not change this basic problem.

For this reason, the BfR has based its opinion on PAH in tattoo inks (Opinion No. 044/2011[3]) **on** two scenarios: one worst-case scenario with five tattoos > 900 cm^2 which assumed that 14 mg/cm^2 of tattoo ink was applied (with a 25 % solution and end concentration of 3.5 mg/cm^2 of tattoo ink in the skin), and a scenario which assumed a tattoo size of 600 cm^2 and an applied amount of 0.6 mg/cm^2 (corresponding to an equivalent of 2.4 mg/cm² of tattoo ink). On that basis an exposure assessment is possible and feasible.

5 Health Risks Resulting from Tattoos

The number of tattooed individuals has significantly increased in recent years (Drews et al. 2000; Laumann und Derick 2006). In the United States of America, 24% of the population have tattoos, in Germany 9% (23% for the age group 16 to 29; Allensbacher Berichte 2003; Laumann und Derick 2006; Klügl et al. 2010). According to a German national study conducted in 2010 which looked at health problems arising in connection with tattoos, 67% of respondents reported skin problems, whereas 6.6% reported systemic reactions immediately following application of the tattoo ink. Four weeks later, 8% still had health problems. 6% experienced permanent non-healing skin problems in the area of the tattoo, 3% reported other health problems in connection with the tattoo (e.g. psychological problems or increased sensitivity to light; Klügl et al. 2010).

(1) Immediate inflammatory reactions

As part of the tattooing process, the tattoo ink is applied into the dermis with a needle. This results in multiple injuries of the skin which trigger the release of inflammatory

[3] http://www.bfr.bund.de/cm/343/taetowiermittel-koennen-krebserregende-pak-enthalten.pdf

mediators (Gopee et al. 2005). Inflammations typically start about 1-2 hours after tattooing and can continue for 1-2 weeks. A Danish study recently published states that the proportion of individuals who have skin problems as a result of the tattoo within the first three months is 15 %. Symptoms included severe itching, ulcer formation, redness and swelling, delayed healing, fever and discomfort as well as local infections (Høgsberg et al. 2012). The participants of a German study additionally reported scabbing, pain, oedemas, bleeding, burning sensations and blistering directly after tattooing (Klügl et al. 2010). In addition, systemic reactions such as dizziness, headaches, sickness, fever, shivering and tiredness have been reported (Klügl et al. 2010).

(2) Infections

It is difficult to gauge the real incidence of tattoo-related infections, since most patients probably do not see a doctor but instead seek advice from the tattoo studio (Mataix und Silvestre 2009). Even though reports on infections following tattooing are often descriptions of individual cases, the increased incidence of specific severe infections such as hepatitis as well as the wide pathogen spectrum clearly show that tattooing poses quite a substantial risk of infection.

Bacterial infections

Superficial skin infections often occur within the first few days after tattooing. They are often caused by Group A streptococci or by staphylococci. Manifestations are Impetigo contagiosa (school sores), Ecthyma or Acne varioliformis (Kazandjieva und Tsankov 2009). However, deep skin infections can occur such as traumatic erysipelas, cellulitis or gangrene and even sepsis (*Pseudomonas aeruginosa, Streptococcus pyogenes*) (an overview is provided in Kazandjieva and Tsankov 2007; Mataix and Silvestre 2009).

Tattoo-associated infections with the syphilis pathogen (*Treponema pallidum*) have been reported, although these have become less significant due to the general decrease of syphilis cases of. However, the number or reported syphilis cases has been on the increase again since the mid-1990s and thus also the risk of being infected with it in the course of the tattooing procedure. Reports on tattoo-associated soft chancre and tetanus are available (Kazandjieva and Tsankov 2007).

Bacterial endocarditis, triggered by infections with *Staphylococcus lugdunensis* and / or *Staphylococcus aureus*, has repeatedly been described in connection with tattoos (Armstrong et al. 2008; Mataix and Silvestre 2009).

Mycobaceteria: the tattooing process poses a risk of infection with typical or atypical mycobacteria. There have been reports on tuberculosis cutis (*Mycobacterium tuberculosis*) and, rarely, on leprosy (*M. leprae*; overview in Kaatz et al. 2008; Kazandjieva and Tsankov 2007; Messahel and Musgrove 2009). Atypical mycobacteria which are diagnosed in connection with tattoos include *M. chelonae*, *M. abscessus* and *M. haemophilus* (Kazandjieva and Tsankov 2007; Kaatz et al. 2008; Messahel and Musgrove 2009).

Viral infections

Papillomavirus, *Molluscum contagiosum* viruses (MCV): the occurrence of warts in tattoos due to viral infections has been repeatedly described (overview in Kazandjieva and Tsankov 2007; Mataix and Silvestre 2009; Messahel and Musgrove 2009).

Herpesviridae: there are reports on the transmission of herpes viruses (zoster and simplex) through tattooing (Goldstein 1979 in: Health Canada Report 1999).

Hepatoviridae: Hepatitis B and C are the best documented infections which can be transmitted in the process of applying a tattoo (overview in Kazandjieva and Tsankov

2007; Mataix and Sylvestre 2009; Messahel and Musgrove 2009). The link between hepatitis and tattoos is well known. For this reason, recently tattooed persons are considered unsuitable as blood donors (guidelines on how to obtain blood and blood components and on the application of blood products (Haemotherapy Guideline, German Medical Association 2010).

Retroviridae: there are reports on infections with the human immunodeficiency virus (HIV) as a result of tattooing (overview in Kazandjieva and Tsankov 2007; Mataix and Silvestre 2009; Messahel and Musgrove 2009).

Mycoses
Even the occurrence of mycoses in connection with tattoos has been described on numerous occasions, including zygomycosis through *Saksenaea vasiformis* and infections *caused by Candida albicans*, *Trichophyton rubrum* and *Epidermophyton floccosum* (overview in Kazandjieva and Tsankov 2007; Mataix and Silvestre 2009).

(3) Hypersensitivity reactions

Tattoo inks can contain a range of active ingredients and their degradation products which can have a sensitising effect. These include inorganic pigments (e.g. chrome, cobalt, cadmium, mercury, nickel salts), but also organic pigments such as quinacridone (CI 73900) and azo colourants or other organic substances which in some cases are mixed in as auxillary substances (Kaatz et al. 2008). The clinical manifestations of these delayed hyper sensitivity reactions are heterogeneous and typically occur after weeks, months or years. There are descriptions of eczema, pseudolymphomas, lichen and contact urticaria (overview in Kaur et al. 2009; Mataix and Silvestre 2009). Another manifestation are granulomas. Apart from the granulomas caused by an allergic reaction, other reactions to tattoos that have been described include foreign object granulomas, for example as a reaction to the pigment which is recognised as foreign to the body or to the auxillary substance glycerine, and sarcoid granulomas. In tattoos, sarcoid granulomas often take the form of early clinical manifestations of a systemic sarcoidosis which can then also affect the lungs and other organs. The pathogenesis is unknown for the most part (Kazandjieva and Tsankov 2007; Mataix and Silvestre 2009).

(4) Other skin diseases

As other diseases in connection with tattoos, granuloma annulare, multiple epidermal cysts and psudoepitheliomatose hyperplasia have been described (Kluger et al. 2011; Koh et al. 2009; Cui et al. 2007).

Photodermatoses have repeatedly been described, notably as a reaction to yellow pigments in tattoo inks. These are often a reaction to cadmium sulfide which causes severe photosensitisation. Even though cadmium and its salts must not be contained in tattoo inks according to the German Tattoo Regulation, photodermatoses as a reaction to yellow pigments continue to be reported, and it is to be assumed that the tattoo products causing such reactions can enter the German market via the Internet (Cruz et al. 2010; Kazandjieva und Tsankov 2007).

There are several reports on tattoo-associated inflammations of the skin and the retina (cutaneous and retinal vasculitis) (Moschos and Guex-Crosier 2003; Jolly and Danila 2007; Hermida et al 2007; Hessert and Devlin 2010).

Associated illnesses such as psoriasis and other tetters (Lichen planus) can also manifest themselves in tattoos: skin lesions caused by the tattooing process spontaneously turn into psoriatic lesions, a so-called isomorphous irritation effect, also known as the Koebner Phenomenon. The tattoo process can reactivate herpes simplex or herpes zoster infections and also additional skin diseases (chronic discoid Lupus

erythematosus) (Kazandjieva and Tsankov 2007; Kaatz et al 2008). One case of toxic shock syndrome due to a tattoo has been reported (Cowan und Martens 1993).

(5) Tumours

The appearance of tumours in tattoos has recently been discussed by Kluger and Koljonen (2012). According to this study, over 50 cased have been reported in the literature in English and French over the last 40 years. Out of these, there were 23 cases of cancer in the tissue (squamous cell carcinoma and benign skin tumours (keratoacanthoma), 16 cases of melanoma and 11 cases of basal cell carcinoma. In most cases, melanomas and skin cancer (basal cell carcinomas) were found in black, dark blue or dark tattoos, whereas cutaneous squamous cell carcinoma and keratoacanthoma and benign pseudoepitheliomatose hyperplasia were largely associated with red tattoos. Black and red are the colours most often used in tattoos. It is possible that the high incidence of tumours in these tattoos are attributable to this fact (Klügl et al. 2010). In case of black tattoos, polycyclic aromatic hydrocarbons (PAH) can contribute to the formation of tumours. In contrast, red tattoo inks are for the most part associated with the occurrence of allergic reactions (Kluger et al. 2010).

The clinical relevance of tumours in tattoos is unclear, and data, for example on the composition of the tattoo inks, are absent from most case studies. Since millions of people are tattooed, the number of 50 reported cases in the course of 40 years must be seen as a coincidence (Kluger and Koljonen 2012; Mataix and Silvestre 2009). However, the composition of tattoo inks has changed over the last 20 years. Inorganic salts containing mercury, cadmium or cobalt are used less often now. In contrast, aluminium, titanium and carbon are frequent ingredients. In many instances inorganic salts are now replaced by organic colours, of which about 60% are azo colourants. Of these, many can release carcinogens (Bäumler et al. 2003).

6 Removal of Tattoos and Associated Health Risks

Methods for removing tattoos include sanding of the skin (dermabrasion), operative excision of tissue, the use of highly concentrated lactic acid and laser treatment. However, in many cases complete removal of a tattoo is not possible.

Dermabrasion means that the skin is ground off until the tattoo is gone. The resulting extensive wound poses a great risk of infection. In addition, there is a high risk of scarring and depigmentation (Kent and Garber 2011).

Removal by surgery (***operative excision***) of tattoos also leads to scarring and again carries the risk of infection. On the extremities, there is often little tissue, meaning that large tattoos cannot be removed in one go without endangering wound healing. Repeated operations increase the risk of infection (Kent and Garber 2011).

For removing tattoos ***concentrated lactic acid*** is used as well. The BfR has issued a detailed opinion on this method of tattoo removal. Due to the irritation effect of lactic acid in high concentration (40%), the use of such tattoo removal substances entails high health risks. Even in a concentration of 20%, lactic acid causes irritation of the skin and mucosa. In the eyes, such irritation can already occur if the eye comes into contact with lactic acid in a concentration of 10%. The use of highly concentrated lactic acid can result in severe inflammatory reactions with scarring (BfR Opinion No. 033/2011[4]).

[4] http://www.bfr.bund.de/cm/343/tattoo_entfernung_einsatz_waessriger_milchsaeure_ist_mit_gesundheitlichen_risiken_verbunden.pdf

So-called quality switched lasers are also used for tattoo removal (***laser tattoo removal***). As part of this process, a highly energised light beam of extremely short duration, in the range of nanoseconds, is emitted. Ideally, only the target object is heated and fragmented and the surrounding tissue remains in tact. There are three types of Q-switched lasers (QS Ruby- QS Alexandrite and QS Neodymium: Yttrium Aluminium Garnet (Nd:YAG) laser) that emit light of different wavelength and therefore can only destroy specific chromophores. Black and dark blue pigments best respond to laser beams, followed by green pigments. For red pigments, the effectiveness depends on the chemical structure. White, yellow, orange, pink and flesh-coloured pigments are often difficult to fragment with laser. For some pigments, laser treatment does not lead to a breakdown of the colour but instead to darkening or blackening. In tattoo inks containing iron, for example, this can be due to the reduction of iron (III) oxide to iron (II) oxide (overview in Bernstein 2006; Kent and Garber 2011). The presence of titanium oxide can mean that a tattoo does not react at all to laser treatment (Ross et al. 2010).

An unwanted effect of laser treatment is the possible destruction of melanocytes whose chromophore melanin also absorbs light of a certain wavelength; this can result in hypopigmentation (overview in Bernstein 2006; Kent and Garber 2011).

Straight after use of the laser, redness, blistering, scabbing and desquamation are possible (Bernstein 1991). In addition, scarring can occur as a result of the laser treatment, although this is rare (Ferguson et al. 1997). In case the work is not done hygienically, infectious germs can enter the wound.

The destruction of the pigment as a result of the laser treatment can lead to genotoxic breakdown products. This has been described, for example, for azo colourants (Taylor et al. 1991; Ferguson et al. 1997; Vasold et al. 2004). The cells containing pigment are also destroyed by the laser treatment. This means that breakdown products are released into the extracellular space, provided that the pigment was not located in the extracellular space anyway. This leads to inflammatory infiltration with macrophages, and a portion of the degradation products are carried away via macrophages. Also in lymph nodes , pigment has been located. Via the lymphatic system, the pigment and its potentially toxic degradation products can be distributed in the body (Kent and Garber 2011).

A further problem of laser treatment is the occurrence of allergic reactions to the pigment or its degradation products which can be as severe as an anaphylactic shock (Ashinoff et al. 1995; Kuperman-Beade et al. 2001; England et al. 2002). Through the destruction of cells and release of pigments and / or breakdown products, the immune system is activated (Kaur et al. 2009). These reactions can be experienced either immediately after the treatment or an hour later (Bernstein 1991). Doctors recommend that patients be treated with oral corticosteroids and antihistamines prior to the laser treatment in order to prevent these reactions. Some doctors are opposed to tattoo removals by laser if the patient is known to be allergic to tattoo inks (Bernstein 1991).

7 Framework of Action / Measure Recommendations

This opinion describes the general criteria according to which safety assessments of tattoo inks should be conducted. However, some points, notably the biokinetics of pigments, require further investigation.

8 References

Armstrong ML, DeBoer S, Cetta F (2008) Infective endocarditis after body art: a review of the literature and concerns. J Adolesc Health 43, 217-225.

Ashinoff R, Levin VJ, Soter NA (1995) Allergic reactions to a tattoo pigment after laser treatment. Dermatol Surg 21, 291-294.

Bäumler W, Vasold R, Lundsgaard J, Talberg HJ (2003) Chemicals use in tattooing and permanent make up products. In: Papameletiou D, Schwela D, Zenie A, eds. Workshop on Technical/Scientific and Regulatory Issues on the Safety of Tattoos, Body Piercing and of Related Practices. Ispra, VA: European Commission, 2003: 21–36.

Bernstein EF (2006) Laser treatment of tattoos. Clin in Dermatol 24, 43-55.
BfR-Stellungnahme Nr. 012/2009
http://www.bfr.bund.de/cm/343/anforderungen_fuer_eine_sicherheitsbewertung_von_taetowiermitteln.pdf

BfR-Stellungnahme Nr. 033/2011 Tattoo-Entfernung: Einsatz wässriger Milchsäure ist mit gesundheitlichen Risiken verbunden.
http://www.bfr.bund.de/cm/343/tattoo_entfernung_einsatz_waessriger_milchsaeure_ist_mit_gesundheitlichen_risiken_verbunden.pdf

BfR-Stellungnahme Nr. 044/2011 vom 1. Juli 2011, Tätowiermittel können krebserregende PAK enthalten.
http://www.bfr.bund.de/cm/343/taetowiermittel-koennen-krebserregende-pak-enthalten.pdf

1. Sitzung des adhoc Ausschusses „Tätowiermittel“ der BfR-Kommission für Kosmetische Mittel
http://www.bfr.bund.de/cm/343/1_sitzung_des_adhoc_ausschusses_taetowiermittel_der_bfr_kommission_fuer_kosmetische_mittel.pdf

Bundesärztekammer: Richtlinien für die Hämotherapie.
http://www.bundesaerztekammer.de/downloads/rilihaemotherapie2010.pdf

Charnock C (2006) biocidal activity of a bioactive glass-protected, preservative-free tattooing solution. Am J Infect Control 34, 290-295.

Cowan K, Martens MG (1993) Toxic shock syndrome mimicking pelvic inflammatory disease presumably resulting from tattoo. South Med J 86, 1427-1431

Cui W, McGregor DH, Stark SP, Ulusurac O, Mathur SC (2007) Pseudoepitheliomatous hyperplasia – an unusual reaction following tattoo: report of a case and review of the literature. Int J Dermatol 46, 743-745.

Cruz FAM, Lage D, Frigerio RM, Zaniboni MC, Arruda LHF (2010) Reactions to the different pigments in tattoos: a report of two cases. An Bras Dermatol 85, 708-711.

Drews DR, Allison CK, Probst JR (2000) Behavioral and self-concept differences in tattooed and nontattooed college students. Psychol Rep 83, 24-27.

Engel E, Santarelli F, Vasold R, Maisch T, Ulrich H, Prantl L, König B, Landthaler M, Bäumler W (2008) Modern tattoos cause high concentrations of hazardous pigments in skin. Contact Dermatitis 58, 228-233.

Engel E, R Vasold, F Santarelli, T Maisch, NV Gopee, PC Howard, M Landthaler, Bäumler W (2010) Tattooing of skin results in transportation and light-induced decomposition of tattoo pigments – a first quantification *in vivo* using a mouse model. Experimental Dermatology 19, 54–60.

England RW, Vogel P, Hagan L (2002) Immediate cutaneous hypersensitivity after treatment of tattoo with Nd:YAG laser: a case report and review of the literature. Ann Allergy Asthma Immunol 89, 215-217.

Ferguson J, Andrew S, Jones C, August P (1997) The q-switched neodymium:YAG laser and tattoos: a microscopic analysis of laser-tattoo interactions. Br j Dermatol 137, 405-410.

Gopee NV, Cui Y, Olson G, Warbriton AR, Miller BJ, Couch L, Wamer WG, Howard PC (2005) Response of mouse skin to tattooing: use of SKH-1 mice as a surrogate model for human tattooing. Toxicol Appl Pharmacol 209, 145-158.

Hermida MD, Otero M, Della Giovanna P, Garcia S, Cabrera HN (2007) Cutaneous vasculitis following an intradermal tattoo. JEADV 21, 1253-1202.

Hessert MJ, Devlin J (2011) Ink sick: Tattoo ink hypersensitivity vasculitis. Am J Emerg med 29, 1237.e3-1237.e4.

Høgsberg T, Hutton Carlsen K, Serup J (2012) High prevalence of minor symptoms in tattoos among a young population tattooed with carbon black and organic pigments. JEADV DOI: 10.1111/j1468-3083.2012.04590.x

Ichikawa H, Armstrong RB, Harber LC (1981). Photoallergic contact dermatitis in guinea pigs: Improved induction technique using Freund's complete adjuvant. Journal of Investigative Dermatololgy **76**, 498-501.

Institut für Demoskopie Allensbach (2003) Körperkult bei Jüngeren: Tattooos und Piercings. Allensbacher Berichte Nr. 24

ISO/FDIS (2009) Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization. International Standard ISO/FDIS 10993-10. Final Draft 2009.

Jolly M, Danila MI (2003) Tattoo: inflicted vasculitis? J Clin Rheumatol 13, 49.

Kaatz M, Elsner P, Bauer A (2008) Body-modifying concepts and dermatologic problems: tattooing and piercing. Clin in Dermatol 26, 35-44.

Kaur RR, Kirby W, Maibach H (2009) Cutaneous allergic reactions to tattoo ink. J Cosmet Dermatol 8, 295-300.

Kazandjieva J, Tsankov N (2007) Tattoos: dermatological complications. Clin in Dermatol 25, 375-382.

Kent KM, Garber EM (2011) Laser tattoo removal: a review. Dermatol Surg 38, 1-13.

Kluger N. (2010) Cutaneous complications related to permanent decorative tatooing. Expert Rev Clin Immunol 6, 363–71.

Kluger N, Godeneche J, Vermeulen C (2011) Granuloma annulare within the red dye of a tattoo. J Dermatol , 191-193.

Kluger N, Koljonen V (2012) Tattoos, inks and cancer. Lancet Oncol 13, e161-e168.

Klügl I, Hiller K, Landthaler M, Bäumler W (2010) Incidence of health problems associated with tattooed skin: a nation-wide survey in German-speaking countries. Dermatology 221, 43-50.

Koh MJA, Teo RYL, Liu TT (2009) Multiple epidermal cysts occurring in a tattoo. Singapore Med J 50, e376-e377.

Kuperman-Beade M, Levine VJ, Ashinoff R (2001) Laser removal of tattoos. Am J Clin Dermatol 2, 21-25.

Laumann AE, Derick AJ (2006) Tattoos and body piercings in the United States: a national data set. J Am Acad Dermatol 55, 413-421.

Mataix J, Silvestre JF (2009) Cutaneous adverse reactions to tattoos and piercings. Act Dermosifiliogr 100, 643-656.

Messahel A, Musgrove B (2009) Infective compliations of tattooing and skin piercing. J Infect Publ Health 2, 7-13.

Moschos MM, Guex-Crosier Y (2004) Retinal Vasculitis and cystoid macular edema after body tattooing: a case report. Klin Monatsbl Augenheilkd 221, 424-426.

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)
http://www.coe.int/t/e/social_cohesion/soc-sp/ResAP_2008_1%20E.pdf

Ross EV, Yashar S, Michaud N, Fitzpatrick R, Geronemus R, Tope WD, Anderson RR (2001) Tattoo darkening and nonresponse after laser treatment. Arch Dermatol 137, 33-37.

SCCS (2010) the SCCS's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation, 7th revision, Adopted by the SCCS during the 9th plenary meeting of 14 December 2010. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_004.pdf

Sullivan TP, WH Eaglstein, SC Davis, P Mertz (2001) The pig as a model for human wound healing. Wound Repair and Regeneration. The International Journal of Tissue Repair and Regeneration **9** (2), 66-76.

Taylor CR, Anderson RR, Gange RW, Michaud NA, Flotte TJ (1991) Light and electron microscopic analysis of tattoos treated by Q-switched ruby laser. J Invest Dermatol 97, 131-136.

Vasold R, Naarmann N, Ulrich H, Fischer D, König B, Landthaler M, Bäumler W (2004) Tattoo pigments are cleaved by laser light -The chemical analysis *in vitro* provides evidence for hazardous compounds. Photochem Photobiol 80, 185-190.