First Meeting of the Ad Hoc Committee "Tattooing Agents" of the BfR Cosmetics Committee

Minutes, 4 November 2009

The ad hoc working group "Tattooing Agents" of the BfR Committee for Cosmetics held its first meeting in November 2009. Just six months after the entry into force of the Tattooing Agents Ordinance in Germany, an expert body has been set up to look at the concrete requirements for safe tattooing agents. Besides scientific foundations and analytical methods, health aspects and the need for further regulatory measures were also discussed at the meeting.

1 Introduction

Tattooing is a process whereby substances or preparations are introduced into or under human skin to permanently influencing its appearance. They include decorative tattoos and permanent make-up (PMU). Whereas in the case of decorative tattoos pigments are applied to the middle dermis, in the case of permanent make-up they are only intended to reach the less deep papillary layer. As, however, the density of skin layers can vary considerably, exact application is not possible technically. Hence, a distinction between tattoos and permanent make-up is not possible on this basis in practice. Henna tattoos, also called temporary tattoos, do not come under the definition for tattoos but are called body paints. They are covered by the Cosmetics Directive and the German Cosmetics Ordinance. Henna has not, however, been approved for this application.

According to the statistics, 9 % of the population in Germany have tattoos. In the group of 16 to 29-year-olds this figure is 23 %. Estimates suggest that roughly 20 % of people with tattoos are minors. For decorative tattoos mostly organic pigments are used like mono-azo laked pigments, di-, tri- and poly-azo compounds and polycyclic pigments like phthalocyanines with great colour brilliance. Iron oxides and carbon blacks are used for permanent make-up. The problematic ingredients in tattooing agents are carcinogenic aromatic amines that may occur as the cleavage products of organic dyes or as impurities, as well as heavy metals and other contaminants, preservatives (for instance benzoisothiazolinone, 2-methyl-4-isothiazoline-3-one, benzoic acid, salicylic acid, phenol) and a number of ingredients with, in some cases, unclear functions like diethylene glycol, methyl salicylate, hexachlorobenzene, shellac or essential oils. Furthermore, some tattooing agents come with special effects like glow in the dark where the ingredients are largely unknown.

Acute adverse reactions in conjunction with tattoos are infections, foreign body reactions, scarring or allergic reactions. The long-term effects of tattooing agents are largely unknown. This raises questions about the possible cleavage of dyes under the skin and the possible transport of pigments and cleavage products to other organs. Pigments have already been detected in the lymph nodes of tattooed individuals. It is likewise unclear whether malignant tumours in tattooed areas of skin have been caused by the tattoos.

The removal of tattoos is not unproblematic either. During laser treatment the pigments are cleaved and, as a rule, rendered invisible. Recently, tattoo removing fluids have become available which are injected under the skin. Cases of intoxication after using fluids of this kind have already been notified to BfR.

Microbial impurities play a major role in tattooing agents. Local health authorities are responsible for hygiene aspects.

2 Regulatory status

Tattooing agents are not regulated by European laws. The Resolutions ResAP(2003)2 on tattoos and permanent make-up and ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up of the Council of Europe are the basis for national regulations in various Member States. The Tattooing Agents Ordinance entered into force in Germany on 1 May 2009. This Ordinance prohibits the use of pigments that can be cleaved into listed carcinogenic aromatic amines. Furthermore, substances which are prohibited (Annex 1 Cosmetics Ordinance – KVO) or restricted for use in cosmetics (Annex 3 Part A Column F, Cosmetics Ordinance – KVO) as well as pigments on a negative list may not be used for tattooing agents.

The planned first amendment to the Tattooing Agents Ordinance may, in future, require manufacturers to undertake a safety assessment of their products. This constitutes the corresponding enforcement of a *Bundesrat* resolution which was adopted when the Ordinance entered into force.

3 Tattooing agents in official monitoring

Analytical methods are currently being developed by the "§ 64 – Working Group" in order to be able to monitor in future tattooing agents in line with the requirements of the Tattooing Agents Ordinance. The focus here is on inorganic (ICP-MS detection) and organic pigments (MALDI-TOF, HPLC-DAD, TLC scanner). Furthermore, methods are being developed to detect nitrosamines (LC-MS), PAHs (HPLC-fluorescence detection) and preservatives. What is particularly important here is a uniform method for the detection of arylamines whereby as realistic as possible conditions are to be simulated for reductive azo cleavage. The recording of photo- induced cleavage constitutes a special challenge, too. As in the case of cosmetics, element analysis is performed using pressure decomposition in the microwave at 200°C under 65 % nitric acid plus 37 % hydrochloric acid.

Within the framework of the Nationwide Monitoring Plan tattooing agents were examined in 2007 for heavy metals (arsenic, antimony, lead, cadmium, chromium, cobalt, copper, nickel, mercury) and for preservatives (benzoic acid, salicylic acid, phenol, phenoxyethanol, 1,2-benzisothiazoline-3-one [BIT]). Eight federal states took part in this project involving a total of 148 samples. 782 sub-samples were examined for a maximum of 23 preservatives. This resulted in a total of 53 positive cases (6.7 %) in which eight preservatives were detected; benzisothiazolone or 1,2-benziso-thiazoline-3-one was detected in 37 cases. When it comes to medium levels benzisothiazolone (26.9 mg/kg) benzoic acid (18.6 mg/kg), 2-methyl-4-isothiazoline-3-one (43 mg/kg) and 2-octyl-2H-isothiazole-3-one (19 mg/kg) are the dominant preservatives. The maximum levels of these four preservatives also produce a relatively similar graduation. 878 sub-samples were examined for a maximum of 13 heavy metals. There were 342 positive cases. For heavy metals copper was dominant with a medium level of 4,652 mg/kg, followed by iron (79.2 mg/kg), chromium (29.5 mg/kg) and zinc (16.3 mg/kg). The other heavy metals tin, lead, manganese, selenium, arsenic, thallium, mercury and uranium were found to have lower levels. Three out of the 32 samples examined for phenol tested positive. Concentrations of between 308 and 4736 mg/kg were detected. Furthermore, studies on the microbiological status of tattooing agents were conducted which are not described in detail here.

Co-ordinated sample collection was undertaken in Switzerland in January and February 2009 whereby samples were taken from opened and unopened original containers with tattooing

agents in tattoo studios and beauty salons. Tattooing agents are regulated in the Swiss *EDI Ordinance for objects that come into contact with mucosa, skin and hair as well as candles, matches, lighters and joke articles (Human Contact Ordinance,* HKV SR 817.023.41*)*. The chemical and microbiological requirements to be met by tattooing agents and PMU dyes were largely derived from the Council of Europe resolution “Resolution RAP (2002) 2 on tattoos and permanent make-up”. In Switzerland preservation by means of a preservative listed in the Cosmetics Ordinance that remains on the skin is permissible; fragrances and aromatic substances are prohibited. A total of 152 samples were examined including 105 tattooing agents from 26 manufacturers and 47 PMU dyes from 18 manufacturers.

Organic pigments were analysed primarily using MALDI-TOF and, for the purposes of validation where appropriate, RP-HPLC after dilution and extraction in dimethyl formamide. In the case of pigments with insufficient solubility in organic solvents, the samples were diluted with sulphuric acid and measured using UV spectroscopy. Besides poor solubility the major problem for analysis was procuring the reference substances. A standardised method for textiles is available for the detection of prohibited azo dyes. The reduced extracts were directly analysed without cleanup using LC/MS/MS. An LC/MS/MS method was used to analyse nine carcinogenic N-nitrosamines whereby extraction was performed with water. To confirm N-nitrosodiethanolamine the positive samples were analysed using a second LC/MS/MS method with column switching in order to separate the precursor substances from the actual separating column. Furthermore, four analytical methods were used to detect preservatives from the cosmetics area. These methods have already been repeatedly tested in tattooing agents and PMU dyes, and their transfer was found to be unproblematic in most cases. The determination of more than 40 UV-active preservatives following extraction with methanolic formic acid was done with HPLC/DAD. This method was also used for screening for dibutyl, benzylbutyl and diethylhexyl phthalate and for UV-active fragrances and other ingredients. The polar preservatives methylisothiazolinone, methylchlorisothiazolinone and benzisothiazolinone were identified and quantified following extraction with aqueous or aqueous-methanolic formic acid again using HPLC/DAD.

Overall, 35 % of the samples examined (30 % of the tattooing agents and 49 % of the PMU dyes) were objectionable; 41 % (54 % of the tattooing agents and 11 % of the PMU dyes) were the subject of an application ban. Objections were made to samples with nitrosamine levels between 10 and 150 µg/kg, with a combination of preservatives, declaration shortcomings and germ contamination. Inadequate labelling was the second most frequent reason for the objections: Substances were declared which could not be detected in the products and substances were detected that hadn’t been declared. Declarations were made that various “surfactants”, “emulsifiers” or “dispersing agents” were used as auxiliaries to maintain colour suspension without the substances used being described in detail. In various products dyes were declared which are only approved in cosmetic products with short-term skin contact (so-called rinse off products) or were not approved at all.

Application bans were issued for prohibited pigments (20 % of samples), levels of octhilinone, benzisothiazolone or phenol > 50 mg/kg (14 % of the samples), detected aromatic amines following azo cleavage > 30 mg/kg (6 % of samples), nitrosamine levels > 150 µg/kg (7 % of samples) and for germ counts higher than 1,000 germs/ml (3 % of samples).

In a study conducted five years ago concentrations of 50 mg/kg cinnamyl alcohol were detected in around 10 % of the samples.

4 Health complications from tattoos

The trauma of material introduction can lead, in the case of predisposed individuals, to exuberant scarring (keloid). Furthermore, there may be formation of foreign body granulomas, sarcoidosis lesions and pseudolymphomas after tattooing. The corresponding ingredients can trigger sensitisation or – in the case of already sensitised individuals – a contact allergy. Phototoxic reactions in conjunction with tattooing have likewise been described. After tattooing, the risk of endocarditis in patients with congenital cardiac defects is elevated. The occurrence of transcribed psoriasis vulgaris or red plane lichen foci has also been observed after tattooing in the area treated. There have been sporadic reports of the occurrence of malignant or semi-malignant tumours in tattoos. Whether the plate epithelium and basal cell carcinomas and malignant melanomas were actually caused by tattooing (isotopic stimulus) or whether this is a pure coincidence is something that in many cases remains open.

Hollow needles (like for instance epidurals) can transport pigments from the lumbar dermis into deeper lying tissue areas. This can lead to subarachnoidal, subdural or peridural neurologically relevant inflammatory or granulomatous tissue reactions.

Several cases of adverse reactions have occurred for instance in the USA in conjunction with the pigment C.I. 73360 (D&C Red 30, Vat red 1). In the case of patients whose lip contours were changed by means of permanent make-up containing this pigment, inflammatory, dispersed immediate reactions occurred as well as positive reactions to the dye in the prick test. The conclusion, therefore, is that the inflammation can be attributed to the pigment. C.I. 73360 is a thioindigo dye which could be reducible and soluble in the skin. As C.I. 73360 is prohibited pursuant to Annex 1 to the KVO, it may no longer be used in Germany for tattooing agents.

All experts see a need for comprehensive epidemiological data on adverse reactions after tattooing. What are missing more particularly are indications of a causal relationship between the effects observed and the trigger substances. When it comes to sensitisation, tattoos have been included by IVDK in the list of contact substance categories.

5 Scientific aspects and current research

Analytical methods

The EN 14362 standard, which was developed for textile dyes, is frequently used to detect aromatic amines. However, the pigments in tattooing agents are frequently not dissolved under the conditions in this standard and are not, therefore, accessible for cleavage. This applies for instance to the pigments C.I. 21095, 21110, 11741, 21115 and 21160 for which, given their chemical structure, highly positive results would have been expected, at best however traces of carcinogenic amines. Here we do not know whether the pigments are more readily soluble in the dermis than in the reaction medium (citrate buffer pH 6). When detecting pigments using HPLC, it should also be borne in mind that only some of the dye is dissolved and can be detected. HPLC tests involving various pigments showed, beside the expected main peak, numerous peaks for further, in some cases unknown, ingredients. Sometimes it was questionable whether the main peak really depicted the main pigment. Work is currently underway on a suitable, harmonised and recognised detection method for aromatic amines.

Impurities and breakdown by cleavage

The aromatic amines 3,3'-dichlorobenzidine, aniline, o-toluidine and 2,4-nitrotoluolene were detected in pigment samples after they had been radiated with laser light. What was notice-

able, however, was that these amines could not be detected at all or only in low concentrations after reductive cleavage. Aromatic amines can be impurities or residues of the starting products or also cleavage products of the corresponding organic pigments. Moreover, further substances can also be formed during laser radiation whose toxicological potential is in some cases unknown. For instance 4-chloro-2,5-dimethyl-acetanilide and 4-chloro-2,5-dimethyl-acetoacetanilide (from CI 21108) are formed after laser radiation. The extent to which enzymes in the skin promote pigment cleavage is not yet known.

Studies with Pigment Red 22 showed that the aromatic amines 2-methyl-5-nitroaniline and 4-nitrotoluolene had been detected in pigment samples. After laser radiation they could be detected in around 20 times higher concentrations. Furthermore, there were other unknown substances. Temperature-induced cleavage of this pigment at 400°C led to a different spectrum from light-induced cleavage using laser. Sunlight and UV-B radiation, in contrast, resulted in a comparable spectrum of cleavage products to laser radiation. A solution of Pigment Red 22 could be decoloured by UVB (13J/cm^2) or through the action of direct sunlight over 100 days. Sunlight-induced cleavage could also be detected for Pigment Yellow 74. This means that in the case of tattoos in skin areas exposed to the sun, the cleavage of pigments can be assumed.

PAHs were determined in various black tattooing agents in concentrations up to 200 µg/g. However, there were also samples where the PAH levels were below the detection threshold. It is pointed out that only approximately six of the 200 carbon blacks from the biggest German manufacturer have FDA approval. For them the PAH concentrations are below 0.5 ppb.

As a rule, nitrosamines in organic pigments are impurities from the coupling reaction of the azo ingredients using nitrosating agents and could be removed by means of additional clean-up steps. The precursors, diethanolamine and morpholine, are nitrosated by inorganic pigments (above all iron pigments).

Nanoparticles

In commercially available black tattooing inks particles of around 40 nm in size could be detected under a transelectron microscope. The extent to which primary particles agglomerate depends on the coating and on the production method. The behaviour of nanoscale particles in the skin is a subject matter of further research. It is likely that these particles could be surrounded by proteins *in vivo* or transported to the lymph nodes.

Laser therapy

By means of laser radiation tattoos can be removed more or less successfully. To this end between six and eight sessions are necessary. Ultra-short laser impulses of 10 ns and an intensity of 10^8 W/cm² lead to temperatures of around 800°C and thermolysis of the target structures. Depending on the pigment, laser rays of between 510 and 1064 nm are used. As a rule, black pigments are easily cleavable, red pigments less so. Yellow pigments do not absorb anything in the radiation range and cannot, therefore, be cleaved. Directly after treatment there may be a short-term formation of vacuoles in the corresponding skin areas and then whitening. What is problematic when it comes to whitening is that inorganic dyes from permanent make-up may even darken in some cases after laser treatment.

Amounts of pigment applied and pigment transport in the skin

The amounts of pigment introduced into the skin during tattooing were determined in an *ex vivo* pigskin model and in human skin samples from skin biopsies (forensic medicine). Depending on the experience of the tattooer the values were between 0.6 and 2.5 mg/cm^2. On average one can assume input amounts of 1 mg/cm^2 whereby pigment properties like, for

instance, covering power play an important role. Dye pigments introduced into the skin are largely stored intracellularly whereby they may also be transported via blood vessels and lymph passages to other areas in the body. In the case of a patient with a melanoma in a tattooed area of skin, the pigments were also found in the lymph nodes. In the meantime comparable case studies have been repeatedly published in the scientific literature. Using a nude mouse model tests were also carried out of the transport behaviour of pigments in the skin. To this end, the mice were given a tattoo on their backs. Immediately after tattooing the skin showed puncture tracks which had, however, healed fully after four weeks. On average 584 µg pigment were applied to each animal. The pigment concentration had fallen by 32 % after 42 days. If the animals were also exposed to sunlight or laser radiation, the pigment concentration fell by 60 and 51 % respectively. Other tests with further skin models, also *in vivo*, should be conducted in order to undertake a better assessment of the situation for man.

At Regensburg University an Internet-based survey was conducted with approximately 4,500 tattooed individuals. Information on the number and tattooed area revealed that on average tattooed skin areas between 300 and 400 cm^2 can be assumed. There are, however, some individuals who have more than eight tattoos or have individual tattoos which are bigger than 900 cm^2. In the case of an average input amount of 1 mg/cm^2 this does indeed lead to toxicologically relevant exposure to pigments and other ingredients from tattooing agents. The survey showed that most customers are happy with their tattoos. 8 % don't want any more tattoos and 5 % (extrapolated in Germany on the basis of 400,000 up to 500,000 persons) would like to have their tattoos removed. Ongoing health disorders occurred in 6 % of the tattooed individuals. They include persistent swelling and constant irritation, e.g. through contact with textiles or also the action of light. What was noticeable was that the health impairments occurred more frequently in the case of colour tattoos than in the case of black ones. One-third of the respondents indicated that they had been sufficiently informed beforehand in the studio about the dyes. But around 40 % missed detailed information.

6 Pigments for tattooing agents

Some experts demand a number of requirements for tattooing agents. They should be easy to process (trend towards watery), offer even coverage and have a brilliant appearance once healed. This means water-soluble, highly colouring, colour-fast products with high light fastness and good dispersity. Tattooing agents must be safe (not phototoxic, no allergenic potential, high purity, no harmful cleavage products). Classical formulations with water, alcohol and glycerine are used as well as "tusche" formulations on a shellac base, formulations on a water base with surfactants or formulations on an acrylic basis (acrylates).

The pigments used are titanium dioxide (white), carbon (black), iron oxide (mainly PMUs), copper phthaloxyanines (green / blue), chromium oxide (green/blue, mainly PMUs), oxazines (violet), monoazo and disazo compounds, aminoketones, anthraquinones, indigoids (yellow, orange red).

The following problems are touched on by some experts:

- Availability of pure pigments (even cosmetic grade is sometimes contaminated)
- Availability of toxicological data
- No validated test methods
- Applicability of DIN EN ISO 10993
- Problem of animal experiments
- No uniform legal provisions in Europe

- No defined requirements in terms of safety assessment
- Unprofessional workers / producers have no knowledge
- Unsuitable, false certificates
- No control of suppliers or studios

When it comes to the requirements to be met by pigments for tattooing agents, it has been proposed that the same procedure should be adopted as for the establishment of criteria for fingerpaints. The following requirements were deemed to be important:

- No use of pigments classified as CMR substances in Categories 1, 2 or 3 or IARC Class 1 or 2;
- No use of dyes which are classified as highly toxic, toxic, harmful, corrosive, irritating or sensitising;
- Limit values for carcinogenic aromatic amines, heavy metals and harmful contaminants and ingredients.
- When drawing up the criteria it should, however, be borne in mind that 100 % bio-availability can be assumed for tattooing agents as they are applied under the skin.

7 Discussion: Requirements to be met by safe tattooing agents

The Tattooing Agents Ordinance is a first step towards regulating tattooing agents in the context of consumer protection. In the long term, however, the experts see a need for further improvements. They concern the following aspects:

Auxiliaries and additives
Only the necessary safe auxiliaries and ingredients should be used for tattooing agents.

Preservation
Tattooing agents must be sterile. This should be ensured by law. Here it may make sense to draw up a list of suitable preservatives. Substances with allergenic potential should not be used. The use of antimicrobial substances that have not been approved for cosmetic products like benzisothiazolinone, octylisothiazolinone and phenol should be banned.

Purity
The values for technologically unavoidable levels of impurities should be specified. Here recourse could be made to the Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up of the Council of Europe where technically unavoidable levels have already been established. These values are based on health requirements and take into account the latest technically feasible status. In some cases they have been taken over from requirements in the field of medicine (e.g. for parenterally administered drugs). Particularly strict requirements should be formulated for nickel which is of major health relevance as a potent allergen.

Cleavage products
It should be borne in mind that pigments can also be cleaved by UV light. To that end, pigments which can form harmful cleavage products should be placed on the negative list. Furthermore, consideration should be given to cleavage products which can be formed from laser radiation.

Negative versus positive lists

In the long term, the negative lists are seen as unsatisfactory as all dyes may be used whose use is not restricted by the Cosmetics Ordinance and which do not release any carcinogenic aromatic amines after reductive cleavage in the tattooing and PMU dyes although they have not been toxicologically tested for tattooing purposes.

In the case of the following pigments, there are specificities concerning their current regulation:

Pigment Yellow 1 (CI 11680), Pigment Green 7 (CI 74260) and Pigment Orange 43 (CI 71105) are prohibited for use in tattooing agents because of restrictions in place for cosmetics (no application to mucosa or eyes).

Pigment Yellow 74 (CI 11741), Pigment Red 22 (CI 12315), Pigment Red 146 (CI 12485), Pigment Brown 25 (CI 12510) and Pigment Orange 34 (CI 21115) are not currently covered by the provisions whereby Pigment Yellow 74 has an o-anisidine building block and Pigment Orange 34 a 3,3´-dichlorobenzidine building block. The -3,3´-Pigments CI 21095, CI 21110, CI 11741, CI 21108 and CI 21160 consist of azo building blocks and are not currently regulated.

Pigment Blue 15 (CI 74160) has been prohibited since October 2009 as a hair dye as no dossier was submitted for evaluation.

Some experts regret the restrictions for Pigment Blue 15 and Pigment Green 7 as these are important pigments which cannot be easily replaced. It was pointed out that the substitutes (thioindigoids and Berlin Blue) are not safer from the toxicological angle and furthermore are not popular because of their low brilliance.

For black pigments (carbon blacks) the extent to which additional requirements e.g. concerning PAH contamination or particle size are necessary should be clarified.

Assessment of tattooing agents

Safety assessments were deemed to be urgently required for tattooing agents. The committee members agreed that given the special application form for tattooing agents more extensive requirements should be put in place than for cosmetics. Suitable test models would also have to be developed which reliably reflect the situation after introduction into the dermis from the angle of kinetics and stability, and where also potential removal e.g. via the lymph system is taken into account. However, this will require further deliberations. The following requirements were initially formulated for all ingredients:

Physico-chemical characterisation (at least in the case of hair dyes)
Purity
Impurities (e.g. heavy metals, aromatic amines)
Auxiliaries
Stability / photostability (UV, enzymes, laser)

Toxicological data
Skin irritation, skin corrosion
Mucosal irritation, mucosal corrosion
Immunotoxicity (sensitisation, photosensitisation)
*In vitro** genotoxicity including cleavage products and photogenotoxicity

Further details
E.g. *in vivo* data following subcutaneous application*
Kinetics

* Minimum requirements