2nd International Conference on Tattoo

18–19 November 2021, Berlin

Imprint

BfR Abstracts

2nd International Conference on Tattoo Safety

All authors are responsible for the content of their respective abstracts.

Federal Institute for Risk Assessment
Max-Dohrn-Straße 8–10
10589 Berlin
Germany

Berlin 2021
28 Pages

Table of Contents

Dear readers,

Much has been achieved since the German Federal Institute for Risk Assessment (BfR) has organised the first symposium on tattoo safety in 2013. Thanks to excellent research works worldwide, we understand better what health risks may be posed by ingredients of tattoo inks. Yet, major data gaps still exist. Taken the growing number of tattooed individuals, especially young people, the establishment of risk assessment measures is needed more than ever.

The BfR is continuously engaged in addressing these topics at scientific and regulatory levels. Our work focuses on the assessment of health risks associated with tattooing, the use of human data and the development and validation of analytical methods for tattoo pigments and their degradation products. Our results are published as expert opinions and scientific papers. At this point, it is our pleasure to announce the establishment of the BfR-Committee on tattoo inks. The committee will assemble international scientists of different areas. It will act independently and advise the BfR with regard to the risk assessment of tattoo inks at the highest scientific level.

The 2nd International Conference on Tattoo Safety of the BfR brings together experts of toxicology and regulation, clinicians, experts in analytics, as well as representatives of the industry. We look forward to insightful reports on the latest scientific achievements and to fruitful discussions towards better consumer protection in the field of tattoo safety.

Sincerely,

Professor Dr Dr Andreas Hensel
President of the BfR

Professor Dr Tanja Schwerdtle
Vice President of the BfR

1 Programme

Thursday, 18 November 2021

Presenter: Katja Nellissen

I Opening

09:00–09:10 am
Welcome
Professor Dr Tanja Schwerdtle, German Federal Institute for Risk Assessment (BfR), Berlin, Germany

09:10–09:20 am
Welcome
Dr Helmut Tschiersky, Federal Ministry of Food and Agriculture (BMEL), Berlin, Germany

09:20–09:30 am
Introduction and objective of the conference
Professor Dr Dr Andreas Luch, BfR

09:30–10:10 am
Art at your own risk: on tattoos and their removal by Christian Warlich
Dr Ole Wittmann, Institut für deutsche Tattoo-Geschichte e.V., Hamburg, Germany

10:10–10:30 am Coffee break

II Health Risks of Tattoos: Clinical Evidence
Session Chair: Dr Peter Laux, BfR

10:30–10:55 am
Tattoo pigments in skin and body –
transportation processes boosted by laser light and UV radiation
Professor Dr Wolfgang Bäumler, University Hospital Regensburg, Germany

10:55–11:20 am
Complications of tattoos and permanent make-up: an overview
Dr Sebastiaan van der Bent, Alrijne Hospital Leiden, Netherlands

11:20–11:45 am
Tattoos and cancer: where are we in 2021?
Dr Nicolas Kluger, MD, Helsinki University Hospital, Finland

11:45 am–12:10 pm
Laser and light tattoo removal: principles, risks and efficacy
PD Dr Maja A. Hofmann, Charité, Germany

12:10–01:10 pm Lunch break

III Epidemiology and Risk Assessment
Session Chair: Dr Michael Giulbudagian, BfR

01:10–01:35 pm
Statistical data on inks used in 2019: a historical benchmark
Professor Dr Jorgen Serup, Bispebjerg Hospital,Copenhagen University, Denmark

01:35–02:00 pm
The evolving topic of tattoos in cancer epidemiology and why studies should be prospective
Dr Milena Förster, International Agency for Research on Cancer, Paris, France

02:00–02:25 pm
Allergens in tattoo inks
Dr Steffen Schubert, Information Network of Departments of Dermatology, Göttingen, Germany

02:25–02:50 pm
Gaining *in vitro* and human data on tattoo ink toxicology
Dr Ines Schreiver, BfR

02:50–03:10 pm Coffee break

IV Risk Assessment and Regulation of Tattoo Inks: Chances and Challenges
Session Chair: Dr Agnes Schulte, BfR

03:10–03:35 pm
United States FDA regulatory and analytical perspectives on tattoo inks
Dr Linda Katz, MD, United States Food and Drug Administration (FDA), Maryland, USA

03:35–04:00 pm
EU new regulation on substances in tattoo inks and permanent make-up
Dr Ana Maria Blass Rico, European Commission, Brussels, Belgium

04:00–04:25 pm
The REACH restriction on substances in tattoo inks or permanent make-up – reflections from a consumer representative
Dr Franz Fiala, Consumer Council, Vienna, Austria

04:25–04:50 pm
Requirements for the risk assessment of tattoo inks: chances and challenges
Dr Peter Laux, Dr Michael Giulbudagian, BfR

Friday, 19 November 2021

Presenter: Katja Nellissen

09:00–09:10 am
Summary of the first day
Professor Dr Dr Andreas Luch, BfR

V Analytics and Enforcement
Session Chair: Dr Ines Schreiver, BfR

09:10–09:35 am
Semi-quantitative analysis of organic pigments in tattoo Inks with HPLC/DAD – work in progress
Dr Urs Hauri, Kanton Basel-Stadt,Kantonales Laboratorium, Basel, Switzerland

09:35–10:00 am
Analysis of tattoo inks in Germany:current status and future challenges
Dr Birgit Gutsche, Chemical and Veterinary Investigation Office Karlsruhe, Germany

10:00–10:25 am
Analytical methods and results on metallic contamination, including nanoparticles, in tattoo inks purchased in Italy
Dr Beatrice Bocca, Beatrice Battistini, Italian National Institute of Health (ISS), Rome, Italy

10:25–10:50 am
Preservatives in tattoo and PMU inks in the frame of REACH regulation: results of an Italian study
Dr Marco Famele, Italian National Institute of Health (ISS), Rome, Italy

10:50–11:10 am Coffee break

11:10–11:35 am
Industry meets authority – different perspectives, common goal
Veit Houben, CTL® GmbH Chemical-technological laboratory, Bielefeld, Germany

11:35 am–12:00 pm
Raman spectroscopy in the measurement of tattoo inks – opportunities and challenges
Dr Katarzyna Karpienko, Gdańsk University of Technology, Poland

12:00–12:25 pm
Identifying tattoo pigments in human skin samples with adverse reactions based on µXRF and LDI-MS imaging and mass spectral library matching
Dr. Corinna Brungs, Carina Wolf, University of Münster, Germany

12:25–01:30 pm Lunch break

VI Stakeholder Positions

01:30–01:40 pm
Regulatory approaches on PMU colours inside & outside the EU
Dr Cornelia Hildebrandt, MT DERM GmbH, Berlin, Germany

01:40 am–01:50 pm
Optimising consumer safety on all stages of the tattooing process
Dr Olaf Seidel, edding TATTOO, Hamburg, Germany

01:50–02:00 pm
Tbc
Sean Brown, General Manager, Eternal Ink, LLC, Michigan, USA

02:00–02:10 pm
Tattoo colours: don't overcolour
Dr Mark Benecke, Pro Tattoo e. V., Essen, Germany

02:10–02:20 pm
REACH – tattoo industry shutdown 2022?
Ralf Michel, Tattoo ink manufacturers of Europe, Neuburg am Rhein, Germany

02:20–02:30 pm
Tattoo 2030
Urban Slamal, Bundesverband Tattoo e. V., Düsseldorf, Germany

02:30–02:50 pm Coffee break

VII Panel Discussion and Farewell
02:50–04:00 pm

2 Abstracts

2.1 Tattoo pigments in skin and body – transportation processes boosted by laser light and UV radiation

Professor Dr Wolfgang Bäumler
University of Regensburg, Department of Dermatology, Germany

Tattooing entails the injection of colourant mixtures into skin mainly using the solid needles of tattoo machines. The major ingredient of tattoo colourants are colouring inorganic or organic pigments, which are black, white, or coloured. One group of inorganic pigments is based on iron oxides, another group contains heavy metals such as cadmium, mercury, or chromium. Its use declined due to hazardousness of such heavy metal compounds. Two important inorganic pigments are still in use: titanium dioxide for tempering and carbon black for black tattoos. About 60 % of tattoos are either completely or partly black. Today, more than 80 % of the tattoo colourants contain industrial organic pigments with azo or polycyclic structures.

Commercially available pigments are tiny, solid state particles with diameters of a few tenths of nanometres (nanoparticles) up to a few micrometres. To achieve a workable tattoo colour, the respective pigment particles are suspended in a complex solvent mixture yielding so-called tattoo suspensions. Tattooists usually purchase ready to use suspensions which may contain up to hundred substances. Thus, the suspensions consist of the respective pigment, educts and decomposition products of pigment synthesis, solvents, emulsifier, anti-foam agents, preservatives and other admixtures and various impurities.

Directly after tattooing, the initial concentration of tattoo pigment in the skin is in the range of 2.5 mg per cm². Subsequently, the concentration of tattoo colourant declines due to exudation via perforated skin and transportation in blood or lymphatic vessels to other organs inside the body. The time response and the extent of both processes are almost unexplored except for a few animal studies. First investigations showed that large amounts of pigments are located in the loco-regional lymph nodes and less amount in the liver. It is assumed that a part of the injected pigments and other ingredients of tattoo colourants could be eliminated by the excretion organs of the body. After days and weeks, the pigment concentration in the tattooed skin seems to reach a final value representing the respective tattoo.

However, it is known that most of the used pigments may decompose upon exposure to solar radiation that includes the high-energy radiation in the ultraviolet spectral range from 280 to 400 nm. This UV radiation may slowly continue to decompose the pigments inside the tattooed skin once it is exposed to any source of UV radiation.

In addition, very short and intense laser pulses are applied in case tattooed individuals regret tattooing and seek for tattoo removal. A major mechanism of tattoo removal is laser assisted fragmentation of pigment particles, which are then transported away from skin. For many years, Q-switched lasers with nanosecond pulse durations at high light intensities have been applied to cause such fragmentation via rapid heating up while sparing the adjacent tissue. Despite the long-lasting use of such laser treatment, the exact mechanisms of laser assisted fragmentation are hardly investigated. Due to short and intense laser pulses applied, non-linear effects of light (e.g. photoacoustic, optical breakdown) and nonlinear thermal properties in tattoo particles may play a crucial role. UV radiation and laser pulses are known to produce new chemical compounds in skin and comparably to situation after tattooing, the same mechanisms may transport these compounds to other organs.

In conclusion, tattooing of colourants into skin as well as any light or radiation process afterwards entail a complex reaction of the skin that triggers the immune system and launches manifold transport processes, which might pose additional health risks not only to skin but also to other organs of tattooed individuals.

2.2 Complications of tattoos and permanent make-up: an overview

Dr Sebastiaan van der Bent, MD
Alrijne Hospital, Tattoo poli (Tattoo Clinic), Department of Dermatology, Leiden, The Netherlands

Background: Worldwide 10–20 % of the population is tattooed. However, tattoo complications can occur, such as allergic tattoo reactions, infections and manifestations of autoimmune dermatoses. Despite the growing popularity of tattoos and changes in tattoo ink composition over the last decades, little is known about these complications, its clinical aspects, pathomechanism and relative occurrence.

Objective: The aim of this study is to describe the types and clinical aspects of dermatological tattoo complications, its relative occurrence and underlying conditions.

Methods: We performed a retrospective cohort study enrolling all patients with tattoo complications. Tattoo complications were categorised into infections, inflammatory tattoo reactions, neoplasms or miscellaneous reactions and correlated to clinical data.

Results: Of the total of 326 patients, 301 patients were included with 308 complications. The majority of the complications were chronic: 91.9 %. In our study, 78.2 % of all complications were inflammatory reactions including allergic reactions, autoimmune dermatoses and chronic inflammatory black tattoo reactions (CIBTR). Allergic red tattoo reactions and CIBTR accounted for 50.2 % and 18.2 % respectively of all complications. Of these CIBTR reactions, extracutaneous involvement was found in 21.4 %, including tattoo-associated uveitis (7.1 %) and systemic sarcoidosis (14.2 %). Of all black tattoo reactions, systemic sarcoidosis was found in 7.8 %. Infections occurred only in 3.3 % and included impetigo, mycobacterial infection and *verrucae vulgares* and *planae*. Neoplasms accounted for 0.6 % of the cases (basal cell carcinoma) and miscellaneous complications 14.3 % including blow-outs, neurosensoric tattoo reactions, photo induced complications, scars and keloids and traumatic tattoos. Laser tattoo removal induced complications included blistering, hypopigmentation and scarring in 4.5 %. Non-laser tattoo removal, such as by caustic products, accounted for 1.3 % of all tattoo complications.

Conclusion: Tattoos can cause a wide range in complications that may start years after getting the tattoo. The most frequent tattoo reactions are allergic red tattoo reactions and chronic inflammatory black tattoo reactions, making these the most relevant for the dermatologist. CIBTR have a high percentage of multi-organ involvement and therefore screening for sarcoidosis, including ocular involvement, is advised.

van der Bent SAS, Rauwerdink D, Oyen EMM, Maijer KI, Rustemeyer T, Wolkerstorfer A. Complications of tattoos and permanent makeup: overview and analysis of 308 cases. Journal of Cosmetic Dermatology. 2021

2.3 Tattoos and cancer: where are we in 2021?

Dr Nicolas Kluger, MD
Helsinki University Hospital, Finland
Bichat Claude Bernard Hospital, Paris, France

The introduction in the dermis of exogenous pigments and dyes to obtain a permanent design (tattooing) represents a unique *in-vivo* situation, where organic dyes and metallic salts remain in the skin for the lifetime of the bearer. The potential local and systemic carcinogenic effects of tattoos and tattoo inks remain unclear. Several studies have shed light on the presence of potential carcinogenic or procarcinogenic products in tattoo inks. Despite those findings, the number of skin cancers arising in tattoos is seemingly low, and this association has to be considered thus far as coincidental.

We will discuss reevaluate the current knowledge regarding carcinogenicity of tattoos in 2021.

2.4 Laser and light tattoo removal: principles, risks and efficacy

PD Dr Maja A. Hofmann
Charité – Universitätsmedizin Berlin, Germany

The abstract was not available at the editorial deadline.

2.5 Statistical data on inks used in 2019: a historical benchmark

Professor Dr Jorgen Serup
Bispebjerg Hospital, Copenhagen University, Denmark

The abstract was not available at the editorial deadline.

2.6 The evolving topic of tattoos in cancer epidemiology and why studies should be prospective

Dr Milena Förster

International Agency for Research on Cancer, Environment and Lifestyle Epidemiology Branch, Lyon, France

It is well known that tattoo inks may contain substances classified as (probably) carcinogenic to humans. However, as studies underlying this classification did not consider subcutaneous exposure, it remains unknown whether tattoos might cause cancer in humans. To investigate this question, epidemiological evidence is needed showing either an elevated or non-elevated risk in a tattooed vs non-tattooed population to develop certain kinds of cancer.

To date, not even a handful of small case-control studies on tattoos and cancer (skin and lymphatic) have been published and their mixed results need to be regarded with caution: To study a potential carcinogenicity of tattoos, it is crucial to choose the correct study design. Taking into account reflections about the prevalence and nature of the exposure, as well as the dose-response relationship and lag-times to cancer development, the assets of prospective studies in epidemiology and regarding tattoo exposure in particular, will be discussed. Furthermore, the study protocol for the assessment of tattoo exposure within the German and French national cohorts will be presented.

2.7 Allergens in tattoo inks

Dr Steffen Schubert[1], Michael Dirks[2], Heinrich Dickel[3], Claudia Lang[4], Johannes Geier[1]

[1] Information Network of Departments of Dermatology (IVDK), Institute at the University Medical Centre, Göttingen, Germany
[2] THE 3 PYLONS GmbH, Sankt Martin an der Raab, Austria
[3] Clinic of Dermatology, Venerology und Allergology, St. Josef-Hospital, Universitätsklinikum der Ruhr-Universität Bochum, Germany
[4] Dermatological clinic, Universitätsspital Zürich, Switzerland

About one third of severe tattoo complications are suspected to be hypersensitivity reactions ("pigment allergy"), as they frequently develop delayed even by years. Allergic reactions to liquid components of tattoo ink occur within days to weeks. Culprit allergens of tattoo ink usually cannot be determined by allergologists as declarations are unreliable or applied inks cannot be traced back. Patients are exposed to various tattoo allergens in other areas as well, which have to be considered as confounders (nickel in piercings, preservatives in cosmetics or industrial products etc.). The epicutaneous patch test (PT) is the gold standard for diagnosis of contact allergy and provided poor significance for tattoo patients in the past. Hence, epidemiological evaluation of positive PT reactions constitutes a major challenge for dermatologists.

Between 08/2018 and 07/2020, the new patch test recommendation of the German Contact Dermatitis Research Group (DKG) for diagnosis of non-infectious tattoo reactions was applied to 57 patients (40 women, 17 men) in 23 of 58 dermatological centres of the Information Network of Departments of Dermatology (IVDK). The complete PT recommendation includes over 80 commercially available PT preparations in four established DKG PT series (standard series, leather and textile dyes, industrial biocides, preservatives in topical preparations) and the new DKG tattoo series. The diagnostic value of this PT recommendation for the diagnosis of "tattoo allergy" has yet to be determined. On day 3, 69 positive reactions to 27 PT preparations were documented [+: 41 (59.4 %), ++: 15 (21.7 %), +++: 13 (18.9 %)]. Positive PT reactions were seen to colourants, metals, preservatives, local anaesthetics and shellac.

The study population is very small and we cannot yet draw far-reaching conclusions. Nevertheless, there is a backlog in the availability of suitable PT substances and the need for an improved anamnesis in order to epidemiologically evaluate confounders properly. For these reasons a tattoo study including an extended body art anamnesis and the option for pigment analysis in skin samples of patients with non-infectious tattoo reactions was already started in the IVDK network. Epidemiological studies with additional pigment allergens are needed.

2.8 Gaining in vitro and human data on tattoo ink toxicology

Dr Ines Schreiver
German Federal Institute for Risk Assessment, Department of Chemicals and Product Safety, Berlin, Germany

Specific tattoo and permanent make up resolutions and regulations in the EU exist for almost two decades. From the beginning, data gaps have been pointed out that prevented a full risk assessment. In addition, methods able to explain visible side effects in tattoos are yet missing. In our laboratories, we are using *in vitro* methods and human data to tackle some of the main questions concerning tattoo ink toxicology.

Starting from this autumn, a short-term biokinetics study for soluble ink ingredients will be conducted with human volunteers. These data can be used to picture a realistic exposure scenario.

In the past, human skin biopsies of allergic patients have been used to identify the most prominent pigments correlating with this seldom but severe side effect. A combination of *in vitro* data and human patch testing shall be used to identify the true allergen.

Recently, we established the first tattooed skin model, TatS, which can be used for phototoxicity testing. At the moment, immune cells are incorporated into the model to fully resemble healed tattooed skin. Next to phototoxicity, also genetic alterations and other endpoints can thus be investigated in future.

Human data and *in vitro* methods can therefore be used to improve our knowledge on tattoo toxicology. By understanding the underlying mechanism of side effects, our research may lead to the developments of tattoo-specific tests to prevent these in the long run.

2.9 United States FDA regulatory and analytical perspectives on tattoo inks

Dr Linda Katz, MD
United States Food and Drug Administration, College Park, Maryland, USA

Tattoos and permanent makeup have greatly increased in popularity over the past several decades. Globally, the safety of tattoo inks has been of interest and has prompted the consideration of regulatory requirements for the tattoo inks as well as their pigment components. Development of analytical methods to determine the composition of the many types of tattoo inks available in the global marketplace has been challenging and complex. This presentation will address some of the challenges in the U.S. for tattoo inks and pigments on both from a regulatory and analytical perspective.

2.10 EU new regulation on substances in tattoo inks and permanent make-up

Dr Ana Maria Blass Rico
European Commission, DG GROW, Brussels, Belgium

With the adoption of the REACH restriction on substances in tattoo inks or permanent make-up on 14 December 2020[1], the European Commission, with the support of the member States and the European Parliament has taken an important step to protect the health of EU citizens from hazardous substances contained in mixtures for tattoo inks and permanent make-up.

This restriction aims to achieve a harmonised high level of protection of human health and to ensure that EU citizens are equally protected independently of the country where they get the tattoo and whether the ink is manufactured in the EU or not. Furthermore, it ensures free movement of goods and harmonised and transparent rules applicable to all inks in the EU market.

The restriction bans substances which are already not permitted in cosmetic products[2], chemicals that are carcinogenic, mutagenic or toxic for reproduction, and substances causing skin sensitisation, skin corrosion or irritation and eye damage or irritation[3]. Maximum concentration limits are established for either groups of substances or for individual substances such as certain azodyes and carcinogenic aromatic amines, polycyclic aromatic hydrocarbons (PAHs), metals (arsenic, barium, cadmium, chromium, cobalt, copper, zinc, lead, selenium) and methanol.

It also provides for harmonised labelling requirements in order to give consumers and tattooists additional information, to facilitate implementation of the restriction, to prevent fragmentation of the internal market and to ensure that investigations can be properly carried out in the event of adverse health effects.

The restriction will become applicable in January 2022, after a transition phase of 12 months. From that point in time, tattoo inks and permanent make-up that contain the substances listed in quantities exceeding the specified limits may no longer be placed on the market and used in the EU.

[1] COMMISSION REGULATION (EU) 2020/2081, of 14 December 2020 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards substances in tattoo inks or permanent make-up

[2] Regulation (EC) No 1233/2009

[3] Regulation (EC) No 1272/2008

2.11 The REACH restriction on substances in tattoo inks or permanent make-up – reflections from a consumer representative

Dr Franz Fiala

Office of the Consumer Council operated by the Austrian Consumer Association, Vienna, Austria

The restriction included in Annex XVII of REACH as regards substances in tattoo inks or permanent make-up published in the Official Journal in December 2020 and applicable from January 2022 generates mixed feelings from a consumer protection perspective. A critical review of the process as well as the final result seems warranted to draw lessons for the future.

The European legislative procedure was triggered by some Member States (Austria and Denmark in 2013, Latvia in 2014) which notified draft regulations for this kind of product based on a Council of Europe Resolution adopted in 2008 (CoE ResAP (2008) 1) following the example of other Member States which had implemented similar regulations before. Following (debatable) objections from the EU Commission the European discussions on the subject resulted finally in a request by the EU Commission to the European Chemicals Agency (ECHA) to prepare REACH restriction proposal in December 2015. The overall duration of the whole process of around 7 years is definitely not a shining example of efficient consumer protection.

The restriction proposal developed by ECHA contained 2 restriction options (RO1 and RO2). The first restriction option (RO1) had a number of shortcomings but was a solid basis for the further debate. The second restriction option (RO2) provided only a very low level of protection and was fortunately rejected by RAC/SEAC. The final proposal was based on RO1 but was significantly strengthened.

The restriction itself is remarkable for various reasons. For the first time since the adoption of REACH substances in a product were restricted in Annex XVII based on CLP hazard classifications in a generic fashion (e.g. ban of CMRs). This is a significant departure of regulating chemicals based on a substance-by-substance risk assessment approach. In fact, the provisions anticipate the new "Chemicals Strategy for Sustainability – Towards a Toxic-Free Environment" by the Commission published in October 2020 which declares that "for the most harmful chemicals the generic approach to risk management becomes the default option, in particular as regards their use in consumer products". Whilst his move is very much appreciated from a consumer perspective it must be borne in mind that the approach is typically limited to substances with harmonised classifications (which sensitising substances often do not have). A further remarkable element of the tattoo restriction is the (automatic) incorporation of restrictions included in another regulation (substances prohibited or restricted in cosmetic products). However, a limitation is that the safety assessment of such substances is not based on intradermal injection of chemicals. Moreover, the Cosmetics Product Regulation (CPR) follows a positive list approach (i.e. for some uses only authorised substances are permitted). Such approach was proposed by the CoE resolution for preservatives but cannot be implemented in REACH. Hence, it would have been preferable to either incorporate the tattoo provisions into the CPR or to create a separate piece of legislation for this product group. It must be also borne in mind that the current approval system for preservatives in accordance with the Biocidal Products Regulation (BPR, considered to complement the REACH restriction) does not take into account the specific characteristics of tattoo inks. A separate product class and associated risk assessment guidelines would have to be created for this product group.

2.12 Requirements for the risk assessment of tattoo inks: chances and challenges

Dr Michael Giulbudagian, Dr Peter Laux
German Federal Institute for Risk Assessment, Department of Chemicals and Product Safety, Berlin, Germany

Today, up to 25 % of the youth are tattooed. Yet possible side effects of the injected tattoo inks are still uncertain. The European chemicals regulation has restricted substances with known and suspected adverse health effects as a major effort. However, as mainly oral or inhalation toxicity data are considered, the specific risk of intradermal exposure remains uncertain. Adverse effects may occur either locally near to the injection site or as systemic reactions. The absence of data, appropriate test methods and criteria for tattoo ink risk assessment represents a major obstacle. This holds in particular true for tattoo pigments as main ink components that persist as a lifelong chemical deposit in the human body. Therefore, the German Federal Institute for Risk Assessment (BfR) has compiled a set of minimum requirements for tattoo pigment characterisation and toxicology as a first step for their risk assessment.

The application of the minimum requirements will reduce potential health risks according to the current state of science and technology. In a second step, the necessary data for a complete health risk assessment of tattoo inks need to be accomplished. However, this requires a further development of test methods in particular with regard to chronic health effects. A prediction of systemic exposure by tattoo pigments and their degradation products following intradermal application should be developed. In addition to analytical and toxicological methodologies, this requires application of *in silico* tools and use of human data.

2.13 Semi-quantitative analysis of organic pigments in tattoo Inks with HPLC/DAD – work in progress

Dr Urs Hauri

Kanton Basel-Stadt, Kantonales Laboratorium, Basel, Switzerland

The new ECHA regulation on tattoo inks has set legal limits for forbidden pigments: 0.1 % for certain azo pigments and a much lower limit of 0.00005 % for pigments that are either forbidden or restricted in cosmetic products. Limits were also set for several dyes. The latter is not the topic of this work, as these dyes are not used in tattoo inks and methods are available.

A major obstacle in the analysis of pigments is the lack of quantitative references for most pigments which is a prerequisite for quantitative analysis. Without such references analytical methods are at best semi-quantitative. Another major analytical problem for a quantitative determination of pigments lies in their poor solubility. Solubility though is a key element for most quantitative analytical methods, e.g. liquid chromatographical methods. Despite this, we chose HPLC instead of other successfully used methods for the identification of pigments e.g. LDI-Tof/MS and Pyrolysis GC/MS.

The pigments responsible for the colour of tattoo inks are usually present in the range of 1 % to 50 % while the legal limits mentioned are up to 1'000'000 times lower. Because of the poor solubility in the extraction solvents and even more so in the LC eluents, samples have to be highly diluted for a quantitative analysis, the degree of dilution depending on the pigment in question. In order to check legal limits however, concentrated extracts have to be analysed too. For this, a screening method is employed where small amounts of ink (10–20 mg) are extracted with small volumes (2 ml) of Dimethylformamide (DMF), N-Methylpyrrolidone (NMP) and Chloronaphthalene (CLN) each using an ultrasonic homogeniser. This method also serves to detect any traces of pigments and will not dissolve the major amount of pigment. If regulated pigments are present above the linear range, the suspensions are thus diluted and reextracted in order to reach the linear range for these pigments. Mono-azo-pigments like e.g. C.I. 12315, C.I. 12477, C.I. 11741 are generally well extracted with DMF and analysed with a standard RP HPLC-DAD method. Other often used pigments are better extracted with either CLN (e.g. C.I. 21095, 21110, 51319 or 74160) or NMP (C.I. 73900, 73915, 56110) and analysed with a RP-HPLC system using NMP/Acetonitrile at 60 °C. For some of the pigments the solubility in the solvent and in the eluent and thus also the linear range is very limited. Therefore, for the pigment C.I. 74260 we use a colorimetric method at the moment which is suitable to detect C.I. 74260 as an ingredient but would fail to detect traces of this pigment.

In the year 2020, we determined organic pigments in 64 "coloured" inks for organic pigments and assessed the results on the basis of the present ResAP2008 regulation (C.I. 74160 being legal). 28 % (18) of the inks contained forbidden pigments in relevant levels, presumably in the percent range. Only in 2 of 18 samples these forbidden pigments were correctly disclosed. Applying the new ECHA regulation to these preregulation samples (C.I. 74160 and C.I. 74260 being legal for the moment), a label check revealed forbidden pigments in 58 % of the samples. Analysis though showed that 78 % of the samples contained relevant levels of these colourants (with a high probability to be above the limit of 0.1 %). Only in two additional cases we detected forbidden impurities around the 0.1 % limit.

In general, analysts and law enforcers alike prefer limits as it simplifies their work and guarantees a certain harmonisation in taking measures. In the case of pigment analysis though, the lack of references, the development and validation of quantitative methods and last but not least the routine testing demand a much higher effort than before, where the main pigments in the percent range were identified without the need of quantitation and small, unintentionally present, concentrations were tolerated.

2.14 Analysis of tattoo inks in Germany: current status and future challenges

Dr Birgit Gutsche
Chemical and Veterinary Investigation Office Karlsruhe, Germany

In Germany, tattoo inks are governed by the regulations of the food and feed code and were regulated by the German Tattoo law since 2009. This regulation as well as Resolution ResAP(2008)1 of the European Council listed several substances that must not be used for tattoo inks. To ensure that tattoo inks comply with the recommendations German official laboratories – responsible for cosmetics and tattoo inks – developed analytical methods for the analysis of e.g. primary aromatic amines, polycyclic aromatic hydrocarbons, metals, preservatives and UV-active compounds of Annex II to VI EU cosmetic regulation and started to standardise some of them.

As requirements changed with REACH regulation of tattoo inks, analysis has to be adapted or completely changed. The presentation will give an overview over performance of existing methods, first adaptions and developments as well as future plans. Analytical results of tattoo inks will be presented.

2.15 Analytical methods and results on metallic contamination, including nanoparticles, in tattoo inks purchased in Italy

Dr Beatrice Bocca[1], Beatrice Battistini[2]

[1] Italian National Institute of Health, Department of Environment and Health, Rome, Italy
[2] Tor Vergata University of Rome, Department of Biomedicine and Prevention, Italy

Recent report by the European Commission found a high number of hazardous chemicals in tattoo inks including metals and metal nanoparticles. Despite these findings, there are knowledge gaps on comprehensive analytical methods capable of characterising tattoo ink metallic constituents including nanoparticles (size less than 100 nm) for proper toxicological evaluation and efficient regulation. The method based on the inductively coupled plasma mass spectrometry (ICP-MS) and acid-assisted microwave digestion was developed to quantify major, trace and ultra-trace elements (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, Sn, Ti, Z) in tattoo inks of different brands and colours purchased in Italy in 2018–19. Additionally, the alkaline extraction of inks followed by the ion chromatographic separation and on-line ICP-MS detection (IC-ICP-MS) were used to detect the Cr(VI) concentration in samples. Moreover, a fast method for the counting and sizing of nanoparticles of Al, Co, Cr, Cu, Hg, Ni, Pb, Ti and Zn using the Single Particle ICP-MS (SP-ICP-MS) was developed. In addition, the coupling of ICP-MS with multi-angle light scattering and asymmetric flow field fractionation (AF4-MALS-ICP-MS) enabled the fractionation and sizing as well as the content of metals in various sizes. Results, also in comparison with those obtained in 2009, showed that despite tattoo ink production have shifted to purer raw materials and better manufacturing practices, the risk of exposure to metals and metal nanoparticles by tattooing remains a matter of concern. This research is a positive step to produce the analytical methods capable of fully monitoring and regulating tattoo ink market to protect tattooed individuals from hazards.

2.16 Preservatives in tattoo and PMU inks in the frame of REACH regulation: results of an Italian study

Dr Marco Famele

Italian National Institute of Health, National Centre for Chemicals, Cosmetics and Consumer's Health Protection, Rome, Italy

Preservatives with a harmonised classification under CLP Regulation as skin sensitisers/irritant or eye irritant or serious eye damage or prohibited by the Cosmetic Product Regulation (CPR) are among the over 4000 substances covered by the new REACH restriction (entry No. 75) on tattoo and PMU inks. Therefore, after 4 January 2022 all the mixtures for tattooing purposes placed on the EU market shall be compliant with the concentration limits set for these substances by the REACH Regulation. According to RAC and SEAC opinions on the restriction, preservatives as part of the tattoo ink mixture are also regulated under the EU Biocides Regulation (BPR) and fall under the authorisation regime of the BPR. Interestingly, up to today no biocidal product (PT-06, preservatives for in-can preservation) has been authorised for its use in tattoo/PMU inks, although preservatives are largely added to the inks to prevent microbiological contamination.

In the framework of a larger research project funded by the Ministry of Health, we developed and validated analytical methods for detection of 15 different preservatives in inks available on the Italian market. According to our results, isothiazolinones are currently the most frequently used preservatives followed by 2-phenoxyethanol. About 26 % of tattoo inks and 22 % of PMU inks would contain benzisothiazolinone (BIT) exceeding the concentration limit for skin sensitisers set at 10 ng/mg by the new REACH restriction. A lesser number of tattoo samples would not be compliant with REACH requirements for the presence of 2-phenoxyethanol and octylisothiazolinone (OIT). In particular, we found out that tattoo inks showed the higher non-compliant rate respect to PMU inks.

Based on our results, manufacturers have to consider new strategies to reduce levels of skin sensistising and eye irritant or serious eye damaging preservatives in the mixtures guaranteeing at the same time an effective preserving action on inks. Moreover, concentration levels of these substances have to be taken into account for a proper labelling and classification under CLP. The new REACH restriction will imply efforts from manufacturers and official laboratories in terms of testing the compliance of inks.

2.17 Raman spectroscopy in the measurement of tattoo inks – opportunities and challenges

Dr Katarzyna Karpienko, Filip Sadura, Dr Katarzyna Januszewicz, Dr Anna Dettlaff, Marcin Ekman, Dr Jarosław Skokowski, Dr Maciej S. Wróbel

Gdańsk University of Technology, Poland

Due to the increasing popularity of tattoos among the general population, to ensure their safety and quality, there is a need to develop reliable and rapid methods for the analysis of the composition of tattoo inks, both in the ink itself and in already existing tattoos. We focused on exploring the possibility of use of using Raman spectroscopy to examine tattoo inks in biological materials. In our study, we used two types of tissue models – pigskin, and self-developed optical tissue phantoms mimicking the optical scattering coefficient typical for the human dermis as a substitute for an *in vivo* study. The material employed herein allows for mimicking the tattoo-making procedure. We investigated the effect of the scattering coefficient of the matrix in which the ink is located, as well as its chemical compositions on the spectra. We studied the ability to detect miniature concentrations for a tattoo margin assessment, by carrying out Raman surface line scanning for each ink in the skin phantom. We also presented an analysis and comparison of the spectra of the inks and the tattooed inks in both phantoms. Additionally, we made a preliminary study on the possibility of distinguishing tattoo ink from other materials in lymph nodes. Finally, we made an extensive discussion on the opportunities and challenges related to using Raman spectroscopy as a method for the measurement of tattoos and tattoo inks.

2.18 Identifying tattoo pigments in human skin samples with adverse reactions based on µXRF and LDI-MS imaging and mass spectral library matching

Dr. Corinna Brungs, Carina Wolf

University of Münster, Institute of Inorganic and Analytical Chemistry, Research Group Uwe Karst, Germany

The popularity of tattoos has grown worldwide. In 2016, about 12 % of Europe's population was tattooed, and up to 24 % in the USA. In some cases, allergic, infectious, or neoplastic reactions occur or autoimmune diseases develop weeks or even years after tattooing. The trigger is often unknown. To follow up on the used pigments and identify the culprits, the analysis of inks and tattooed human skin samples is needed. In this study, pigments, tattoo inks, and tattooed human skin samples were investigated by micro X-ray fluorescence (µXRF) and laser desorption ionisation-mass spectrometry (LDI-MS) for elemental and molecular imaging, respectively. The skin samples were obtained from patients with adverse reactions in tattooed skin regions. Especially for the complex LDI-MS imaging datasets, an analysis workflow is needed to overcome tedious manual interpretation. Addressing this bottleneck, we developed a workflow for the identification of tattoo pigments based on queries against mass spectral libraries, which contain LDI-MS^1 and LDI-MS^2 spectra of "pure" pigments. The open source software MZmine was employed for data processing and identification. To achieve this, we have implemented new modules into MZmine to generate spectral libraries and to match experimental spectra. Similar mass spectra were clustered to reduce the amount of data and speed up the library matching.

The µXRF results gave a first hint on which pigments were used. Especially the presence of copper, chlorine, iron, and titanium in pigment regions in human skin thin sections was of great interest. Titanium dioxide is often used as a brightener in tattoo inks and in this study, titanium was found in many skin samples and tattoo inks. In reddish inks or tattooed skin, iron oxide is often used as an inorganic pigment. In some skin samples, iron can be detected by µXRF in higher intensities. Copper and chlorine can be part of the pigments (e.g., phthalocyanines) or contaminations, which are abundant due to the overall low purity of the pigments. These findings can guide the following LDI-MS analysis if specific elements are detected, such as chlorine. The samples were analysed as well as the pigments and were matched against the mass spectral pigment library. The matching of MS^1 spectra yields annotations for putative precursor ions of pigments facilitating the subsequent acquisition of MS^2 spectra if needed. A final structural identification relies on the similarity of the isotopes, ion adducts, and fragmentation patterns based on MS^1 or combined with MS^2 library matching. As a first result, some of the "pure" pigments were contaminated with other pigments. Alarmingly, we found incorrectly labelled commercial inks that were used in tattoo studios. This included multiple findings of hidden pigments that are banned for the use in tattoo inks in Germany and other countries. Among others, those banned pigments were identified in human skin thin sections.

The developed workflow significantly boosts the numbers and speed in which samples can be analysed, with increasing confidence in pigment identification. With more samples of adversely reacted tattooed skin, we seek to correlate and identify problematic tattoo ink components and reveal yet unknown signals in complex mass spectrometric data with new computational tools. The workflow annotated a variety of pigments in about 60 tattooed human skin samples that were analysed by LDI-MS imaging.

3 Index of Authors