# Sessions

# Opening & Plenary Talk

**THE ARCHAEOLOGY OF TATTOOING: NEW DISCOVERIES AND UNDERSTANDINGS**

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As the popularity of tattooing continues to grow over the early decades of the twenty-first century, there is a corresponding rise of interest regarding the history of the practice. Scholars studying past societies, professional tattoo artists, indigenous researchers, and tattooed individuals are all now seeking out information on the motifs, origins, cultural significance, and methods of ancient and historical tattooing traditions. Archaeological interest in tattooing has led to recent studies illuminating regional, cultural, and diachronic trends in pre-electric tattooing technologies and techniques, and revealed new information regarding the antiquity, material culture, and social framework of tattooing traditions around the globe.

Over the past decade, my research into the archaeological evidence for ancient tattooing has included using microscopic wear signatures to identify archaeological tattooing tools, efforts to record tattoos on mummified human remains, and experiments to recreate and test ancient and historic tattooing implements. This presentation will incorporate the results of that research and of collaborations with other archaeologists, professional tattooists, and indigenous scholars, to describe recent discoveries and share current knowledge regarding the deep history of tattooing as a global practice.

# 1.5 years after REACH: Part I: Challenges in implementation

## WHY CHEMICALS POLICY MATTERS FOR TATTOO-PIRATES

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The REACH regulation is a central pillar of how the EU regulates chemicals. It sets down important rules on the marketing, usage, manufacturing and import of substances and mixtures. One of its regulatory instruments are restrictions, which can be found in annex XVII of the REACH regulation. One of the restrictions, namely entry 75, regulates substances in tattoo inks and permanent make-up. This restriction was included in annex XVII in December 2020 and entered into force on 4 January 2022, while allowing a slightly longer transition for Pigment Blue 15:3 and Pigment Green 7 until 4 January 2023.

In my presentation, I would like to briefly discuss some basics of the REACH regulation, continue with looking back into the restriction process for tattoo inks and permanent make-up and end with an overview of the status quo, consequences, and possible options within the framework of the REACH regulation.

**SAVE THE PIGMENTS!**

Erich Mähnert[1]

[1]*Tattoo Artist & "Save-the-pigments" activist, Austria*

On January 26th, "Save the Pigments" had the opportunity to speak a second time in front of the European PETI Committee and the Parliamentary Representatives. The aim of this hearing was to point out the problems of REACH, which causes massive problems especially in the European tattoo and pigment industry.

But what are actually these problems, with which the European tattoo and pigment industry has to fight?
What are the motivations that led Save the Pigments to become active on the European stage?
How do the authorities and especially European politics react to these problems?
Why is a European representation of the industry's interests so important?
What new insights did the visit to Brussels bring?
What does the future of the industry in Europe look like?
All these answers to these questions, but also new approaches, we will try to explain to you at the 6th World Congress on Tattoo and Pigment Research (WCTP 2023) on 24-26 May 2023 in Vienna, Austria.

**IMPACT OF REACH REGULATION ON TATTOO INKS' SUPPLY: SURVEY OF FRENCH TATTOO ARTISTS**

Karine Grenouille[1]

[1]*Syndicat National des Artistes Tatoueurs, ET des Professionnels du Tatouage, France*

**Aim:** As part of the dialogue with its national authorities, French union of tattoo artists and tattoo professionals (SNAT) faced professional tattooers in order to collect data on the economic impact of the European Regulation on tattooing activities.

**Method:** Three online surveys were shared with French professional tattooers in February, May, and December 2022. Forwarded by e-mailing and social networks, we collected 768, 600 and 579 respondents respectively, after eliminating duplicate forms or incomplete answers.

**Results:** Although the proportion barely dropped between February and December, almost 7 out of 10 tattoo artists say they work with non-compliant inks, due to the entry into force of EU Regulation 2020/2081 on 14 December 2020. Supply distresses since January 2022, lack of knowledge about the products and their quality, professionals' reluctance to test these new inks, and price increase (x2 to x4) are the most recurrent reasons for this situation.

**Conclusion:** REACH has turned the European market and brands positioning upside down, temporarily putting one manufacturer in a monopoly-like situation (but unable to supply the entire market); New brands have appeared, in particular for black inks; Some brands that were previously not very present have now imposed themselves; Historical brands are struggling to maintain themselves and/or to offer compliant products.
All these brands are still unable to supply the European market (quantity and quality), which is now characterized by the predominant use of inks that are no longer compliant.

**DATA COLLECTION ON TATTOO INKS UNDER REACH**

Stefanie Lamm[1]

[1]*Extend the Scope, Bundesverband Tattoo e.V., Göttingen, Germany*

REACH plunged the EU-tattoo world into a period of great uncertainty and existential fears for the future. In the tattoo industry, it is not common for tattooers to be given information about the ingredients in tattoo equipment before purchasing.
In order to provide the tattooers with information about the declared properties of tattoo inks and to reduce fears and financial risks, the BVT (Bundesverband Tattoo e.V. = German Federal Association for Tattooing) has started its initiative for data collection on tattoo inks.

Using an existing dataset from 2009-2022, members were invited to donate their ink-data for all colours they have purchased since 2022. As an ongoing process, the BVT records and structures all information in these sections:

- Overview of all declared C.I.s since REACH, to find in which brand and whether this pigment is new or has been in use for tattooing for a long time.
- Overview of all brands that produce (or announce) coloured inks that comply with EU regulations; their peculiarities and their strategy in dealing with blue and green.
- List of all inks which are already available and, according to declaration, can be used under REACH.
- List of all inks that had to be discarded.

To date the collection of tones that can still be used includes 232 inks of the entire colour spectrum. An assessment of suitability for use as a tattoo ink (processing, healing, optical result, stability) has to be carried out in a differentiated manner and still needs to be done.

**SHOW HOW THE RESTRICTIONS OF REACH HAS IMPACTED THE DANISH TATTOO INDUSTRY?**

Esben Hammershøy[1], Bjørn Severin[1]

[1]*Inkbase, Skanderborg, Denmark*

**Aim:** Show how the restrictions of REACH has impacted the Danish tattoo industry?

**Methods:** From data collected with InkBase, we will put light on the impact of REACH.

**Results:** The timeline of new inks entering the scene and the variety of inks available to the industry compared to before REACH.

Suppliers of tattoo inks before and after REACH
Supplies of tattoo inks after REACH
The range of pigments before and after REACH January 2022
The range of pigments before and after REACH January 2023
Ingredients in general used in inks before and after REACH.

**Conclusion:** Has REACH impacted the tattoo industry in a disastrous way?
How does the future look?

**REACH VS. NATIONAL LAWS - CURRENT CHALLENGES**

Michael Dirks[1]

[1]*The 3 Pylons GmbH, Austria*

From the previously published declarations ResAP(2003)2 on tattoos and permanent make-up and ResAP(2008)1 requirements and criteria for the safety of tattoos and permanent make-up, seven European Member States implemented elements of the resolutions in their tattoo product regulations. In an 18-month European Commission project called "Tattoos and Permanent Make-up," the Joint Research Center began collecting data on existing regulatory frameworks, consumer behavior, distribution patterns, manufacturers, ingredients, and chemical composition of tattoo inks and potential health problems associated with tattoo inks and permanent make-up. The collected data resulted in a final report, which was subsequently forwarded to ECHA to draft a unified, substance-related legislative proposal while safeguarding the European principles of harmonized movement of goods.

On December 14, 2020, the Official Journal of the European Union has been published with Commission Regulation (EU) 2020/2081 amending Annex XVII of Regulation (EC) No 1907/2006 of the European Parliament and the Council on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) concerning substances in tattooing dyes or permanent make-up. In general, the regulation is the sum of all the resolutions and the seven tattooing products regulations implemented from them, as well as the parts of the Cosmetics Regulation, the Regulation on Classification, Labeling and Packaging (CLP) of Substances and Mixtures and REACH.

The intention of the European Commission was noble. The consumer should be protected from potential health hazards. Above all, under the premise of safeguarding the European principles of harmonious movement of goods. The presentation will show if the ECHA succeeded in fulfilling the demands of the Commission.

**LEGAL ISSUES AFTER TWO YEARS OF ENTERING INTO FORCE OF REGULATION EU 2020/2081 AND THE CJEU DECISION ABOUT CLASSIFICATION AND LABELLING OF TITANIUM DIOXIDE**

Luca Viola[1]

[1]*Lexpertise Legal Network, Milan, Italy*

Two years after the EU Regulation came into effect, so many questions remain about how the restrictions should have been evaluated and enforced. Several opinions have questioned how impact assessments and technical choices have been carried out the delegated commissions. Many stakeholders have applied for the amendment of the Regulation in order to allow the industry to have a real possibility to be compliant even in a so short period of time. At the moment nothing has changed leaving all industry in a state of uncertainty and great difficulty.

In particular ink manufacturers are facing enormous R&D expenses to try to come up with products that comply with the new limits but also to maintain an acceptable artistic standard - or at least similar – as per old blends but to date there are just question marks.

The authorities must monitor compliance with the new rules, but control plans are still wait-and-see.
To date beyond the important initiative represented by the petition no. 1072/2020 (Manhert-Dirks) and the BfR studies on minimum requirements for harmonized standard tests and positive list of ingredients, no concrete possibilities for revising the new EU Regulations are on track.

Despite the rigid EU position two initiatives seem particularly interesting: (i) The British government Agency HSE has analized - as a starting point - the EU Regulation 2020-2081 and has concluded to disregard it; (ii) CJEU has convicted for the changing of the EU Regulation 12722008 (CLP) that has been challenged regarding the labeling of titanium dioxide as a carcinogen substance. The Court has examined the procedures and principles followed by the legislator and has concluded for the modification of the Regulation.

These two cases lead to the conclusion that the scientific and drafting basis of the Regulation 2020/2081 seems to show several flaws underlining how important could be to involve the CJEU on evaluating the consistency of the Regulation with the TFEU and with the 2003 Interinstitutional Agreement on Better Law-making as well as compliance with the principles of proportionality, legal certainty, and proper assessment of the impact of legislation.

At the moment no sue cases against manufacturers and tattooers are known.
The turning point - to have a real balance between all the stakeholders' interests - could be appealing to the CJEU on the challenge against the EU Regulation.

# 1.5 years after REACH: Part II: Future of chemicals in tattoo inks

**ESTP perspective of the REACH restriction**

Steffen Schubert[1], Ines Schreiver[2]

[1]*Information Network of departments of Dermatology (IVDK), Institute at the University Medical Center Göttingen, Göttingen, Germany*

[2]*German Federal Institute for Risk Assessment (BfR), Department of Chemical and Product Safety, MaxDohrn-Str. 8-10, Berlin, Germany*

Since January 2023, the EU-wide REACH regulation on inks for tattooing and permanent make-up (annex XVII to EC 1907/2006) is fully implemented. It aims at consumer health protection and harmonization of the EU tattoo market by introduction of definite limits for a variety of substance classes. These concentration limits derived from a worst-case exposure scenario (full body tattoo) and should be revised by a reasonable tattooed surface area as suggested by other ECHA guidance documents. Some of these limits are technically not achievable as e.g., pure raw materials of pigments and chemicals are often not available. Furthermore, certain pigment bans are not supported by scientific data and harmonized analytical methods are lacking for certain substances where extraction methods may be variable, which complicates the responsible work of manufacturers and national market surveillance laboratories. Moreover, clients and in particular tattooist were left in a confusing situation during the corona pandemic characterized by miscommunication about REACH such as “a ban of all tattoo colors”. Now more than ever, we call on all stakeholders in the tattoo industry to focus on future, common goals, which are 1) safe application of tattoos and permanent make-up, 2) minimization of risk, and 3) to keep tattooing legal. Therefore, the ESTP and WCTP community is encouraged to work together to collect scientific data as a base for future regulatory improvements and to unite attempts and successes in the development of analytical methods. The ESTP will continue communicating research and to translate results between artists, researchers and authorities.

**MINIMUM REQUIREMENTS ON TATTOO INKS - REPORT ON THE NEW BfR COMMISSION**

Peter Laux[1], Michael Giulbudagian, Frank Bierkandt, Stefanie Seifert, Ajay Vikram Singh, Loryn Theune, Andreas Luch

[1]*German Federal Institute for Risk Assessment (BfR), Department of Chemical and Product Safety, Berlin, Germany*

Currently there are no criteria according to which a safety assessment of tattoo inks should be performed. Carcinogenic, mutagenic or reprotoxic (CMR) as well as sensitizing or irritating substances are restricted by entry 75 of Annex XVII of the REACH regulation. However, non-classified substances of unknown toxicity may still be used in tattoo inks. Moreover, there is a lack of suitable test methods for tattoo inks, in particular for pigments. The German Federal Institute for Risk Assessment (BfR) has developed minimum requirements for tattoo inks. The use of pigments that meet the minimum toxicological requirements will reduce potential health risks according to the current state of science and technology. However, due to the absence of appropriate test methods and data, no complete risk assessment and no recommendation for the use of pigments in tattoo inks is possible at present. A new commission for tattoo inks, comprising experts from relevant disciplines, was established to advise the BfR with regard to the composition of tattoo inks, their manufacturing and analysis, as well as on the toxicological testing.

**THE MODERNIZATION OF COSMETICS REGULATION ACT OF 2022 (MoCRA)**

John Misock[1]

[1]*Ceutical Laboratories, Inc., Texas, USA*

The first major change to US cosmetic regulatory law since 1938 has been enacted by Congress and signed by President Biden. The FDA has been given new authority under the FD&C Act which sets significant new requirements for cosmetics manufacturers and brand owners. FDA is mandated to promulgate multiple new regulations over the next couple of years that will create sweeping requirements across the cosmetic industry. The regulations will be fully enforceable by December 29, 2025.

The new law requires registration, listing, adverse event reporting, safety substantiation and GMP compliance for tattoo and permanent makeup (PMU) (cosmetic tattooing) ink. Heretofore, registration has been voluntary and GMP requirements did not exist. Tattoo ink and PMU are cosmetics. Although the definition of cosmetics has not changed, the new law includes mention of "injected" cosmetics.
By December 29th of this year, all tattoo ink manufacturers must register their facilities. The law created a new definition of "responsible person" who is the "person" who's name is on the label. The law assigns compliance to the responsible person. A "facility" is defined as any establishment that manufactures or processes cosmetics distributed in the United States, but specifically excludes establishments that "solely perform" labelling, relabelling, packaging, repackaging, holding, and/or distributing cosmetic products. Several other types of entities are also expressly excluded from the definition of a facility, such as cosmetic retailers and beauty shops/salons and tattoo parlours and permanent makeup facilities (unless they're engaged in manufacturing).

The presentation will cover all of the new requirements that apply to tattoo and PMU ink as well as an update on efforts to file Colour Additive Petitions (CAPs) for pigments in the US.

# TATTOO INKS: U.S. REGULATORY PERSPECTIVES

Linda Katz[1]

[1]*Food and Drug Administration, Cfsan Office of Cosmetics and Colors, College Park, Maryland, United States*

**Aim:** Provide an overview of ongoing activities at the U.S. Food and Drug Administration (FDA) regarding tattoo inks and pigments.

**Results:** The safety of tattoo inks has been the subject of numerous discussions occurring both domestically and internationally. Further, these discussions have prompted consideration regarding regulatory requirements for both tattoo inks and pigments from a microbial and heavy metal safety standpoint.
A summary of recent research undertaken at the FDA will be presented.

**Conclusions:** Tattoos and permanent makeup have continued to increase in popularity over the past several decades both in the United States and globally. Polling indicates that the percentage of adults with at least one tattoo is now approximately 30% and is 40% for individuals between 18-40 years of age. Validated analytical methods to determine the composition of the many types of tattoo inks are under development, as are risk assessments for potential adverse reactions, but identifying a uniform approach has been challenging and complex. This presentation will address some of the key issues that the U.S. FDA is focusing on to address safety.

**THE REGULATION OF TATTOOS, TATTOOING AND TATTOO REMOVAL IN GERMANY IN HISTORICAL PERSPECTIVE**

Claudia Schmidt[1]

[1]*Heinrich-Heine-Universität Düsseldorf, Department of the History, Philosophy and Ethics of Medicine, Düsseldorf, Germany*

With the implementation of REACH, tattoo artists and the whole industry are confronted with a set of rules that is a novum in the history of the regulation of tattooing. However, regulations of tattoos, tattooing and even tattoo removal are not new. In my talk, I will therefore give a historical outline about this topic, using the example of Germany.

One of the first regulations on tattoos after 1945 was the prohibition of the removal of the SS blood group tattoos, initiated by the allied forces. Tattoos itself were never forbidden, neither in Western nor Eastern Germany, but certain motives were. To this day, tattoos showing anti-constitutional symbols such as Swastikas, the insignia of the 3rd SS Panzer Division Totenkopf and similar motives are not allowed to be shown in public. In the GDR, the range of forbidden symbols was even greater. After all, prisons were a place where tattooing was, of course, forbidden and a set of rules was enacted to that effect. All those regulations were accompanied of a progressing medicalisation of tattooing in the meantime.

Based on a qualitative analysis of literature and archival sources I will give a brief history of the regulation of tattoos, tattooing and tattoo removal in Germany with special reference to the above examples. My goal is to put REACH in a historical context and to clarify the importance of interdisciplinary research on tattoos and pigments.

# Tattoo complications and clinical insights

**TATTOO COMPLICATIONS: DIAGNOSIS AND TREATMENT**

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[1]Alrijne Hospital, Leiden, Netherlands

In the Tattoo Clinic in the Netherlands (Tattoo poli, Alrijne Ziekenhuis Leiden)* hundreds of patients with tattoo complications have been consulted. The Tattoo Clinic is currently regarded as a nationwide highly specialized referral centre in The Netherlands. A great variety of complications in tattoos, including permanent makeup, is observed.

Tattoo complications can be categorized into inflammatory tattoo reactions, infections, neoplasms and miscellaneous reactions. In a hospital setting, inflammatory tattoo complications are the most frequently observed. The majority of these reactions are chronic and include allergic tattoo (pigment) reactions, chronic inflammatory black tattoo reactions and manifestations of autoimmune dermatoses such as psoriasis, lichen planus, non-segmental vitiligo and lupus erythematosus.
Allergic red tattoo reactions can even account up to 50.2% of all complications.** Treatment options include local or intralesional corticosteroid, CO2-laser, dermatome shaving or systemic therapy.

Although bacterial infections are the most frequent tattoo complication, they only present a minority of the total of tattoo complications in the Tattoo Clinic as they are generally treated by the general practitioner. Other more rare infections include mycobacterial or fungal infections and manifestations of the herpes simplex virus, human papilloma virus and molluscipox virus. Neoplasms in tattoos, such as basal cell carcinoma, are rarely observed. Miscellaneous complications include blow-outs, scars, keloids, neurosensoric and photosensitive tattoo reactions. The last decade, a (renewed) trend of alternative tattoo removal has emerged including caustic removal crèmes. For this reason, tattoo removal induced complications are increasingly observed in the Tattoo Clinic.

* www.tattoopoli.nl
** 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. J Cosmet Dermatol. 2021 Nov;20(11):3630-3641.

**TATTOOS AND "PANDEMICS": COVID-19 AND MONKEYPOX**

Nicolas Kluger[1, 2]

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[2]*Tattoo consultation, Hôpital Bichat - Claude Bernard, Paris, France*

SARS-CoV-2 infection and COVID-19 have had profound impact on our life for the past 3 years. On January the 6th 2023, there have been 657 977 736 confirmed cases of COVID-19, including 6 681 433 deaths, reported to WHO.
As of 21 December 2022, a total of 13 073 712 554 vaccine doses have been administered. Besides, since May 2022, multiple cases of monkeypox were identified in several non-endemic countries. In this presentation, we will discuss the impact of both infections on tattooists' activity as well as the possible adverse events reported among tattoos customers during the past years.

**ANAESTHETIC CREAMS – COMPLICATIONS**

Walter Lieszewski[1]

[1]Northwestern Memorial Hospital, USA

Topical anesthetic creams are used by some tattooists to control the pain and discomfort of tattooing. However, the health implications and safety of these products amongst tattooed individuals is poorly defined. This session will review the major categories of topical anesthetics, their impact on human health, and their allergenic potential.

**TATTOOING IN PSORIASIS: A QUESTIONNAIRE BASED ANALYSIS OF 150 PATIENTS**

Patrycja Rogowska[1]

[1]*Medical University of Gdańsk, Poland*

Aim: Among populations of Western countries, tattoos have become an accepted form of skin ornamenting. With tattoos growing in popularity, also patients suffering from chronic dermatoses may more often be willing to get tattooed. Psoriasis is not considered as a strict contraindication for tattooing; however, it is not advised to get a tattoo while undergoing immunosuppressive treatment and during an active stage of the disease. We attempted to assess the knowledge level of tattooed psoriatic patients about the potential risks connected with tattooing, as well as to explore their attitudes and tendencies towards this procedure. Moreover, we analyzed the frequency and type of tattoo complications in this study group.

Methods: An anonymous, online questionnaire was performed among online communities dedicated to psoriasis. Data from 150 tattooed psoriatic patients have been scrutinized.

Results: Eight percent of the surveyed psoriatic patients sought medical advice before getting a tattoo. While undergoing the tattooing procedure, 23 (15.3%) of the respondents received systemic psoriasis treatment: 8 (5.3%) being treated with methotrexate, 5 (3.3%) with cyclosporine A, one (0.7%) acitretin, and 9 (6%) patients were under biological treatment. Thirteen (8.7%) of the participants experienced complications associated with their tattoos, among which, the insurgence of the Koebner phenomenon on the tattoo, was the most frequent one (8 cases- 5.3%). Getting tattooed improved patients' self-esteem in 76 (50.7%) of the cases.

Conclusions: An increased level of education among patients, medical practitioners, and tattooists concerning general precautions of tattooing in psoriasis is advisable.

# Basic science: Dermal in-vitro/in vivo studies

## ASSESSMENT OF CYTOTOXICITY AND SENSITIZATION POTENTIAL OF INTRADERMALLY INJECTED TATTOO INKS IN RECONSTRUCTED HUMAN SKIN

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*[4]Department of Oral Cell Biology, Academic Centre for Dentistry (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, The Netherlands.*

**Background**: The number of people within the European population having at least one tattoo has increased notably, and with it the number of tattoo-associated clinical complications. Despite this, safety information and testing regarding tattoo inks remains limited.

**Objective**: To assess cytotoxicity and sensitization potential of 16 tattoo inks after intradermal injection into reconstructed human skin (RHS).

**Methods**: Commercially available tattoo inks were injected intradermally into RHS (reconstructed epidermis on a fibroblast-populated collagen hydrogel) using a permanent make-up device. RHS biopsies, tissue sections and culture medium were assessed for cytotoxicity (MTT assay), detrimental histological changes (H&E) and for the presence of inflammatory and sensitization cytokines (IL-1α, IL-8, IL-18; ELISA), respectively.

**Results**: Varying degrees of reduced metabolic activity and histopathological cytotoxic effects were observed in RHS after ink injection. Five inks showed significantly reduced metabolic activity and enhanced sensitization potential compared to negative controls.

**Discussion**: Using the RHS model system, five tattoo inks were identified as highly cytotoxic and classified as potential sensitizers, suggesting that allergic contact dermatitis could emerge in individuals carrying these inks. These results indicate that RHS-based assessment of cytotoxicity and sensitization potential by intradermal tattoo ink injection is a viable analytical tool to determine ink-induced deleterious effects.

**PHOTOTOXICITY IN VITRO: A PERSPECTIVE ON 2D AND 3D SKIN MODELS**

Susanne Kochs[1], Anja Schreiber[2], Aseel Alsarahni, Sandra Schiewe[1], Helena Huynh[3], Andreas Luch[1], Ines Schreiver[1]

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**Aim:** Tattoo ink pigments are internalized *in vivo* primarily by dermal macrophages and fibroblasts. Many tattooed people experience reactions such as swelling, itching, pain or redness during and after sunbathing (approx. 20%, Hutton Carlson & Serup, 2013). In addition, allergies in the red colour spectrum often occur on sun-exposed skin areas (Bent et al., 2019). A connection with photo-irritation effects is therefore likely. Hence, we aim to develop test methods to predict phototoxicity of pigments.

**Methods:** For 2D cell culture experiments, we modified the validated OECD 432 phototoxicity guideline for testing pigments with human fibroblasts and macrophages. Applicability of the modified test was proven by correct prediction of proficiency substances given in the guideline. Pigment titanium dioxide anatase showed phototoxicity in fibroblasts and macrophages, as expected. Other pigments (carbon black, pigment red 22) were negative in fibroblasts. Benzo[*a*]pyrene, a common phototoxic contaminant of carbon black, was tested as pure substance and bound to the pigment.
Contrary, previous 3D experiments with tattoo pigment-containing skin models (TatS) showed protective effects of pigments on skin cells due to shading cells from UV light. An immuno-competent skin model (iTatS) is currently under development to include the possible impact of macrophages on phototoxicity.

**Outlook:** The modified OECD 432 test strategy for tattoo pigments is promising for prediction of general phototoxic properties. However, other mechanisms seem to add to the *in vivo* reactions. This or similar test strategies will be further developed to also cover cytokine release, photosensitization and –genotoxicity in future.

**HISTOPATHOLOGICAL PATTERNS RELATED TO PIGMENT EXPOSURE: AN OVERVIEW**

Nataly Portilla[1]

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**Importance:** Reactions to tattoo simulate common dermatosis or skin neoplasms. Histopathology allows diagnosis and determining the degree of inflammation associated, orientating treatment.

**Objective:** To describe histological features found in biopsies of cutaneous reactions to tattoo.

**Design:** multicenter case series.

**Setting:** All consecutive histopathological samples of tattoos referred to the Hospital General de Catalunya, Hospital Germans Trias i Pujol and a private practice, all in Barcelona, Spain and from the Kempf und Pfaltz Histologische Diagnostik in Zurich, Switzerland were retrieved.

**Participants and exposure:** The inclusion criteria were all cosmetic/permanent make-up, artistic/professional, and traumatic tattoos associated to inflammatory reactions and/or with tumors and/or infections. Exclusion criteria were cases without any associated pathologic finding in the place of the ink, amalgam tattoos, and medical or temporary tattoos.

**Main outcomes and measures:** clinical features (age, sex, location, tattoo color, and presentation) were recorded. Histological features evaluated included ink color, tumors or infections, and inflammatory reaction pattern.

**Results:** From 477 biopsies, 230 cases from 226 patients met the inclusion criteria. Corresponded to 107 males, 120 females, and 3 of unknown gender. Median age was 39 years [from 9 to 84 years]. 53 samples were referred from centers in Spain and 177 from the center in Switzerland. The series was analyzed in two parts: tattoos associated only to inflammatory reactions (117/230) and tattoos associated to tumors or infections (113/230). The most common form of inflammatory pattern associated to tattoo was the fibrosing reaction (79/117, 68%). Combined features of two or more types of inflammatory patterns were seen in 64% cases.

**Conclusions and relevance:** Our series confirms that cutaneous reactions to tattoos are polymorphous. Inflammation tends to present with combined patterns. Infections are tending to decline. And pathologic findings are not specific to ink color, or clinical features.

**SIMULATION OF PIGMENT METABOLISM BY ELECTROCHEMISTRY-MASS SPECTROMETRY (EC-MS)**

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**Aim:** Despite the popularity of tattooing, little is known about the fate of colour-giving pigments in the human body. Due to the unique exposure pathway and pigment insolubility, it is challenging to monitor or simulate biotransformation. Therefore, large data gaps on degradation and metabolism within the skin and in following excretory routes exist. The occurrence of skin reactions, such as allergic reactions, further stresses the need for investigation and understanding of pigment metabolism.

**Methods**: Electrochemistry (EC) is known as a tool for a purely instrumental approach to simulate the metabolism of xenobiotics, i.e., chemicals compounds that are foreign to the organism. Using an electrochemical flow-through cell, oxidative or reductive potentials can be applied to a sample solution, mimicking, for example, enzyme-mediated reactions. Electrochemically formed products can thereafter be analysed by high-resolution mass spectrometry (MS), leading to chemical formula annotations and structure proposals.

**Results**: In oxidative and reductive mode, reaction products of red organic pigments were formed and identified. Some products moreover conjugated to the oligopeptide glutathione, leaving the assumption of highly reactive compounds forming through the applied potential. However, as pigments are not available as clean standards, interfering compounds need to be separated by HPLC prior to analysis.

**Conclusion**: EC-MS as a widely used tool in metabolism simulation has been applied successfully to red organic pigments. As a purely instrumental approach, the method provides a low-cost and time-efficient tool while providing first ideas on possible biotransformation of pigments in the human body.

**TOXICOLOGICAL IMPACT OF PHOTODEGRADED ORGANIC TATTOO PIGMENTS ON HUMAN CULTURED KERATINOCYTES**

Lise Aubry[1], Lucie Breuillé[1], Thierry Douki[1]

[1]*Univ. Grenoble Alpes, Cea, Cnrs, Irig, Symmes, Cibest, France*

**Aim:** Tattooing is very popular around the world. However, the safety of tattoo inks remains to be fully evaluated. These products contain pigments, which are insoluble compounds that persist in the skin dermis. Organic pigments including azo and polycyclic pigments are increasingly used for tattoos. These compounds may be altered by sun exposure or dermic macrophage activity. These processes may lead to generate soluble molecules, which could diffuse into the epidermis and affect the major cells of this layer, keratinocytes. Interestingly, a link is suspected between tattooed skin and some cutaneous diseases like basal-cell carcinoma. Only few articles deal with the toxicity of degraded pigments in epidermis. This topic is the basis of ongoing work in our group.

**Methods:** High-performance liquid chromatography coupled to mass spectrometry (HPLC-MS) is used to investigate the degradation of pigments. *In vitro* studies using the HaCat keratinocytic cell line allow us to unravel the effects of photodegradation and degradation by macrophages on the toxicological profile of pigments.

**Results:** First results show different cytotoxic profile between a series of pristine organic pigments and suspensions exposed to heat and simulated sunlight. Analysis by HPLC-MS show that exposure of some pigments to ultraviolet light can generate photoproducts.

**Conclusions:** Simulated sunlight exposure alters some organic pigments and it may modify their toxicological properties on keratinocytes. Further studies will investigate the production of oxidative stress and generation of DNA breaks. Similar studies will be performed with pigments aged in solution mimicking the lysosomic condition in macrophages.

# Allergies & epidemiology

**GERMAN TATTOO PATCH TEST PANEL - RESULTS OF THE IVDK**

Steffen Schubert[1]

[1]*Information Network of Departments of Dermatology (IVDK) – Institute at the University Medical Center Göttingen, Germany*

**Introduction:** Tattoo inks consist of pigments, binders, solvents and additives (e.g., preservatives). The German Contact Dermatitis Research Group (DKG) recommends extensive patch testing of patients with suspected allergic reactions to tattoos or permanent make-up.

**Objectives:** To revise the current DKG tattoo patch test recommendation, which consists of 79 test preparations contained in 5 DKG test series.

**Methods:** Retrospective analysis of patch test results with the DKG tattoo patch test recommendation in 58 patients tested between 08/2020 and 12/2022.

**Results:** Most frequent sensitizers not contained in the DKG baseline series were: ammoniated mercury (9.3%), *p*-phenylenediamine (PPD, 7.7%), several disperse dyes (8.0% - 3.7%), propylene glycol (4.2%) and shellac (3.9%). Furthermore, single reactions to isothiazolinones, meth-/acrylates, naphthol AS, aluminum chloride and iron sulphate were observed. In contrast, no positive reactions were documented with o-phenylphenol, formaldehyde (-releasers) and abietic acid.

**Discussion:** Red cinnabar is not used in tattoo inks. Therefore, the clinical relevance of reactions to ammoniated mercury remains elusive. Incompatibility to hair dye or black henna has to be considered as confounder for sensitization to PPD and disperse dyes. Results obtained with propylene glycol (20% aq.) and shellac have to be carefully interpreted.

**Conclusion:** To facilitate patch testing, 5 substances were removed from the tattoo series and several preservatives and 3 disperse dyes were added. The reviewed DKG tattoo panel comprises 28 test preparations to be tested in addition to the DKG baseline series. Due to the lack of appropriate test preparations, negative patch test results do not rule out pigment allergy.

**PATCH TESTING IN PATIENTS SUSPECTED OF HAVING A TATTOO INK ALLERGY**

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[4]*Federal Institute for Risk Assessment, Chemical and Product Safety, Berlin, Germany*

**Aim:** Tattoo inks are composed of many ingredients such as pigments, solvents, preservatives, and emulsifiers. Although allergic reactions to red tattoo ink are frequently seen in dermatology clinics, finding the culprit allergen remains very challenging. Based on analyses of tattoo inks and the literature, a tattoo patch test series was developed. The aim of the study was to assess the frequency and relevance of contact sensitivity in patients with allergic reactions to tattoo ink.

**Methods:** 8 patients with suspected allergic reactions to tattoo ink who underwent patch testing with the European baseline series and tattoo series at the Amsterdam University Medical Centres between May 2022 and December 2022 were included.

**Results:** Of the 8 patch tested patients, 3 (37.5%) reacted to red ink, 3 (37.5%) to pink ink, and 2 (25%) to green ink. Six patients (75%) were female and the median age was 38 years. Seven patients (87.5%) were atopic. In 4 patients (50%) sunlight exacerbated the skin symptoms. Six patients (75%) had positive patch tests to one or more allergens. Metals (nickels sulphate n=2; copper sulphate n=1; potassium dichromate n=1; cobalt chloride n=1), preservatives (methylisothiazolinone n=2; 1,2-benzisothiazolin-3-one n=1), and fragrances (limonene hydroperoxide n=2; linalool hydroperoxide n=1) were most frequently positive. One reaction to colophony and one to pigment red 57:1 was classified as clinically relevant for the tattoo reaction.

**Conclusions:** These preliminary data show that more research is needed to reliably identify the culprit allergen in patents with allergic reactions to tattoo ink.

**LOCAL ENRICHMENT OF CHEMICAL-SPECIFIC T CELLS IN SKIN FOR TATTOO ALLERGEN IDENTIFICATION**

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[3]*Queen's University, Department of Medicine, Kingston, Canada*

**Aim:** We present a novel strategy to confirm or exclude suspect tattoo allergens in allergic skin reactions.

**Methods:** Chemical-specific T cells were detected by activation-induced marker (AIM) T cell assay using peripheral blood mononuclear cells (PBMCs). Sorted blood T cells and matching biopsies from inflamed and healthy skin of the same person were submitted to T cell receptor (TCR) high-throughput sequencing to quantify specific T cell clonotypes in both compartments.

**Results:** PBMC incubation with contact allergens induced the surface expression of CD154 and CD137 on chemical-specific CD4+ and CD8+ T cells after 5 h or 16 h, respectively. High frequencies of metal-specific T cells were observed in non-allergic individuals, e.g., ~0.1% CD154+CD4+ T cells with 200 µM $NiSO_4$. This signal exceeds by far that of protein allergens in the absence of immune responses and impedes a blood-based allergy diagnoses since only few allergic individuals top that background. A possible solution is TCR repertoire analysis. We show a local enrichment of $Ni^{2+}$-specific CD4+ T cell clonotypes in $Ni^{2+}$-induced allergic contact dermatitis. A skin biopsy from an allergic tattoo reaction lacked such $Ni^{2+}$-specific T cell enrichment excluding $Ni^{2+}$ as allergen.

**Conclusions:** A combination of AIM T cell assay and TCR sequencing can reveal chemical-specific T cell frequencies in blood and skin. The local enrichment of $Ni^{2+}$-specific T cells in a small biopsy identified nickel as culprit allergen in situ. Our approach may be transferred to other chemical sensitizers.

**DEVELOPMENT AND VALIDATION STUDY OF THE EPIDEMIOLOGICAL TATTOO ASSESSMENT TOOL (EpiTAT)**

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**Aim:** Understanding of long-term adverse health effects, including cancer, associated with tattooing is limited. To advance this knowledge, epidemiological studies are needed for which good exposure assessment is the main prerequisite. Therefore, we developed the Epidemiological Tattoo Assessment Tool (EpiTAT), a questionnaire to self-assess tattoo ink exposure in tattooed populations, which validation study is presented here.

**Methods:** A validation study of the EpiTAT questionnaire (N=97) was conducted in Lyon, France. Its main aim was to compare three measurement units of tattoo self-assessment, namely hand surface, credit card, or a combination of body schemes and Likert scales. Self-reported measurements of tattoo surface, colour, and coverage using one of these units were compared with physical validation measurements by trained interviewers and digital surface analysis.

**Results:** Of the three tested measurement approaches, the hand surface provided the most trustworthy estimation of tattoo size. However, we noticed a substantial overestimation of self-assessment compared to the validation measurement (mean tattooed body surface 1768, SD 1547, cm2 vs 930, SD 1047, $cm^2$; P<.001). Moreover, the single tattoo surface was slightly overestimated in validation measures compared to digital image analysis. For measurement of tattoo coverage and colours, Likert scales were the most appropriate method.

**Conclusions:** The final EpiTAT questionnaire assesses visual and contextual tattoo factors on 22 items and is a useful tool to assess tattoo exposure in health research. However, further studies to determine the pattern of tattoo size estimation are needed.

**IDENTIFYING TATTOO PIGMENTS IN HUMAN SKIN SAMPLES WITH ADVERSE REACTIONS BASED ON MXRF AND LDI-MS IMAGING AND MASS SPECTRAL LIBRARY MATCHING**

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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 ionization-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 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. 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 workflow annotated a variety of pigments in about 60 tattooed human skin samples that were analysed by LDI-MS imaging.

# Tattoo complications and removal

## BLUE TATTOOS: COMPLAINTS AND COMPLICATIONS NOTICED REAL LIFE

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Pigment blue-15 hitherto was the universal blue pigment in tattoo inks. The Tattoo Clinic from 2008 to January 2023 diagnosed 1008 patients with a broad range of tattoo complications. 22 (2.2%) had chronic inflammatory complications in purely blue tattoos. Some appeared a few days after tattooing, others after a few months or years.; some late reactions at colour shift toward greenish. This record matched the finding in our previous study from the STH clinic; 1.3% (1). However, our “beach study” noted reactions in 5.6% dominated by photosensitivity (2). A Danish study of self-reported tattoo problems among 5.914 tattooed individuals found problems in 3.2% of purely blue tattoos (3). A Polish study of 53/40 tattooed from a dermatology clinic found reactions in blue tattoos in 4% (4). Records mostly do not distinguish transient mild complaint and chronic medical complication. There appears to be three mechanisms involved, early irritant/infectious reaction, delayed inflammatory reaction allergic and chronic in nature and, being commonest, the photosensitivity complaints. A small percentage only seeks medical advice, spontaneous recovery is common and allergic reactions are exceptional also relative to the percentage of persons having blue tattoos. The tattoo clinic noted no case of premalignant or malignant lesion in blue tattoos.

Conclusion: It is not supported by clinical data that the pigment blue-15 colourant is of special concern.

1. Høgsberg T et al. JEADV 2013;27:846-852
2. Hutton Carlsen K et al. JEADV 2014;28:231-237
3. Friis K et al. Dermatology in press
4. Rogowska P et al. Dermatology 2022;238:799-806

**Tattoos as a target of natural UV radiation and laser removal - facts and myths**

Wolfgang Bäumler[1]

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Tattooed skin contains numerous solid particles of tattoo pigments. These pigments particles consist of aggregated pigment molecules, which have inorganic or organic structures. The pigments are black, white or coloured. The black pigments absorb optical radiation in a broad spectral range from the Ultraviolet (UV) to the Infrared (IR). Coloured pigments absorb radiation in the UV and in specific narrow ranges in the visible spectrum.

Depending on the location of a tattoo, the pigment particles in skin are persistently exposed to optical radiation. This optical radiation is solar radiation, which comprises on the earth surface wavelengths from about 290 to 4000 nm, but also emission of artificial light sources. Depending on the wavelength of the radiation, pigment molecules absorb radiation and the absorbed energy may subsequently cause a chemical alteration of the molecules. This alteration may change the colour of the pigment up to a complete fading. It is known that most of the used pigments may decompose upon solar radiation exposure and a persistent UV exposure (sun, tanning booth) may slowly decompose the pigments inside the tattooed skin.

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. However, a complete removal of a tattoo is usually not achievable, in particular when the tattoo is multi-coloured or cover a large skin area. On the one hand, the exact mechanisms of laser-assisted fragmentation are hardly investigated despite the long-lasting use of such laser treatments. On the other hand, tissue-resident macrophages in skin may take up the fragmented pigment particles and thereby hamper the transportation process after fragmentation.

**WATER DISPERSION PIGMENT PG7 AND GREEN CONCENTRATE TREATED BY FEMTOSECOND LASER**

Marilena Carbone[1]

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**Aim:** Investigating the effects of femtosecond laser treatments on PG7 and GC for removal purposes.

**Methods:** Dispersions of the same concentrations of PG7 and GC were treated with a Ti:sapphire femtosecond laser operating at 800 nm, at different peak and total powers. Treated samples were investigated by UV-Vis spectroscopy, SEM, and gas-chromatography/mass spectrometry coupled to PC analysis.

**Results:** The variation of absorption intensity of the treated samples is not linear with the irradiated power. The same types of fragment molecules are generated in all samples, but the concentration distribution is un-trended with the treatment.

**Conclusions**: Provided that femtosecond laser technology can be transferred to skin treatments, doses must be carefully evaluated, due to the non-linearity of processes

# History of Tattooing & Tattoo Organisations

## THE VIEW OF A TATTOOER ON THE LAST 35 YEARS

Maik Frey[1]

[1]*Tattoo Artist, Germany*

An inside look of an active tattooer with a focus on the changes in hygiene, equipment, tattoo colours and the art of tattoo.

**INTRODUCING THE COUNCIL OF EUROPEAN TATTOO ASSOCIATIONS (CETA)**

Dolores Murray[1]

[1]*CETA - Council of European Tattoo Associations*

**Aim:** With our presentation at the WCTP2023, we want to introduce the CETA to European tattoo related professionals from different fields.

**Introduction:** The Council of European Tattoo Associations (CETA) sees itself as an interest group in which all European Tattoo Associations come together to stand united for our tattoo industry. Furthermore, we act as an interface to share knowledge and information with tattoo artists in Europe.

**Current members of CETA:**
In alphabetical order

- ABMAI (Ireland)
- ASBL (Belgium)
- ATPR (Romania)
- BTAF (UK)
- BVT (Germany)
- CTNP (Italy)
- DOT (Germany)
- DTL (Denmark)
- FTAA (Finland)
- NBTK (Netherlands)
- NTU (Norway)
- ÖTPV Vienna (Austria)
- SNAT (France)
- SRT (Sweden)
- TK (Germany)
- UNTAP (Spain)

**Foundation:** In January 2021, Bruno Menei contacted Yuri Basso and both quickly came to the conclusion that, due to the imminent threat of REACH regulation and the consequences of the Corona Pandemic, it was time to unite European tattoo associations to jointly support the interests of tattooists in Europe.

**Activities:** At the WCTP, we will give insights in the following previous activities:

1. The Council of European Tattoo Associations formulated as the acting tattoo associations a “Letter of Support the EU-Petition” under the leadership of Austria.
2. SAVE THE TATTOO INKS! Poster designed by the Council of European Tattoo Associations, that links directly to EU-Petition “Save the Pigments”.
3. As a backup, possible accelerator and leverage to influence EU decisions, Save the Pigments has decided to take a separate legal route in the matter and the Council of European Tattoo Associations (CETA) supported the legal assessment financially.

**SRT - THE SWEDISH ASSOSIATION FOR TATTOO ARTISTS**

Rebecca Ryrberg[1]

[1]*Srt (Sweden's Registered Tattooists), Kingsroad Tattoo/Srt, Kristinehamn, Sweden*

**Aim:** We aim to inspire other tattoo associations with the work our association has done in Sweden since we started in 2007.

**Methods:** We have worked with authorities, not against. We try to be one-step ahead and have a serious approach towards the media and our members.

**Results:** Swedish tattooists are able to get official journeyman certificates and mastership diplomas in our trade and we have our own insurance that only members are allowed to get. We have also written a book to help guide the teacher and apprentice through the education and developed an application that make it easy for our members to share information with the client and follow the ink regulations.

**Conclusions:** SRT have put in a lot of time and energy creating an understanding and cooperation regarding related governmental issues. SRTs main objectives have always been to further the understanding and importance of a safe way of practice. Our model has been very beneficial for both the tattooists, the general health and the consumers.
Sweden’s Registered Tattooists have come a long way in the work to become a force to be reckoned with.

# Cosmetic tattoos & Breast reconstruction

**BREAST RECONSTRUCTION, RETOUCHING OF SCARS AND COLOUR RESTAURATION**

Andy Engel[1]

[1] *Andy Engel BWK, Germany*

Andy Engel utilizes photorealistic tattooing for different aesthetic medicine applications.
Customized kits with exclusive inks ensure the highest quality of the used products during and after tattooing. Standardized processes, quality management and retention samples ensure the safety of the patients. The talk covers different methods of skin reconstruction: breast reconstruction with and without plastic reconstruction of nipples and areolas in (male and female) breast cancer patients, scar correction after accidents and surgeries, as well as colour restorations in sex reassignment surgery patients.

**IMPACT OF REACH RESOLUTION OVER EUROPEAN TATTOO ARTISTS: GREYING THE HORIZON OF BREAST CANCER SURVIVORS**

Alexia Cassar[1]

[1]*The Peony Company Sas, The Tétons Tattoo Shop, France*

**Aim:** Nipple Areola Complex (NAC) reconstruction helps patients closing breast cancer journey.
NAC medical tattoo can be disappointing and semi permanent pigments require multiple touch-ups.
Artistic tridimensional NAC tattoo with traditional tattoo inks mimics nipple protrusion, and lasts longer without evidence of carcinogenic properties.

**Methods:** Artistic 3D tattoo with tattoo inks gives more satisfactory and long-lasting results as compared to semi permanent pigments.


With appropriate training, tattooing can happen one year after surgery and stay 5-10 years.
Before REACH resolution, NAC tattoos set of colours comprised 60+ different shades, adaptive to individual variability and reaching realism, with less of 15% touch ups.

Since January 2022, compliant ink set has shrunk to less than 10 from same American manufacturer and dropped to 6 after recalling Blue 15:3 and Green 7 pigments.

Tattoo precision and actual nipple resemblance have reduced, impacting overall quality and rising need for touches up as colours fade more and details vanish over time.


**Results:** Unavailable compliant inks full colour ranges, stock shortages and unreliable new formulas have measurable impact on customers confidence and technique requests. Customers and artists worry about untested formulas, few durability evidence, additional touch-ups costs and aesthetics.

**Conclusions:** Unavailability of colour ranges for compliant tattoo inks and loss of 90% of colours impact European cancer survivors 'access to self-esteem restauration and force them renouncing or getting tattooed unsafely with non-compliant inks. Revision of REACH resolution could give additional time to manufacturers, artists and customers as no large-scale health issues have been published about former inks.

# Analytics: Market surveillance & ingredients of inks

## 'REACH-COMPLIANT' TATTOO INKS UNDER SCRUTINY

Urs Hauri[1]

[1] *Cantonal Laboratory Basel, Switzerland*

**Introduction:** At the end of 2020, an analysis of twenty tattoo inks of nine producers demonstrated that none of the samples complied with the upcoming REACH regulation. Two years later, an analytical investigation of 'REACH-compliant' tattoo inks should reveal whether these products are truly compliant with the regulation.

**Methods and Samples:** Thirty-one self-disclosed «REACH-compliant» inks of eight brands were sampled at the end of 2022 on the European market. At the time, only five brands offered coloured inks. Inks were analysed for pigments, preservatives, isopropyl alcohol and contaminants like primary aromatic amines, form- and acetaldehyde, polyaromatic hydrocarbons, diethanolamine or nitrosamines.

**Results:** Of all 31 inks, only one black ink complied with the restriction regarding the analysed parameters. In general, black inks had fewer issues with the legal requirements. For coloured inks, three brands only had issues with contaminants, while the others still use forbidden pigments, one brand even openly disclosing them on their labels.
Non-compliance rates: acetaldehyde (at least 90%), diethanolamine (at least 50%), forbidden pigments (37% of coloured samples), benzisothiazolinone (16%), formaldehyde (at least 13%), isopropyl alcohol (13%), benzoic acid (10%), iodopropinylbutyl carbamate (10%), aniline (6%).

**Conclusion:** As anticipated in the 2020-study, producers had problems to resolve all compliance issues. Banned pigments were replaced by some producers and consequently there was less contamination with aromatic amines and fewer forbidden pigments detected. Too many inks still contained undisclosed sensitizing preservatives. Contaminants like acetaldehyde, formaldehyde, diethanolamine or benzoic acid still pose a major problem for all producers. In some cases, it is difficult to completely avoid traces of these substances.
Therefore, we re-emphasize that specific limits for contaminants must be set, based on toxicological evaluation and risk assessment, as was done for substances like aromatic amines or methanol.

**TATTOO INK ANALYTICS AND MARKET SURVEILLANCE**

**Case report: tattooing with new coloured tattoo inks**

Dr. Birgit Gutsche[1], Andrea Keck-Wilhelm[1], Maren Hegmanns[1], Cora Hannes[1], Claudia Baumung[1], Pascal Schneider[1], Tobias Peter[1], Jürgen Geisser[1], Susanne Neu[1], Sarah Hofmann[1], Jürgen Held[1], Carola Jagota[2], Jana Günther[2], Patricia Hipke[2]

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Since January 4, 2022, the legal regulations for tattooing products according to article 67 in conjunction with Annex XVII entry 75 Reach regulation have applied throughout the EU. Since then, tattoo inks available on the market have changed continuously, but up to now not necessarily for the better. We present the developments of the market in Germany as well as the results of the market surveillance study 2022.

We also report on a current case of complaints about unwanted skin reactions. Five customers showed serious skin reactions over a period of a few weeks after tattoo piercing. CVUA Karlsruhe and the LUA Dresden analysed the relevant five new colourful tattoo inks of the tattoo studio as well as comparative original samples collected at the same time. The results of the investigations are presented. We used various multi-methods to detect preservatives and pigments as well as known impurities such as elements, formaldehyde or primary aromatic amines. In addition, further prohibited ingredients and impurities were detected using screening methods such as HPLC diode array detection, HPLC high-resolution mass spectrometry and nuclear magnetic resonance spectroscopy. Finally, the currently emerging challenges for market surveillance and toxicological assessment of tattoo inks are discussed.

**COMPLIANCE OF TATTOO AND PMU INKS RELATED TO THE CONTENT OF PRESERVATIVES AFTER ONE YEAR OF REACH RESTRICTION APPLICATION**

Roberta Lavalle[1], Marco Famele[1], Carolina Ferranti[1], Luca Palleschi[1], Sonia D'Ilio[1], Rosa Draisci[1]

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Our previous study conducted on 138 inks purchased from the Italian market before the date of applicability of REACH restriction, pointed out the isothiazolinone derivatives as the most frequently detected preservatives in both tattoo and PMU inks. Their presence, commonly undeclared on labels, would have accounted for the 26.1% of inks non-compliance with the requirements of REACH restriction for skin sensitizing substances. Moreover, the isothiazolinone derivatives were quantified at concentrations that might cause reactions in already sensitized individuals in the 30.4% of samples. Due to the impact these substances may have on consumer's health, the quantification of isothiazolinones and other preservatives with hazardous properties (eg. phenoxyethanol) in inks has been included in the Italian National Plan for Chemical Products as priority target for the official controls carried out by the network of REACH/CLP laboratories since 2022.

In the framework of research activities aimed at enhancing the official controls system, an ongoing investigation, conducted as the follow-up of previous results, focuses on the evaluation of compliance of inks available on the market concerning the presence of skin sensitizing or eye damaging preservatives after one year of REACH restriction application.

In addition to isothiazolinones, phenoxyethanol and parabens, a new analytical method has been developed and validated for the identification/quantification of further preservatives such as formaldehyde-releasers and benzoic acid (RSD%: 4.9-6.2; recovery%: 86.0-92.0), which is classified under CLP as skin irritant/eye damaging. The presence of formaldehyde-releasers will be correlated with the levels of formaldehyde, which is classified as mutagenic and carcinogenic.

# POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN TATTOO AND PERMANENT MAKE UP INKS AND COMPLIANCE TO REGULATION (EU) 2020/2081: AN INVESTIGATION

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[2]*Sapienza Università DI Roma, Rome, Italy*

**Aim:** Huge diffusion of the practice of tattooing and permanent make up (PMU) led EU Member States to set concentration limits for carcinogenic polycyclic aromatic hydrocarbons (PAHs) in tattoo and PMU inks through the Regulation (EU) 2020/2081. The aim of this study was to quantify these substances in tattoo and PMU inks commercially available in Italy and investigate the compliance with the concentration limit of 5 ng/g and 500 ng/g for Benzo[a]pyrene and other PAHs, respectively, set by the restriction.

**Methods:** Benzo[a]pyrene, Benzo[e]pyrene, Benz[a]anthracene, Chrysene, Benz[b]fluoranthene, Benz[j]fluoranthene, Benz[k]fluoranthene and Dibenz[a,h]anthracene were determined in tattoo inks by Gas Chromatography/Mass Spectrometry. In house validation studies were conducted in accordance with ISO/IEC 17025. The method was applied to 134 commercial inks of different brands in different colours.

**Results:** Limits of detection and quantification ranged between 0.2 – 1.9 ng/g and 0.5 – 6.2 ng/g. Intermediate Precision and Recovery were 2.2 – 7.7 % and 98 – 112 %, respectively. Results showed that only four PAHs were contained at detectable levels and 7% of inks would not be compliant with tattoo restriction as regards the Benzo[a]pyrene, a classified carcinogenic substance.

**Conclusions:** Results obtained suggest that this reliable method could represent an effective work tool to be employed in the control analysis. Further work will be focused on the determination of other PAHs in tattoo and PMU inks that may be hazardous for human health.

**A STATISTICAL JOURNEY THROUGH THE FIRST YEAR OF THE NEW EU REGULATION**

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The new EU regulation of tattoo- and PMU colours has forced everybody in the business to reinvent themselves. Therefore, common knowledge from before is only partially applicable anymore. During the year 2022 more than 500 samples of ink, pigments and other ingredients with 700+ substances, either forbidden, found or requested were tested and statistically evaluated. Around 100 different forbidden substances were found in lab tests. The results of these tests give a unique insight of changes and future chances and challenges.

Four blocks were tested, free amines, heavy metals, PAHs and a screening block which contains all other substances. The screening block has the most findings. However, two similar substances cover more than 50 % of them. The Reach regulation has a method to address the few substances posing major challenges for manufacturers. Using appendix 13 of the Reach regulation is a perfect way to address critical substances after a specialised risk evaluation.
“A statistician can have his head in an oven and his feet in ice, and he will say that on the average he feels fine.”
That is why the limitations of the data set have to be stated. The source of the data is not an average of all manufacturers or traders in the EU. The companies looking for our service have confidence in their colours. Therefore, it can be assumed that inks on the market are more prone to have impurities or forbidden substances.

# Biokinetics & Biodistribution of tattoo inks

## SEMI-QUANTITATIVE HPLC-ANALYSIS OF BANNED ORGANIC PIGMENTS IN TATTOO INKS

Urs Hauri[1]

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**Introduction:** The REACH regulation sets legal limits for banned pigments. These are 0.1 % for certain azo pigments and 0.00005 % for hitherto popular cosmetic pigments such as C.I. 51319, C.I. 73900 or C.I. 73915. Therefore, quantitative methods are required for market controls.

**Methods:** Pigments are analysed with two different HPLC methods in which one is a 'standard' RP HPLC method and the second uses an eluent composition with N-methylpyrrolidone (NMP), acetonitrile and water at 80°C. Inks are screened by a consecutive extraction with three different solvents Dimethylformamide (DMF, Chloronaphthalene and NMP). If banned pigments are detected, a quantitative determination by a second HPLC method follows after extracting the pigment with specific solvents. Mono-azo-pigments like C.I. 11710, 12315, C.I. 12477 or C.I. 11741 are extracted with DMF and quinacridones with NMP. Diazo pigments, C.I. 51319 and C.I. 74160 are extracted with chloronaphthalene. C.I. 74260 is determined by colorimetry after extraction with sulphuric acid.

**Results:** For several pigments, a comparison with colorimetry showed good agreement. LOQ was in the range of 0.0005% to 0.005% for most pigments. A major obstacle remains the lack of quantitative references for the majority of pigments. Of 27 coloured tattoo inks, which were self-declared as 'REACH-compliant', at least 40% contained banned pigments above the legal limits. Seven inks contained C.I. 11710, C.I. 21108, C.I. 51319 or C.I. 73915 as the main pigments, which was correctly disclosed on the ingredient list in three cases. Undisclosed C.I. 74160 and C.I. 74260 were detected in two inks. Traces of C.I 73900 and C.I. 73915 were often found, particularly in inks containing C.I. 73907 and Pigment Violet 55.

**Conclusion:** Determination of banned pigments is feasible with the presented HPLC methods but the limit of 0.00005% is not met. Compared with studies before the implementation of REACH, fewer brands contained undisclosed banned pigments. It is strange that one company openly disclosed banned pigments and still called the products 'REACH-compliant'.

**IN VIVO-EXPOSURE TO TATTOO INKS - A SHORT-TIME HUMAN BIOKINETICS STUDY ON SOLUBLE TATTOO INK INGREDIENTS**

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**Aim:** Following the implementation of the REACH restriction, new limits have been introduced on substances in tattoo inks. However, these are based on *ex vivo*-experiments with pigments and might not accurately represent an *in vivo*-scenario. The aim of this study is therefore to determine the real-life exposure to tattoo ink per cm² as well as the quantification of soluble tracer substances that were added to the ink prior to tattooing.
**Methods:** Twenty-four subjects were tattooed with a design of their choice using black or red ink. The three tracer substances 4-aminobenzoic acid, 2-phenoxyethanol and potassium iodide were added to the commercially available tattoo inks. 4-Aminobenzoic acid was selected due to the structural similarity to aryls with an amino group, 2-phenoxyethanol as a representative for preservatives used in tattoo inks and iodide to determine the amount of intradermally applied ink. Blood, urine, ink and the consumables used for tattooing were collected and subsequently analysed. The study period was 24 hour before and after start of tattooing. Liquid chromatography coupled to time-of-flight mass spectrometer (HPLC-QTOF) was used to determine 4-aminobenzoic acid, 2-phenoxyethanol and their metabolites. Iodine was quantified by elemental analysis using inductively-coupled plasma mass spectrometry (ICP-MS). All methods were validated beforehand.
**Outlook:** The obtained exposure data can contribute to risk assessment and may translate into new limits for substances in tattoo inks. Furthermore, the data of 4-aminobenzoic acid metabolism during tattooing might be transferable to substances of high concern, which cannot be tested due to their hazard profile.

**BIOKINETICS OF TATTOO PIGMENTS - A STUDY IN PIGS**

Janos Cambiaso-Daniel

Nowadays, the general prevalence of tattoos worldwide is 20% and as demonstrated this tendency continues to increase. However, data on ink kinetics as well as long-term or systemic effects are still poorly understood and insufficiently studied. Since tattoos have already found medical applications, the aim of this project was to investigate the kinetics of tattoo ink once the color has penetrated the skin to better understand potential ink migration. Four female porcine were enrolled. Three animals except one (control) were tattooed. Skin biopsies were taken on day 7,14 and 28. On day 28, after euthanasia, of each animal homogenates probes of liver, spleen, kidney, and brain as well as of the local lymph nodes were obtained. Tattoo ink and all probes underwent then inductively coupled plasma-mass spectrometry (ICP-MS) and method dynamic light scattering (DLS) analysis. In the tattoo ink titanium (211.499mg/kg), copper (5.681mg/kg), aluminum (1.195mg/kg), zirconium (1.285mg/kg) and chromium (3mg/kg), were found. Statistically significant deposits of the tattoo ink elements were found in the tattooed skin when compared to non-tattooed skin. Titanium values increased (+238mg/kg), Copper (+92 mg/kg), aluminum (+108mg/kg), Zirconium (+23mg/kg), and chromium (+0.5mg/kg) also raised compared to the control. Furthermore, statistically significant deposits of titanium, copper, aluminum, zirconium, and chromium were respectively 42$\pm$2mg/kg, 69$\pm$25mg/kg, 49$\pm$18mg/kg, 0.3$\pm$0.2mg/kg, 0.5$\pm$0.2mg/in the lymph nodes. No significant deposits of ink elements were found in the liver, spleen, kidney, and brain. In conclusion several elements contained in tattoo ink in the skin as well as in the local lymph nodes, certain elements were up to 60 times higher when compared to non-tattooed animals.

**INVESTIGATING THE BIODISTRIBUTION OF IRON OXIDE TATTOO PIGMENT: AN EXPERIMENTAL MURINE STUDY**

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**Aim:** To examine MRI-induced skin reactions and the biodistribution of iron-oxide pigment in mice using high-resolution MRI and advanced bioimaging techniques, with an emphasis on metallic ink constituents.

**Methods:** Twenty hairless mice were tattooed with a commonly used magnetic cosmetic ink, while seven served as controls. Ten tattooed mice were exposed to a static magnetic field (SMF) from an MRI scanner to examine reactions in tattooed skin. At day 90, the mice were euthanized, and skin samples were analysed using ICP-MS, LA-ICP-MS, light microscopy (LM), and transmission electron microscopy (TEM). The dissected whole organs, including the liver, kidney, and cerebrum, were evaluated with high-resolution MRI and cross-sectionally by LM.

**Results:** No skin reactions were observed after exposure to a SMF, contrary to previous reports in the medical literature. No macroscopic organ pathologies were noted during dissection. LA-ICP-MS of tattooed skin showed significant levels of metallic isotopes even 90 days after tattoo application. MRI evaluation on dissected organs showed no significant difference between tattooed and untreated controls. However limited amount of pigment particles was detected in the liver by LM.

**Conclusion:** This study demonstrates that even highly magnetic tattoo pigment does not trigger skin reactions when exposed to a static magnetic field, even though significant amounts of iron and other metals are deposited in the skin. MRI evaluation of full organs could not detect any significant difference, indicating no evident deposition of inorganic tattoo pigment in peripheral organs. By light microscopy, only minute particles of pigment were visualized in the liver.