POSTER ABSTRACTS

[P1] LONG-TERM EFFECTS OF TATTOO INK PIGMENTS ON IMMUNE SKIN CELLS

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Tattooing is becoming increasingly popular across generations. E.g. in Europe, about 100 million people are estimated to be tattooed. Although tattoos have been around for centuries, the question of their health impact has really arisen rather lately. Tattoos represent an intradermal injection of pigments (i.e. coloured insoluble micro- and nano-particles), which remain in place throughout life. Despite medical complications listed, there is a lack of knowledge of the cellular and molecular long-term effects of pigments on immune skin cells. In the TATTOOINK Project, we will investigate a wide range of tattoo pigments on macrophages. Macrophages play a key role in the persistence of pigments via their phagocytic ability and capture-release-recapture cycles. They also play a key role in the inflammatory response and tissue homeostasis. Consequently, disorders in macrophages functionalities can have a significant health impact. In order to detect long-term effects, we tested pigments using two exposure schemes: an acute exposure for 24 hours and a 3-days post exposure recovery period. We already showed that cobalt (PV14, PB28, PG19) and zinc (PW4) pigments induced short and delayed functional effects, most of which were pigment-dependent. To pursue our investigations, we will focus on iron-containing pigments, which are still used in tattoos. The objectives will be i) test the long-term effects on macrophages up to 20 days after exposure by improving the cell culture conditions, ii) compare the effect of pristine and aged pigments as pigments undergo external “attacks” (e.g. by light or through in-cell degradation).

[P2] DEVELOPMENT OF A NEW SCREENING METHOD FOR TATTOO ANALYSIS USING DIRECT PROBE COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY

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To protect European citizens, thousands of hazardous substances in tattoo inks are restricted in the EU under the REACH Regulation from January 2022.

The most used techniques for the determination of organic substances in inks are usually gas and liquid chromatography coupled with different detectors. The main limitations of these approaches are time-consuming sample preparation, difficult chromatographic separation conditions and the requirement for highly skilled personal.

Due to the breadth of different substances under this restriction, screening methods as fast alternatives analysis with no or minimal sample preparation are highly desirable.
The purpose of this project is to evaluate a quick and simple procedure for the identification of insoluble pigments and other contaminants using direct sample introduction and high resolution Orbitrap mass spectrometry.

The aim of this project is to demonstrate that the direct insertion probe, in combination with high-resolution mass spectrometry, offers a powerful tool for the analysis of insoluble pigments and other contaminants in tattoo inks. Using this ionization, a fast screening of insoluble pigments can be performed without complicate sample preparation, with the possibility of fast distinguishing between compliant and non-compliant samples.

Electron ionization spectra can provide immediate clues on the presence of forbitten chemicals in tattoo inks thanks to spectral libraries. Moreover, the accuracy of high resolution (120.000 FWHM), together with isotopic pattern, can further increase the confidence in compound recognition. The instrument offers the possibility of performing soft ionization, such as PCI or NCI, for additional compounds identification.

[P3] WHY PERMANENT MAKE UP (PMU) IS NOT A LIFETIME APPLICATION

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Aim: The aim is to answer the question about the semipermanent character of PMU application compared with the lifetime applications of decorative tattoos.

Methods: PMU applications are not for a lifetime as the decorative tattoos. The reason is hiding in the colorants and not the machines or the methods used. PMU colorants have bigger particles than nanoparticles find in the tattoo ink. In our study we applied tattoo ink and PMU colorants on SKH-1 mice with a PMU machine to find out the ink's penetration depth in day one,seven and 60th after application.

Results: All the applications with tattoo ink were found to be much deeper into skin from the first day of application but this difference was the same after one week and eight weeks' time (table 1). The amount of colorants as seen in biopsies is the reason why the PMU "disappears" after a couple of years.

Conclusions: This study gives a new aspect of the PMU application compared to the tattoo ink which has a lifetime duration. Although the depth that the colorants stay after a while is almost the same, the amount of the colorants is the reason why the tattoo application lasts forever.

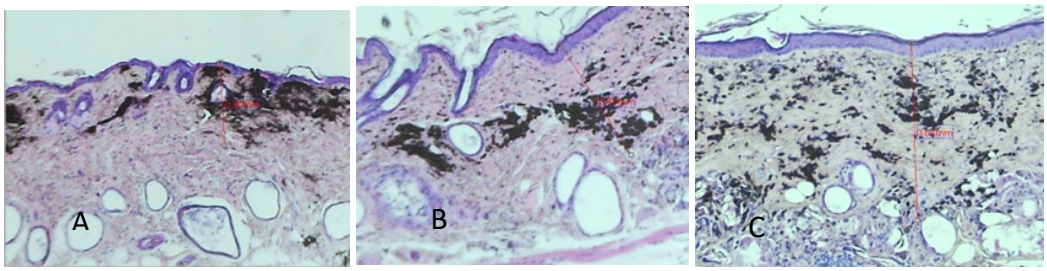

Figure A: Black Tattoo ink 1 day-1 (A) 4x, day-7 (B) 10x,day 60 (C) 10x.

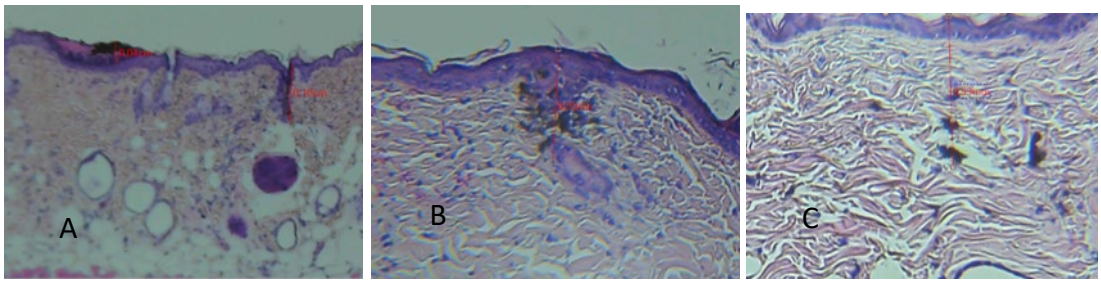

Figure 2: PMU colorants day-1 PMU (A) 4x, day-7 (B) 10x and day 60 (C) 10x.

[P4] REBUILDING LIVES: PMU AREOLA AND NIPPLE COMPLEX RECONSTRUCTION

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How can we help breast cancer woman to continue after a mastectomy and the significance that this could mean for their healing process. What the reconstruction of the nipple areola complex could retribute to de patient, not only in the physical part, but also in the psychological aspects of their life's, like the self-concept, self-esteem, femineity, body image, between others. The concept rebuild is meaningful to every woman in a different way and that´s why is so important to consider in the treatment of PMU.

[P5] MEDICAL TRAINING TO BECOME A STATE CERTIFIED TATTOO ARTIST

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Introduction: Becoming a state-certified tattoo artist in Austria involves practical, oral and written examinations taken mostly after 3-5 years of apprenticeship in a registered tattoo studio. While in many other European countries no training or examination is required.
In Austria Darwin skills, technical aspects of different tattoo guns are tested but also medical knowledge. But what medical knowledge is relevant for a tattoo artist?

Main: Ideally it should allow the tattoo artist to identify clients better, who should not be tattooed at all, who should get a dermatological checkup first and which anatomical area should be avoided in certain clients.
Thus, medical training for tattoo artist should exceed the protocol for needlestick injuries and bloodborne pathogens.
However, knowing the names of the different epidermal layers and special immune cells, as was tested in the past, is not relevant in reducing tattoo complications.
We propose a case-based examination, in which different tattoo clients are presented with their tattoo request (colour, size, anatomical region) and the examinee has to ask for possible contraindications.

Conclusion: Relevant medical knowledge for tattoo artist might help to reduce tattoo complications and should be taught and tested in more European countries.

[P6] AREOLA TATTOOING AFTER REACH

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Introduction: Like all areas of tattooing, also medical tattooing has been affected by the REACH regulation of tattoo pigments. However, after all, the color palette necessary for areola pigmentation did not decrease as much as initially feared. Even after the ban of the pigments blue 15 and green 7 in January 2023, used in brown and pink tones.

Main/Discussion: We were able to finalize patients´ areola tattoos, which were not finished by the end of 2022, with great outcome even after the 4th of January 2023. That shows that this important part of breast reconstruction is available for patients with REACH conform inks.
However, since the implementation of the REACH regulation we noticed an increased inquiry for information what inks to use now for areola tattooing. Medical professionals and permanent make-up artists performing areola tattooing are now unable to order their usual ink. Many contacted us, not knowing about REACH or Rapex, thinking this does not concern them.

Conclusion: There is still a great lack of information in some professional groups performing areola tattooing. However, this change bears a chance to bring awareness and comparable quality standards to all groups of professionals performing areola tattooing.

[P7] TASCC, A TOOL FOR RELEVANT STATISTIC

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Since the start of ESTP we have from TPE and the industry had a feeling that the way we collect information could be more organized and effective. Any law submitted and pointed towards our practice should be based on sound and accurate statistics. After the meeting in 2019 I and a college decided to put thoughts in motion, and we created a very easy to use tool to gather relevant statistics. Our tool can be implemented very fast thru our social media channels and reach thousands of tattooed people as well as practitioners in just days. Any question can be asked. In the trial run we had almost 10.000 interactions in one week, giving data in both Swedish and English. We asked around 50 questions regarding tattoo prevalence and other tattoo related matters. We call this tool TASCC, Tattoo statistics and complication collective. Any research facility in Europe will be given a possibility to ask relevant questions thru TASCC and we hope you see the benefit of this.

[P8] ELECTROPORATION FOR PMU REMOVAL

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Aim: The aim is a new approach in the field of PMU removal with a combination of the electric field of electroporation and mesotherapy technique. This was achieved with the use of a machine which combines both characteristics.

Methods: We used an electroporation machine with nano needles and a PMU machine, in order to remove PMU with plant-based extracts. The application was made on SKH-1 mice which were tattooed with PMU colorants for eyebrows and lips. (Figures 1,2)

Results: The results in histological samples showed the same amount of PMU colorants in both decolorization methods used. The electroporation showed no histopathology damage (Figures 1B,2B).

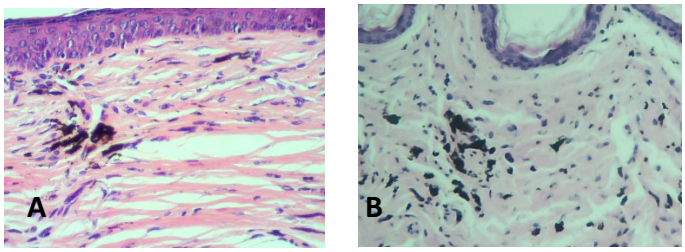

Figure 1: Biopsy from SKH-1 mouse skin with brown PMU colorant used for eyebrow applications 40x.(A) Treated with PMU machine and (B) Treated with electroporation with mesotherapy nano needle. In both cases the decolorization method used was plant-based material.

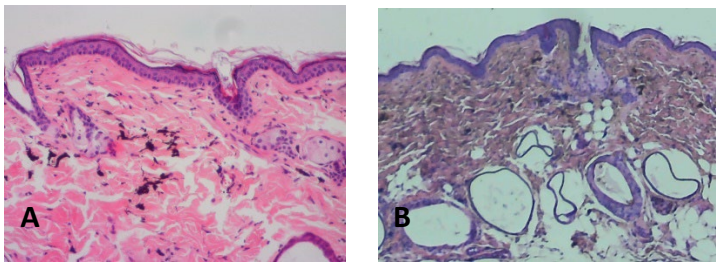

Figure 2: Biopsy from SKH-1 mouse skin with red PMU colorant used for lip applications 10x.(A)Treated with PMU machine and (B) Treated with electroporation with mesotherapy nano needle. In both cases the decolorization method used was plant-based material.

Conclusions: This study gives a new aspect for the use of electroporation as a non-laser treatment for PMU removal. Although the amount of PMU ink was not reduced with the plant origin materials after the six weekly treatments applied in 72 days, electroporation with nano mesotherapy needles is a new approach for PMU removal.

[P9] NEEDLING & CAMOUFLAGE FOR SCAR MANAGEMENT

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The practice of needling scars prior to tattooing has become increasingly popular in recent years.
This technique involves using a specialized needle to create micro-punctures in scar tissue, which can help to break up scar tissue and promote the growth of new collagen. This technique can be particularly effective in covering scars or stretch marks with camouflage pigments, as the scar tissue often makes it difficult for ink to adhere properly. The goal is to create a smoother, more even surface for the tattoo artist to work on, resulting in a more aesthetically pleasing final product.
Ultimately, the decision to needle a scar before tattooing should be made on a case-by-case basis, considering the individual's medical history, the location and severity of the scar, and the desired outcome of the tattoo.

[P10] A PRELIMINARY STUDY ON VOLATILE ORGANIC COMPOUNDS (VOCS) DETERMINATION IN TATTOO INKS

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Aim; Exposure to some volatile organic compounds (VOCs), classified as hazardous for human health in tattoo inks, may occur through skin absorption and inhalation. A preliminary study was performed to quantify benzene and toluene, by headspace gas chromatography coupled with mass spectrometry (HS-GC/MS), in order to ensure the enforcement of Regulation (EU) 2020/2081, which sets concentration limits for these two substances to 0.5 µg/g and 10.0 µg/g respectively.

Methods: A specific *in-house* method validation procedure was developed according to ISO/IEC 17025 for simultaneous quantitative determination of benzene and toluene in tattoo inks. Bottom-up approach was used to evaluate measurement uncertainty. This validated method was applied to 30 different commercially available tattoo inks.

Results: Analytical performance characteristics for target analytes, as limits of detection (LOD 0.1 – 2.0 µg/g), limits of quantification (LOQ 0.4 – 7.0 µg/g), intermediate precision (12% - 27%), recovery (116% - 72%) and measurement uncertainty (11% - 31%), were determined. Analyses of commercial samples showed that toluene was not detected, while benzene was determined above the Regulation (EU) 2020/2081 limit in 43% of inks, with concentrations up to 55 µg/g.

Conclusions: A rapid in-house method for simultaneous quantification of benzene and toluene by HS-GC/MS, in mixtures to be used in tattooing practise, was developed and successfully applied to commercial samples. This preliminary research highlighted that further studies are required for the determination of other VOCs.

[P11] AN ITALIAN STUDY ON CHEMICAL SAFETY OF COMMERCIAL TATTOO AND PERMANENT MAKEUP INKS

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Aim: Millions of people have painted their body with black or colored tattoo inks although some inks may contain hazardous substances for human health classified as carcinogenic, mutagenic, or toxic for reproduction (CMR) according to regulation n.1272/2008 (CLP). Among these, some aromatic hydrocarbons (PAHs) and phthalates were selected as chemicals of concern since are ruled by Regulation (EU) 2020/2081.

Methods: Eighty commercial inks of 23 different brands were collected in Rome and selected according to the following criteria: ink type, brand, manufacturing and colour. Eleven phthalates and eight PAHs were determined in samples by Gas Chromatography/Mass Spectrometry applying in-house previously validated analytical methods.

Results: In this study, 74% of collected samples were tattoos inks, while the remaining 26% were permanent make up inks. As regards the producers' countries, 26% of samples were from Europe and 74% from non-EU countries. Most inks, 66 %, were of different colours with a minor percentage of black inks, 34%. Outcome of the study highlighted that 55% of analysed samples were not aligned with Regulation (EU) 2020/2081 for phthalates. The simultaneous presence of PAHs and phthalates above permitted levels was measured in 9% of samples collected, all of them came from non-EU countries and consisted of 50% dark colours.

Conclusions: This investigation showed that a relatively limited percentage of the analysed samples contained both phthalates and PAHs at levels not complying with Regulation (EU) 2020/2081. Further studies will be performed focusing on other hazardous substances of interest as volatile organic compounds.

[P12] OVERVIEW OF ITALIAN PROJECTS ON HAZARDOUS SUBSTANCES IN TATTOO AND PERMANENT MAKE-UP INKS

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Aim

In the last decade, the number of people getting tattoo has drastically increased, being a matter of concern for human health for European and national Authorities due to the content of hazardous substances in inks. Italian Ministry of Health (financier), Istituto Superiore di Sanità and two national Public Health Laboratories, ARPA Piemonte and APPA Bolzano, joined forces in five research projects and collaborated to the regulatory process that would have led Europe to the REACH restriction on tattoo inks.

The framework of laboratories

Between 2013 and 2021, the projects focused on the development of analytical methods for quantification of a number of hazardous substances present in tattoo inks and evaluation of possible risks for human health. In the meanwhile, the formal procedure for the proposed restriction in Annex XVII on the use of substances in inks for tattoos and PMU was launched. The project of 2017 contributed to support for some aspects the "Justification Document", being developed at EU level to outline the restriction under REACH.

Outcome and future perspective

These projects contributed to support the restriction proposal for aspects relating to development of new analytical methods for substances quantification to be employed restriction enforcement on national territory.
Regulation 2020/2081 limited the use of about 4000 substances in tattoos inks. The ongoing project, started on December 2022 and financed by Ministry of Health, in collaboration with the two above partner laboratories, assists in the implementation of the restriction assuring the development of new analytical methods on additional substances.

[P13] IN VIVO AND IN VITRO EFFECTS OF BLUE TATTOO INK ON REDOX HOMEOSTASIS

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Aim: It is hypothesized that oxidative stress is responsible for the adverse effects of tattooing and tattoo inks; however, there is currently no experimental evidence for tattoo ink-related oxidative stress in humans. The aim was to investigate *in vivo* effects of blue tattoo ink on the biomarkers of redox homeostasis in an N-of-1 human trial. A follow-up *in vitro* study of blue tattoo ink was conducted to explore the alterations observed *in vivo*.

Methods: Skin surface oxidation-reduction potential (ORP) was analyzed with a PH60F flat probe. Antioxidant capacity, H_2O_2 dissociation rate, 1,2,3-trihydroxybenzene autoxidation, thiobarbituric acid reactive substances, and protein and free thiol groups were measured in the interstitial fluid extracted from the tattoo and the control area. The effects of blue tattoo ink constituents on catalase and superoxide dismutase activity were analyzed *in vitro* in the normal skin matrix.

Results: Unexpectedly, surface measurements and redox biomarkers from the interstitial fluid revealed reduced levels of oxidative stress in the tattooed skin. Blue tattoo ink increased antioxidant (ORP, NRP) and H_2O_2 dissociation capacity, and decreased lipid peroxidation possibly due to the presence of (recently banned) tattoo pigment copper(II) phthalocyanine (CuPC). In vitro analyses revealed that CuPC-based tattoo ink can act as a dual mimetic of catalase and superoxide dismutase; however, tattoo ink fractions without CuPC also modulated the activity of both enzymes.

Conclusions: CuPC-based blue tattoo ink can act as a dual functional mimetic of catalase and superoxide dismutase and reduce biomarkers of oxidative stress in human skin in vivo.

[P14] TATTOO COMPLICATIONS SEEN AT THE DERMATOLOGY DEPARTMENT

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[1] *"The Tattoo Clinic", Dermatology Department, Bispebjerg University Hospital, Denmark.*

Aim: Tattoos are popular and approximately every 5th European person is tattooed. People of both genders and from all social classes have followed the tattoo trend. Among the tattooed, every 5th person experiences a tattoo complication. Reactions are troublesome and patients are heavily burdened by itching, swelling, pain and/or sores.

Methods: "The Tattoo Clinic", at the Dermatology Department, Bispebjerg University Hospital in Denmark, is highly specialized in diagnosis and treatment of tattoo complications. More than 1500 reactions have been seen at the clinic since it was established in 2008. The commonest types of tattoo complications will be illustrated and shortly described on the poster.

Results: Tattoo complications often seen are infections, papulo-nodular reactions, allergic reactions, neuro-sensitivity reactions, light induced reactions, tattoo technical hazards (needle trauma, pigment overload, infected ink) and tattoo removal hazards (by laser, caustics, surgery).

Conclusions: General practitioners and dermatologists should be introduced to this newer subspecialty of dermatology. Patients are easily neglected and not offered optimal treatment despite the disease burden being comparable to other cumbersome dermatological diseases. Tattooists should also be aware of these complications to reduce the frequency of reactions hereby infections, tattoo technical hazards and tattoo removal hazards.

[P15] MONKEYPOX AND TATTOOS: A SCOPING REVIEW IN VIEW OF THE RECENT OUTBREAK

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Aim: Monkeypox is a viral zoonosis with symptoms comparable to those of smallpox. The most frequent method of monkeypox virus transmission so far has been through sexual contact.
The aim of the present study is to summarize and assess the recent evidence regarding the link between tattoos and the recent monkeypox outbreak.

Methods: EMBASE, PubMed, and the Web of Science were searched on January 2023. No language restriction was applied.

Results: In total, three male patients with at-risk sexual behaviors who developed tattoo-associated monkeypox virus lesions were identified (mean age: 39,3 years).
The diagnosis of monkeypox infection was confirmed by a PCR test in all patients (skin).
The mode of transmission was attributed to the tattoo application process in 2/3 of the patients.
Monkeypox-related tattoo pseudokoebnerization was reported in one patient.

Conclusions: The findings of this study should remind us of the importance of proper hygienic practices in tattoo parlors, which can avoid the spread of bacterial and viral agents during the tattoo application process. Furthermore, despite the low number of cases reported in the medical literature, possibly because of underreporting, further studies are needed to assess the magnitude of tattoo-related monkeypox transmission in comparison to other viral infections.

[P16] PRELIMINARY SURVEY ON TATTOO AFTERCARE: INDICATIONS AND MANAGEMENT

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Aim: In Italy, approx 7 million tattooed people represent a growing "phenomenon" which, due to the practice's invasiveness and possible risks, implies the need for safety rules and regulations.
The recently published European standard EN 17169 specifies the hygiene requirements applicable before and during tattooing and for post-tattoo care.
The standard defines the post-tattoo or aftercare phase as the care process to promote optimal healing. Currently, in Italy, in the absence of national legislation, there is a tendency to issue laws with regional scope. The primary objective of this preliminary survey is to carry out, through simple questions a follow-up of the post-tattoo phase, starting from the collection of information communicated to the client for the procedure followed after the execution of the tattoo.

Methods It was decided to follow a CAWI (Computer Assisted Web Interviewing) methodology based on the compilation of a web questionnaire provided through a link, a panel or a website. The data collected is saved and made available for a real-time analysis of the collected data and statistics directly within the software.

Results and conclusion: At the moment: (a) The tool has been developed (the link to the questionnaire translated in to English is the following: https://forms.office.com/Pages/ResponsePage.aspx?id=_ccwzxZmYkutg7V0sn1ZEvPNtNci4kVMpoVUounzQ3tUMlY4MjBBVlVHUk9PQ0tQRFdKR1lNVUNVUS4u).(b) It has been submitted to a properly selected community, and will be accessible for 2 months (c) At the end of the submission, it is expected that useful data will be available for understanding whether professional tattoo artists know the standard and which products they use.

[P17] CAN THE USE OF DIGITAL TECHNOLOGIES ENHANCE THE SAFETY OF TATTOOING PRACTICES?

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The application of the principles of telemedicine to dermatology is referred to as teledermatology. Classically, it has been classified into real-time teledermatology and store-and-forward teledermatology [1]. The Covid-19 pandemic demonstrated that Teledermatology, in the both approaches could be an accessible, accurate and cost-effective substitute for conventional face-to-face dermatological consultations [2]. Artificial intelligence, also is becoming increasingly important in dermatology, with studies reporting accuracy matching or exceeding dermatologists for the diagnosis of skin lesions from clinical and dermoscopic images [3]. The last frontier is that relating to the use of Apps based on artificial intelligence for the automatic detection of dermatological pathologies.

Recently, for example, an App called DermAssist has been made available for this purpose. It is a CE-marked as a Class 1 Medical Device, and is currently undergoing further market testing through a limited release [4-6].

The application of digital techniques typical of teledermatology could allow for better interaction of all the players involved in the tattoo sector. The use of applications like DermAssist could be a useful precautionary tool for both the tattoo artist and the person who wants to get tattooed.
In fact, in case of skin lesions, if the App detects a suspected pathology, the execution of the tattoo could be postponed to a moment after the consultation of a dermatologist, who could give the green light to the tattoo if he does not ascertain pathologies, or advise against it in case of pathologies, by indicating its contraindications.

[P18] OUTCOME OF SURVEILLANCE FOR TATTOO INKS IN ITALY: THE SCENARIO AFTER REACH INTRODUCTION

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Aim: The goal of the study is the evolution about the determination of aromatic amines (AA) and polycyclic aromatic hydrocarbons (PAH) in tattoo inks according with Resolution ResAP(2008):1 and Regulation (EC) n. 1907/2006 (REACH).

Methods: Between 2016 and the first half of 2022, about 500 ink samples were analyzed for the determination of AA and PAH. An elaboration of main results of AA and PAH obtained in the past years will be confronted with the new legislation.

Results: 35% of the samples were found to be non-compliant, equal to 126 samples analysed; 15 non-conformities concern the PAH, 111 instead concern the AA. For PAH, a decrease in non-conformities is observed: in 2016 and 2017 from 22% and 24% respectively to values below 5% in the following years. A decreasing trend also for AA were observed: from 2020 only 18% of samples are non-compliant versus the average of 42% in previous years.

Conclusions: The results obtained show that the introduction of a new regulation and more specific inspections within the supply chain produce greater attention towards the choice of raw materials used for production and the technologies used for the treatment of pigments. A significant variability of non-compliance after REACH implementation is observed.