

First International Conference on Tattoo Safety

BfR-Symposium, Berlin, June 6 – 7, 2013

Impressum

BfR Abstracts

First International Conference on Tattoo Safety
BfR-Symposium

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Opening

Written in Skin: The Symbol, Significance, and Practice of Indigenous Tattooing

Lars Krutak, Ph.D., Smithsonian Institution, National Museum of Natural History, Washington D.C., USA

In 1777, the word 'tattoo' was defined as 'an indelible mark of figure fixed upon the body by insertion of pigment under the skin or by the production of scars.' For thousands of years before that date, however, indigenous peoples around the world practiced various forms of tattooing not only to beautify themselves or mark significant life achievements, but also to please or seek protection from particular spirits, which inhabited their world. Of course, there were additional forms of tattooing that were utilized for therapeutic purposes and to mark tribal identity, amongst other things.

Drawing upon fifteen years of fieldwork across the indigenous world, this illustrated lecture will explore the indelible legacy of indigenous body marking to reveal the complex system of tools, techniques, and beliefs that ancient and more recent cultures utilized to control their bodies, lives, and experiences.

Programm

Thursday, June 6, 2013

Opening

9.00–9.10 a.m.

Federal Institute for Risk Assessment (BfR)

Prof. Dr. Dr. Andreas Hensel

President of BfR

9.10–9.20 a.m.

The Free University of Berlin (FU-Berlin)

Prof. Dr. Monika Schäfer-Korting,

Vice President of FU-Berlin

9.20–9.30 a.m.

**Federal Ministry of Food, Agriculture and
Consumer Protection (BMELV)**

Martin Köhler,

Department of Consumer Policy

9.30–9.45 a.m.

Introduction & Objective of the Conference

Prof. Dr. Dr. Andreas Luch,

Head Department of Product Safety, BfR

9.45–10.45 a.m.

**Written in Skin: The Symbol, Significance
and Practice of Indigenous Tattooing**

Lars Krutak, Ph.D.,

Smithsonian Institution, National Museum of Natural History, Washington D.C., USA

10.45–11.15 a.m. Coffee break

I Analytics & Exposure

11.15–11.30 a.m.

Introduction by Session Chair

Dr. Jutta Tentschert, BfR

11.30–11.55 a.m.

The Fate of Tattoo Pigments in the Skin

Prof. Dr. Wolfgang Bäumlér,

University of Regensburg, Germany

11.55–12.20 p.m.

Heavy Metals in Tattoo Inks

Dr. Beatrice Bocca,

National Institute for Health, Roma, Italy

12.20–12.45 p.m.

**Pigments, Preservatives & Impurities
in Tattoo Inks**

Dr. Urs Hauri,

Kantonales Laboratorium Basel, Switzerland

12.45–2.00 p.m. Lunch break

II Toxicology

2.00–2.15 p.m.

Introduction by Session Chair

Prof. Dr. Thomas Platzek, BfR

2.15–2.40 p.m.

Allergies and Tattooing

Prof. Dr. Jørgen Serup,
Bispebjerg Hospital, Department of
Dermatology, Copenhagen, Denmark

2.40–3.05 p.m.

Tattoos, Inks and Cancer

Nicolas Kluger, M.D.,
University of Helsinki, Finland

3.05–3:30 p.m.

Toxicity & Phototoxicity of Tattoo Inks and Associated Materials

Videoconference
Paul Howard, Ph.D.,
National Center for Toxicological Research (NCTR)
U.S. Food & Drug Administration (FDA)
Jefferson, AR, USA

3.30–3.55 p.m.

General Toxicity of Pigments

Dr. Wera Teubner
BASF – The Chemical Company

3.55–4.25 p.m. Coffee break

III Hygiene & Microbiology

4.25–4.40 p.m.

Introduction by Session Chair

PD Dr. Sascha Al-Dahouk, BfR

4.40–5.05 p.m.

Microbial Infections through Tattoos

Dr. Christa De Cuyper,
AZ Sint-Jan AV Hospital, Brugge, Belgium

5.05–5.30 p.m.

Risks of Tattooing: Mycobacter Infections

Victoria Scott-Lang, M.D.,
Royal Infirmary of Edinburgh, UK

End of First Day

Guided Sightseeing of Berlin (6.30–10.30 p.m.)

Walking Dinner (*for speakers only*)

Friday, June 7, 2013

9.00–9.15 a.m.

Summary of First Day

Prof. Dr. Dr. Andreas Luch, BfR

IV Technology

9.15–9.30 a.m.

Introduction by Session Chair

Dr. Peter Laux, BfR

9.30–9.55 a.m.

Microencapsulation of Dyes & PigmentsPD Dr. Lars Dähne,
Surflay GmbH, Berlin, Germany

9.55–10.20 a.m.

Development of Permanent but Removable TattoosBruce Klitzman, Ph.D.,
Duke University Medical Center,
Durham, NC, USA

10.20–10.45 a.m.

Perspective of the DermatologistVideoconference
Eric Bernstein, M.D., Ph.D.,
Main Line Center for Laser Surgery
Ardmore, PA, USA*10.45–11.15 a.m. Coffee break***V Risk Assessment & Regulation**

11.15–11.30 a.m.

Introduction by Session Chair

Dr. Annegret Blume, BfR

11.30–11.55 a.m.

Risk Assessment & Regulation of Tattoo Inks in the USLinda M. Katz, M.D.,
Office of Cosmetics and Colors (CFSAN)
U.S. Food & Drug Administration (FDA)
College Park, MD, USA

11.55–12.20 p.m.

Risk Assessment & Regulation of Tattoo Inks in New ZealandHelen Colebrook,
New Zealand Ministry of Health
Wellington, New Zealand

12.20–12.45 p.m.

**Risk Assessment & Regulation
of Tattoo Inks in the EU**

Paul J. Janssen, Ph.D.,
National Institute for Public Health and the
Environment (RIVM), Bilthoven, The Netherlands

12.45–2.00 p.m. Lunch break

VI Stakeholder Positions

2.00–3.20 p.m.

Chaired by Lars Krutak, Ph.D.

2.00–2.10 p.m.

**The Ecological and Toxicological
Association of Dyes and Organic Pigment
Manufacturers (ETAD), Basel, Switzerland**

Dr. Pierfrancesco Fois

2.10–2.20 p.m.

Pro Tattoo e.V., Essen, Germany

Dr. Iris Eschenbacher

2.20–2.30 p.m.

**Deutsche Organisierte Tätowierer e.V.,
Siegen, Germany**

Andreas Schmidt

2.30–2.40 p.m.

**Information Network of Departments of
Dermatology (IVDK), Hannover, Germany**

Prof. Dr. Axel Schnuch

2.40–2.50 p.m.

Haema AG, Berlin, Germany

Bernhard Fuchs

2.50–3.00 p.m.

MT.DERM GmbH, Berlin, Germany

Dr. Henrik Petersen

3.00–3.10 p.m.

**TIME – Tattoo Ink Manufacturer of Europe,
Neuburg, Germany**

Ralf Michel

3.10–3.20 p.m.

Chemical and Veterinary Inspection Office Karlsruhe, Germany

Dr. Gerd Mildau

3.20–3.50 p.m. Coffee Break

VII Podium Discussion

3.50–4.50 p.m.

Chaired by Lars Krutak, Ph.D.

VIII Farewell

4.50–5.00 p.m.

Professor Dr. Dr. Andreas Hensel,
President of BfR

I Analytics & Exposure

The Fate of Tattoo Pigments in the Skin

Prof. Dr. Wolfgang Bäuml, University of Regensburg, Germany

In recent years, tattoos have become very popular worldwide and millions of people have black or colored tattoos. Despite the increasing number of tattooed individuals, presently there are few requirements, legislation and criteria for the safety of tattoos and permanent make-up. The list of ingredients on tattoo inks is usually missing or incomplete.

Using tiny needles, tattooists place various tattoo inks in the dermis along with numerous unknown ingredients. Most of tattoos consist only of black inks, which predominantly are composed of soot products like carbon black or polycyclic aromatic hydrocarbons (PAH). We found high amounts of PAH as well as other hazard substances such as phthalates in black tattoo inks, which are injected into skin during the tattoo procedure. After establishment of an appropriate extraction method, the analysis of tattooed skin specimen revealed high amounts of carbon black and PAH in skin even years after tattooing. Carbon black and PAH could be also quantified in regional lymph nodes of tattooed individuals.

Many of the colored tattoo inks are azo- or polycyclic compounds, which are intrinsically produced to stain consumer goods. During laser removal of tattoos or under ultraviolet exposure, these azo pigments can be cleaved to form carcinogenic amines. The dispersion of the tattoo inks, its admixtures and possible decomposition products in the human body is unexplored so far, in particular whether other organs like spleen or liver are involved.

A survey revealed that most of the people received the first tattoo at the age of 16 – 20 years. Thus, azo pigments, carbon black, PAH and other constituents tattoo inks can stay for decades in the tattooed body of humans. The survey also showed that many people have many and large tattoos. It must be taken into account that about 1 mg is placed in skin for each cm² of a tattoo. Risk assessment should be an essential part for protecting human health, and this applies also with tattoos.

Heavy Metals in Tattoo Inks

Dr. Beatrice Bocca, Francesco Petrucci, Alessandro Alimonti, National Institute for Health, Roma, Italy

Titanium, barium, aluminum and copper are often used as colorants in tattoos; more worrisome, inks using nonmetal colorants may include traces of antimony, arsenic, cadmium, chromium, cobalt, lead and nickel. Some metal oxides (aluminium oxide, titanium oxide) in the nanoscale size are also utilized to reach the desired colour, transparency or fluorescence. Metal and metal nanoparticles toxicity of inks is a casually observed problem and information about pigments in products is usually a trade secret. Anyway, tattoo inks have caused dermal rashes, infection and inflammation, but more concerning, there are some unanswered questions about long-term risks because pigments are believed to remain in the body for life, whether within the skin or in the lymph nodes.

To provide more information which is scientifically based, our research activity covered: *i) in-vivo* studies on patients that presented pseudolymphomas on their tattooed areas in order to identify the metals responsible for the reactions; *ii)* quantification of metals and characterization of nano-sized metals in tattoo inks in order to better understand the scenario and entity of human exposure.

These studies embraced different separation and quantification techniques, or combination of them, as Field Flow Fractionation, Dynamic Laser Scattering, Multi-Angle Light Scattering, and Inductively Coupled Plasma Mass Spectrometry.

Based on the results obtained so far, different topics are discussed: *i)* the safety concern (local and systemic) for humans exposed to metals and nano-metals contained in inks; *ii)* conformity of inks available on the market to existing guidelines; *iii)* practical measurement issues to be still improved.

Pigments, Preservatives and Impurities in Tattoo Inks

Dr. Urs Hauri, Kantonales Laboratorium Basel, Switzerland

In Switzerland, legal restrictions for inks for tattooing and Permanent Make up (PMU) exist since 2006. These restrictions base upon the European Council resolution ResAP(2003)2.

From 2008 to 2012, our laboratory analysed 416 samples of 73 brands for organic pigments, preservatives and impurities such as N-nitrosamines, primary aromatic amines and recently also for poly aromatic hydrocarbons (PAH). Two nationwide market surveillance studies were conducted (2009:105 tattoo inks, 47 PMU; 2011:167 tattoo inks, 23 PMU). In 2009, alarmingly 54% of the tattoo and 11% of the PMU inks and in 2011 still 37% of the tattoo and 9% PMU inks were banned.

39 Organic pigments were identified with MALDI-TOF and HPLC. The lack of a positive list and the adoption of pigment bans from the cosmetics regulation, led to an increasing occurrence of non-restricted pigments (2009: 39%, 2011: 56%) which never were meant or tested for usage in contact with the human body. The occurrence of azo pigments, prone to splitting off carcinogenic amines when tested according to the textile azo norm (limit of 30 mg/kg) declined from 6% in 2009 to 1% in 2011. Release of such amines under UV or laser irradiation, however, was observed for pigments that comply with the azo norm. As an example, pigments of the diarylide type released 3,3'-dichlorobenzidine under UV as well as laser irradiation.

In Switzerland, the regulation on the use of preservatives in tattoo inks corresponds to the regulation for leave-on cosmetics. Most tattoo inks are not preserved with classical micro biocides for cosmetics. Banned preservatives found were benzisothiazolinone (56 samples; 0.4 - 245 mg/kg), octhlinone (15 samples; 40 - 450 mg/kg) and phenol (12 samples; 40 - 4300 mg/kg). Inks were banned if the limit of 50 mg/kg was exceeded (46 samples). Besides formaldehyde (55 samples, 0.004 - 0.23%), the allergenic preservative Methylisothiazolinone/methylchlorisothiazolinone (MI/MCI) was the most frequently found cosmetic preservative (21 inks; 0.5 to 82 mg/kg). Nine inks had to be banned because the limits of the Cosmetics Regulation were exceeded (phenoxyethanol (2), MI/MCI (5), glyoxal (1) and formaldehyde (1)).

N-Nitrosamines were analysed with LC/MS/MS and found in 68 samples (16%), the most frequent congener being N-Nitrosodiethanolamine (NDELA; 56 samples; 6 - 24000 µg/kg). Undisclosed triethanolamine was the reason for these findings. Nine samples contained N-Nitrosomorpholine (9 - 625 µg/kg), two samples N-Nitrosodibutylamine (53 - 93 µg/kg) and one sample N-Nitrosodimethylamine (17 µg/kg). Inks were banned if they exceeded 150 µg/kg (11 samples).

Polycyclic aromatic hydrocarbons (EPA & EFSA) were analysed in 24 samples using pressurised microwave extraction with toluene at 120°C. 13 samples contained less than the sum of 0.5 mg/kg PAH and thus complied with the ResAP(2008) of the European Council. 6 samples contained more than the sum of 5 mg/kg PAH (18 - 93 mg/kg), eight samples more than 10 µg/kg Benzo(a)pyrene (30 - 900 µg/kg).

A lot of samples contained other undisclosed ingredients in the percentage range, e.g. β-naphthol ethoxylate (15 samples), nonylphenol ethoxylate (7 samples) or octylphenol ethoxylate (8 samples).

II Toxicology

Allergies and Tattoing

Prof. Dr. Jørgen Serup, Bispebjerg University Hospital, Department of Dermatology, the “Tattoo Clinic”, Copenhagen, Denmark

Reactions of tattoos supposed to be allergic in nature really dominate the clinical spectrum of chronic adverse events in tattoos as accumulated in the “Tattoo clinic” since 2008. Skin cancer was never observed. Red is the predominant problem colour. Reactions appear months or years after the tattoo is made, thus, after a remarkably long period of sensitisation. The entire field in a person tattooed with the culprit colour reacts. Reactions typically are inflammatory or so called “lichenoid” but may range from ulceration to excessive epidermal hyperplasia. Itching is advanced. The hallmark histology is inflammation and interphase dermatitis with infiltrating T-lymphocytes.

Allergy patch test on the back with a standard battery of 43 allergens, a textile dye battery of 32 allergens, mostly dispersed azo dyes with variable content of primary aromatic amines (PAA), and a tattoo battery of 8 stock bottle tattoo inks (brand name “Tattoo”, Taiwan; known to cause troubles and withdrawn from market by Danish authority; holding the PAA anisidine among others) was conducted in 58 patients with tattoo reactions. 16 reacted to nickel. 2 reacted to textile dyes (urea formaldehyde and disperse yellow), and 4 reacted to the tattoo battery, all to red, one to three additional colours. Thus extensive patch testing came out negative except for sporadic cases. Patients with additional testing against their individual culprit colour failed to react to the patch.

The patient material included cases with cross reactivity coming up in old tattoos parallel to reaction in a fresh tattoo, all in red. These allergy clue lesions shared clinical signs and histology with the recent problem tattoo of the individual. From such observation the phenomenology of tattoo reactions due to allergy could be concluded. Raman spectroscopy of 3 biopsies from tattoo reactions to red failed to identify the respective PAAs of the known culprit colours (vs positive control of illuminated ink).

Conclusions: The allergen is not found directly in the tattoo ink stock bottle. The allergen apparently is formed inside the skin, probably through haptensisation with proteins. The epitope itself also might be formed in the skin from variable sources. Impurities of inks are manifold. It is unlikely to be some simple PAA split product of the azo pigment as known from the laboratory or registers. Findings are accordant with recent studies on disperse azo dyes. Negative outcome of PAA patch testing of humans with textile dye allergy did not confirm the allergenic potential of individual PAAs. The European Council resolution 2003/08 needs a critical revisit re. the postulated allergenic potential of the listed PAAs supposed to be allergenic in tattoos. The effectiveness of screening PAAs in tattoo ink stock products to distinguish allergy safe and unsafe inks is controversial. The resolution was not validated specifically in relation to tattoos but nevertheless implemented in some countries as a regulatory intervention to prevent allergic reactions in tattoos.

Tattoos, Inks and Cancer

Nicolas Kluger, M.D., University of Helsinki, Finland

The introduction in the dermis of exogenous pigments and dyes to obtain a permanent design (tattooing) represents a unique in-vivo situation, where a large amount of metallic salts and organic dyes remain in the skin for the lifetime of the bearer. The potential local and systemic carcinogenic effects of tattoos and tattoo inks remain unclear. Several studies have shed light on the presence of potential carcinogenic or pro-carcinogenic products in tattoo inks. We extensively reviewed the literature and found approximately 50 cases of skin cancer on tattoos: 23 cases of squamous-cell carcinoma and keratoacanthoma, 16 cases of melanoma, and 11 cases of basal-cell carcinoma. The number of skin cancers arising in tattoos is therefore seemingly low, and this association has to be considered thus far as coincidental. We will discuss the “classic” pros and cons argues for a potential carcinogenic risk of tattooing and tattoo inks, but also the clinical relevance of current toxicological and chemical studies that have been conducted thus far on such topic.

Toxicity & Phototoxicity of Tattoo Inks and Associated Materials

Paul C. Howard, Ph.D., National Center for Toxicological Research (NCTR), U.S. Food and Drug Administration (FDA), Jefferson, AR, USA

Tattooing has become a widespread, culturally acceptable, form of artwork. Tattooing in its simplest form is the semi-permanent introduction of inorganic and organic colored pigments into the dermis of the skin using a needle with repetitive injections. Several studies point to potentially toxicological hazards associated with the components of tattoo inks. First are the studies from this laboratory and others that have shown that many tattoo ink pigments photodecompose in the presence of sunlight to form cytotoxic and potentially genotoxic products. Some of these decomposition products are suspect, or known, human carcinogens. Second, the skin possesses metabolic systems that can metabolize ink pigments to multiple products. Using isolated enzyme systems, Pigment Yellow 74 is metabolized by cytochromes P450 to oxidized products and is reduced at the nitro group to reactive aryl nitroso and hydroxylamines. Third is the immunotoxicological hazard associated with tattoo ink use. Tattooing is essentially controlled wounding of the skin. The response in the skin is predictable and has been shown to involve recruitment of phagocytic and inflammatory cells to the wound site. Data will be shown that demonstrates an inflammatory response in the regional lymph nodes and skin to tattooing. These processes result in tattoo ‘wound’ healing, but at the same time result in the presentation of tattoo pigments to the immune system. Using a modification of the local lymph node assay, many inks were found to induce a measurable proliferation response in the regional lymph nodes, again confirming the immunogenicity of many tattoo inks. The final consideration will be the essential data required for risk assessment of tattoo inks. A considerable amount of research has been accomplished, and while there appears to be a daunting amount of data further required to understand the toxicology profile of tattoo ink components, it would seem prudent that the combination of epidemiological investigations and laboratory studies could be used to address the most problematic ink components (This presentation should not be considered official US Government opinion or policy).

General Hazard Profile of Pigments

Dr. Wera Teubner, BASF Schweiz AG, Basel, Switzerland

This presentation summarizes human health hazard data on more than 10 chemical classes of pigments that has been gathered and used for the registration as an industrial chemical in the European Union.

Briefly put, the European chemical legislation (EC 1907/2006) requires registrants to share available data, to search public databases for relevant information and to review it for adequacy and reliability. Filling of data gaps requires GLP and OECD testing guideline compliant studies, unless certain criteria laid out in the regulation are met. The hazard data is disseminated in the form of robust study summaries on the website of the European Chemicals Agency.

BASF produces pigments for the use in industrial applications such as automotive coatings or coloration of plastics. The presented toxicological data reflects this use pattern.

The hazard data refers to pigments alone. It is not representative of pigment formulations containing dispersing agents, fillers, solvents, etc. It may also not be representative of pigment products containing a high level of impurities as these may contribute significantly to the hazard profile. For example, the disseminated dossier of Pigment Red 112 displays an extra classification for skin sensitisation for products containing more than 1% of the unreacted starting material naphthol AS-D (3-hydroxy-N-(2-methylphenyl)-2naphthalenecarboxamide).

Pigments are non-toxic in rats upon single oral dosing as well as upon acute dermal application. They do not cause irritating effects on skin and in eyes and lack a potential to induce skin sensitization. No genotoxic properties have been observed in bacteria, cultivated mammalian cells and experimental animals. Repeated dose testing by the oral route consistently shows absence of effects for those pigments that are stable in stomach acid. Repeated-dose studies with oral dosing have been disseminated for more than 24 pigments representing more than ten chemical classes.

BONA-metal laked pigments such as Pigment Red 57:1 are susceptible to acid-catalyzed dissociation and show adverse effects on kidneys upon gavage application of high doses. For some pigments, toxicokinetic investigations are available and these show absence of systemic uptake after ingestion and dermal application. This is expected for substances that are of very poor solubility both in hydrophilic and hydrophobic solvents, a basic property of pigments. In general, systemic uptake after ingestion and dermal application is not expected. Reliable experimental data with intradermal application that would support such uses are not available.

III Hygiene & Microbiology

Microbial infections through tattoos

Dr. Christa De Cuyper, AZ Sint-Jan AV Hospital, Brugge, Belgium

Body art in its different forms dates back to ancient times. All body-modifying methods can lead to complications which depend on the conditions in which the procedures are performed, on the training and the skills of the practitioner and on the materials used. Poor hygienic standards and careless procedures can result in localized infections but can also lead to severe life-threatening conditions or even result in irreversible damage and scarring. Materials can be contaminated and devices are often difficult to sterilize.

Bacterial infections are more common following piercing than tattooing procedures. The most frequent are local bacterial infections at the site of the procedure. They are often caused by common pathogens such as *Streptococcus pyogenes* and *Staphylococcus aureus*. Nevertheless, severe secondary infections have been reported such as erysipelas, cellulitis, sepsis, and spinal abscesses, due either to *Streptococcus pyogenes*, *Staphylococcus aureus* or *Pseudomonas* species. Severe bacterial infections can occur when the procedure is performed in poor hygienic conditions or in patients at risk and can be serious and life-threatening such as gangrene and endocarditis. Tattoo infections due to typical and atypical mycobacteria have been reported, the first dating back to 1895 when a tattooist with pulmonary tuberculosis was responsible for the transmission to his fellow-prisoners of tuberculosis through his saliva, licking the tattoo needle. A recent outbreak of *Mycobacterium chelonae* infection was identified in several patients after the placement of a tattoo in the USA due to contaminated tattoo ink.

Inoculation of leprosy after religious ritual tattooing in an endemic area in India has been described. Inoculation of Syphilis was documented from the 19th century. Transmission of Syphilis from a tattoo needle moistened with the tattoo artist's saliva occurred in a group of US soldiers. Unusual cutaneous lesions in two patients with visceral leishmaniasis and HIV infection have been reported. Different types of viral infections can be transmitted such as warts, mollusca contagiosa, herpes simplex, blood-transmitted diseases such as hepatitis and HIV have been linked to tattooing. Reactivation of viral infections, such as herpes simplex and herpes zoster has been observed in association with tattoos. The risk of Hepatitis B and C, as well as HIV transmission by tattooing and piercing should not be underestimated. Moreover not only the clients but also the tattooists are at risk. Preventive hygienic measures and Hepatitis B vaccination are highly recommended for professionals involved in body modifying techniques. In some countries persons who have several tattoos or extensive skin areas tattooed or/and have piercings cannot be blood donors. Some rare cases of systemic mycoses such as *Candida endophthalmitis*, sporotrichosis and zygomycosis following tattooing procedures have been reported.

The increasing popularity of body adornment through piercing and tattooing in its different forms has raised many questions in particular about the safety of the techniques and the materials used. Regulation of the composition of the products and recommendations to ensure that procedures are carried out under appropriate hygienic conditions offer a big step forward to promoting consumers health. Body art practitioners and health care professionals should be aware of the complications that can arise from these procedures.

Risk of Tattooing: Mycobacter Infections

Dr. Victoria Scott-Lang, Department of Dermatology, Royal Infirmary of Edinburgh, Edinburgh, UK

Mycobacterium chelonae is a rapidly-growing nontuberculous bacteria which is an environmental pathogen, found in natural water, tap water, soil and sewage. It is not transmittable from human to human: cutaneous infection results from environmental exposure. With the use of systemic immunosuppressive drugs cutaneous mycobacterial infections have been increasingly recognised in immunosuppressed individuals. Infections have also been seen in immunocompetent individuals, usually following penetrating trauma, invasive medical procedures, and cosmetic procedures and tattooing. Skin manifestations may include papules, pustules and ulceration of affected sites. Prolonged antibiotic therapy for a minimum of three months is recommended to clear the infection.

Over the past two years our department has seen four confirmed cases of *Mycobacterium chelonae* skin infection secondary to tattooing, and five further suspected cases in which histology was highly suggestive of infection but skin culture was negative. To date the clusters of patients from South-East Scotland are the only cases to be reported in the United Kingdom but large series have also been published from France and the United States. All cases seen in South-East Scotland were patients who had undergone tattooing with grey ink: all had very similar eruptions comprising red papules and pustules confined to areas of grey shading. All published cases have described cutaneous eruptions confined to areas of grey shading, and this is likely to be highly relevant to the aetiology of the infection. The Scottish Skin Piercing and Tattooing Order of 2006 legislated the legal requirements for tattoo parlours in Scotland. The Act requires tattoo artists to use only sterile single use disposable needles for skin piercing and tattooing, and to use only single pigment or ink pre-packaged in single use vials. There is, however, no requirement for tattoo artists to use sterile water for rinsing needles, or for diluting black ink to grey: tattoo artists may use tap water for this process. In one tattoo parlour in Edinburgh *Mycobacterium chelonae* was cultured from an opened bottle of ink and from rinsings of the ink-bottle nozzle, but not from unopened bottles. Other published cases of tattoo-related *Mycobacterium chelonae* suggest that the source of the infection may be the use of nonsterile water to dilute black ink into grey, and to rinse instruments.

Further to the tattoo outbreak in South-East Scotland a communication letter was issued to all GPs, Consultant Dermatologists, Microbiologists and Plastic Surgeons and Pharmacists working in the area to highlight the problem and to encourage referral of possible cases. Public Health and Environmental Health were in close contact with the relevant tattoo parlours in the area. An advice letter was also sent to all tattooists in the area recommending that they avoid diluting ink, use grey ink from a UK supplier and to use sterile water for needle cleaning. Following the outbreaks seen in our area of the United Kingdom it may be necessary to establish national guidelines on sterile techniques in tattoo parlours, in particular including the avoidance of nonsterile water.

IV Technology

Microencapsulation of Dyes and Pigments

PD Dr. Lars Dähne, Surflay Nanotec GmbH, Berlin, Germany

The Layer by Layer (LbL) technology uses the stepwise electrostatic assembly of charged polymers (polyelectrolytes) from aqueous solution on surfaces. Due to the self-limiting adsorption process the thickness per layer is few nanometers. Each layer of an LbL-film can consist of a different synthetic or natural polyelectrolyte as e.g. Chitosane, Alginate, DNA, peptides or proteins. Instead of charged polymers also nanoparticles could be stably immobilized in LbL-films. Hence this technology allows the tuning of the surface properties and the introduction of multi-functionality on almost every planar, bended or colloidal substrate.

In the talk we will show some possible applications of this process for tattoo pigments. Due to different surface properties of dye pigments (surface potential, chemistry, ion distribution, surface topography) the recognition of the pigments by the immune system is quite different and lead to deviations in the transport behaviour out of the skin. A few nm thick LbL film is able to equalize these properties and could lead to more stable colour tones. Also the possibility to create a negative or positively charged pigment just by coating can lead to advantages in the skin immobilization. Furthermore, toxic properties of the pigments, like radical formation (TiO₂) or ion diffusion into the tissue can probably reduced or prevented by specific LbL coatings

Development of Permanent but Removable Tattoos

Bruce Klitzman, Ph.D., Associate Professor of Surgery, Biomedical Engineering, and Cell Biology, Duke University, Durham, NC, USA

Studies have shown that 50% of tattoo recipients have regret. All tattoos are removable, although the removal process may have serious side effects. Surgical excision, sometimes after tissue expanders generate extra adjacent skin for local flaps, is the most aggressive technique and has the ability to completely remove tattooed tissue. Noninvasive laser irradiation of skin can effectively cause removal of a fraction of tattoo pigment. Numerous alternative techniques can encourage removal of some pigment, often through inducing a cutaneous inflammatory response. However, rigorous well controlled studies of removal efficacy are severely lacking. Our interest tattoo ink removal had its beginnings in breast reconstruction. Some patients need to return a tattooed nipple-areola on a reconstructed breast to an aesthetically pleasing position following shifting of the breast mound. We designed a more removable tattoo ink by starting with colorant that would be easily cleared by the body, but stabilized it through microencapsulation. Removal could then be accomplished by disrupting the encapsulating shell instead of requiring more extensive disruption of an entire pigment particle. Our first failed attempt relied on microencapsulating water soluble dyes in polyolefin. Then, a team at Harvard University, led by Dr. Rox Anderson, challenged our patent. After many months of legal maneuvers, it was determined that we were indeed the inventors of microencapsulated tattoo ink. The two groups then merged. With leading experts in microencapsulation, we attempted microencapsulation of water soluble dye in polymethylmethacrylate. A fundamental challenge in the use of water soluble dye is the generation of very high osmotic pressure. Low dye volume percentages (about 10% or less) could successfully retain the dye. Higher payload volumes led to generation of osmotic pressure that disrupted the capsule and released the dye. Next, we investigated using insoluble pigment

particles. Preclinical studies in hairless rats and guinea pigs showed significantly increased removability. One laser treatment effectively removed 80% of tattoo intensity, while only 20% of conventional ink was removed in a single identical laser treatment. While these results proved our concept, effective removal in humans with any technique continues to be highly variable and difficult to predict because of variability in the inflammatory response.

Disclosure: Dr. Klitzman has less than 0.1% ownership in Nuvilex, Inc., producer of InfiniInk.

Modern Laser Tattoo Removal and Emerging Technologies to Enhance Efficacy

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For as long as humans have been applying decorative tattoos, they have probably been desirous of their removal. Selective methods of destroying tattoo ink within the skin, without destroying the skin along with it, have been available for over 2 decades. The 3 types of lasers used for selective tattoo removal are the Q-switched ruby, Nd:YAG, and alexandrite lasers. Incremental improvements to these devices have been introduced over the last decade, with anywhere from 4-10 or more treatments often required for complete tattoo removal. In some case, particularly multi-colored tattoos, complete removal is never achieved. Significant challenges to tattoo removal are inks containing zinc and titanium oxides, allergic and photoallergic tattoo reactions, and vibrant colors that span the rainbow. Recent advances in tattoo removal include investigation of index-matching compounds to enhance penetration of laser energy while minimizing its absorption in non-target structures such as the epidermis, picosecond lasers, perfluorodecalin to enable multiple laser passes in a single treatment session, and finally, designer inks that enable easier removal. Despite the worldwide adoption of decorative tattoos by a large percentage of the population, there is shockingly little known about the composition or origin of pigments in a particular tattoo. Enhanced knowledge, characterization and standardization of tattoo inks should enable better strategies for removing tattoos in the future. I am proud and honored to be a part of the First International Conference on Tattoo Safety, and I thank and applaud the organizers.

V Risk Assessment & Regulation

Regulation of Tattoo Inks in the United States

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Under the Federal Food, Drug, and Cosmetic Act (FD&C Act, the Act), tattoo inks are considered to be cosmetics, while the pigments used in the inks are color additives. The way in which cosmetics and color additives are regulated in the United States are different. Cosmetics are defined in the Act as any article “intended to be rubbed, poured, sprinkled, or sprayed on, introduced into or otherwise applied” in order for “cleansing, beautifying, promoting attractiveness or altering the appearance.” Cosmetics are not subject to pre-market approval, with the exception of color additives which require approval and batch certification as indicated in the Act. The law, however, requires that cosmetics and their ingredients not be adulterated nor misbranded, which means that they cannot contain poisonous or deleterious substances or unapproved color additives, or be manufactured or held in insanitary conditions, or be falsely labeled. In the past ten years, as the usage of tattoos has become more mainstream, there have been a number of outbreaks of infections as well as possible allergic reaction to the dyes. FDA’s response has been to alert consumers and health professionals and industry to address these issues, in addition to re-evaluating our current position with regard to the regulation of tattoos.

The challenge of regulating tattooing in New Zealand

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New Zealand is one of the most tattooed nations in the world; it is estimated that 1:5 New Zealanders have tattoos. Tattooing has deep cultural significance for Māori and Pacific peoples living in New Zealand and has become mainstream in youth culture. The prevalence of tattooing, combined with cultural values associated with tattooing, poses challenges for government agencies seeking to influence (or regulate) tattooing practices to better protect public health.

The Ministry of Health is responsible for designing, managing, regulating and funding the New Zealand public health system. This includes regulatory functions associated with public health, including communicable diseases and environmental health. The Ministry is interested in improving practices associated with skin piercing and tattooing to reduce health hazards such as transmission of blood borne viruses and skin infections.

However, the Ministry of Health cannot provide a complete regulatory solution to managing tattooing risks. Several other agencies or statutory officers play a role in reducing risks associated with tattooing. Medical Officers of Health located in District Health Boards, have responsibility for investigating actual and potential causes and cases of infectious disease in their area. Local authorities can make bylaws to control environmental and health risks in their districts. In 2011 the Environmental Protection Authority, introduced standards for the chemical constituents of tattoo ink.

This presentation will discuss health conditions attributable to unregulated customary tattooing and the challenge of better regulating tattooing within New Zealand’s social, political and

legal framework. To date New Zealand might be seen as having embarked down a “soft regulation” route; regulating partially and indirectly. In the absence of new or amended primary legislation, the Ministry of Health, Environmental Protection Authority and local government authorities have implemented work-around solutions (guidelines, standards and bylaws).

The presentation will outline existing initiatives to improve the safety of tattooing (including the most recent Auckland City Council bylaw) and discuss potential legislative proposals that might offer a more comprehensive approach to improving the safety of tattooing.

Risk assessment and regulation of tattoo inks in the EU

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At present there is no regulation for tattoo inks at the EU-level. Within the EU Consumer Product Safety Network (CSN) of the DG Health and Consumers discussion about this issue is ongoing. Several member states have indicated EU-regulation is needed. Over the past decade the Council of Europe (CoE) within its Committee of Experts on Cosmetic Products (P-SC-COS) has been doing work to support regulation on the tattoo issue. CoE resolution ResAP (2008)¹ includes several lists of chemicals that should not be present in tattoo inks. These lists include azo dye-related aromatic amines and all chemicals classified as CMR category 1,2 and 3. In addition ResAP (2008)¹ lays down maximum levels for contaminants, including polycyclic aromatic hydrocarbons. The future aim of P-SC-COS is to perform risk assessments for individual pigments and auxiliary chemicals in tattoo inks in order to establish a positive list of substances proved safe for use in tattoos. As a step towards this goal a P-SC-COS working group has explored the issue of which toxicological data would be required for the safety assessment of individual substances present in tattoo inks. Tattoo products can be seen as a kind of half-way station between cosmetics and medicinal products. Therefore the working group used the guidance for the safety evaluation in these two areas as relevant input. For a number of items in the envisaged safety dossiers for tattoo ingredients, the data requirements in principle do not differ from those for cosmetics or medicinal products. For genotoxicity for instance the usual battery approach can be applied (in vitro testing when needed followed by in vivo testing). For other items specific tests relevant for the tattoo situation should be included, for instance the Intra-cutaneous Reactivity Test as a test for dermal irritation potential and the Magnusson Kligman Guinea Pig Maximisation Test for dermal sensitization potential. An area where current knowledge is insufficient for risk assessment concerns the fate of the pigment in the body after tattoo application. The degree of pigment leakage from the tattooed skin site into the blood stream and the transport of pigments to the draining lymph nodes or other compartments needs to be elucidated. This information is needed for exposure assessment and calculation of the margin of safety. On an case by case basis testing must be considered to determine if toxic photo-degradation products are formed from the pigments, including toxic photo-degradation products formed under laser light in view of possible laser removal treatment of tattoos.

In the Netherlands the tattoo ink requirements as specified by the Council of Europe resolutions have been included in the ‘Warenwet’ (consumer product safety regulation). More generally the Dutch legislation includes a system of 3-year licences for tattoo shops meeting the appropriate rules as laid down in the Warenwet. This system is enforced by the Netherlands local health services (GGD) and the Netherlands Food and Product Safety Authority.