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Allergy and Tattoos

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Preface

In 2016 the EU commission requested the European Chemical Agency (ECHA) to work out an Annex XV dossier under REACH in order to propose regulation of harmful substances in tattoo inks. In this relation the Danish EPA will contribute a description of allergic reactions in permanent tattoos and together with the Norwegian EPA prepare a generic assessment for how to regulate skin sensitizers in the REACH Annex XV restriction.

The present project “Allergy and tattoos” was initiated by the Danish EPA. The project was carried out from September 2016 to December 2016 at the National Allergy Research Centre, Department of Dermatology and Allergy, Herlev-Gentofte Hospital, University of Copenhagen, Denmark.

The Danish Environmental Protection Agency (EPA) investigated in 2012 tattoo inks on the Danish marked (http://mst.dk/service/publikationer/publikationsarkiv/2012/jun/kemiske-stoffer-i-tatoveringfarver/). In relation to this investigation some reactions were observed, which to a high degree looked like allergic reactions - especially in red tattoo ink.

The studies objective was to describe allergic reactions in tattoos based on current scientific knowledge.

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The report has undergone review and discussion with Dorte Bjerregaard Lerche and Grete Lottrup Lotus, the Danish Environmental Protection Agency. The draft report was commented on by the Norwegian Institute of Public Health and the Norwegian Environment Agency.

Summary and conclusions

Summary

With the increasing popularity of tattooing the immunological reactions from modern organic inks has become a new disease entity representing a public health issue. It has been suggested that some of these reactions may be due to contact allergy toward ingredients in the inks.

Contact allergic reactions are typically seen to small molecular weight chemicals, which come into contact with the skin by use of consumer products or exposures at the work place. However contact allergy can also be both induced and elicited by injection of contact allergens into the dermis, where tattoo ink is deposited. It is known from several investigations that tattoo inks may contain contact allergens as metals, colorants and preservatives. The symptoms of an allergic reaction are itchy redness, swelling and possibly vesicles at the site of the tattoo. Typically the reaction will spread outside the area of the tattoo.

A short review was made concerning knowledge from population-based studies. In total seven population-based studies concerning adverse skin reactions to tattoos were identified. The studies employed different methodologies. In some of these investigations questions were asked concerning allergic reactions in tattoos, which were reported by 2.9%-8% of those with tattoos. No definitions were given of what was meant by an allergic reaction and no allergy tests were performed, which is a perquisite of diagnosing allergy. Chronic skin reactions in permanent tattoos, defined as lasting more than 3 to 4 months were reported by at least 5.9%-6.0% of random samples of tattooed persons and transient more acute reactions in 4.3% to 12.5%. Even higher numbers were obtained if subgroups of tattooed persons were studied such as sunbathers or tattooists. Sun-induced complaints were reported with a frequency of 15%-23% of investigated subgroups. The severity of reactions was in most cases unknown. Contact allergic reactions may be among both acute and more chronic adverse reactions, but cannot be more precisely estimated, as it requires medical investigation to make the diagnosis.

In addition to the population studies, an analysis of published series of cases was undertaken. Two case studies were identified, where more than two patients with adverse skin reactions to tattoos were collected and where there has been a systematic approach to obtain exposure information and perform patch testing. In one study 79 patients with tattoo reactions were tested with expected problematic inks, while 74 of them were tested with selected textile azo dyes, 7 (8.8%) and 4 (5.4%) had a positive reaction. The compositions of the inks were unknown and therefore a causal relationship could not be firmly established. In another study 6 patients with severe tattoo reactions were tested and one had a positive reaction to an ingredient of the ink. It qualifies that contact allergic reactions exist, but also demonstrates the gap in knowledge concerning ingredients in the tattoo inks, which has caused reactions or are under suspicion.

Published individual cases are often persons with very severe reactions, while the population data is based on self-reports and will cover very mild and transient symptoms, too. There is no way to link results from the population studies with the information from these individual cases.

It is clear from the description of the individual cases that some of the reactions are not typical for contact allergy and in many cases also patch testing is negative. It may be due to limitations in the patch test methodology, but it may also be that these reactions are due to other kinds of immune activation than contact allergy. One such possibility is foreign body reaction

where macrophages may be overstimulated leading to long-lasting severe reactions. The basic understanding of the non-allergic immune reactions to tattoos is at the research level and no diagnostic test is available.

Transparent information concerning all ingredients in used tattoo inks is required for diagnosing contact allergy or other immune reactions. It is also the basis for expanding the knowledge of other potential toxicological effects of tattoo ink ingredients.

The considerations presented in this note are relevant for all the substances that have an EU harmonized classification as skin sensitizer Cat. 1A or 1B (H317: may cause an allergic reaction) according to CLP and are applied in tattoo inks. Since CMR substances in tattoo inks will be addressed in parallel, of particular interest are the non CMR substances that have a harmonized classification as skin sensitizers.

Substances can be classified according to CLP criteria based on human data or non-human data. In total 1151 substances have an EU harmonized classification as skin sensitizer 1A or 1B. This report focuses on the 22 non CMR substances that have a harmonized classification as skin sensitizers. Among these substances 9 have been related to tattoo inks according to Piccinini P et al., 2015, given in Table A. These 9 substances are all well-known allergens based on clinical experience. Such substances may cause allergic contact dermatitis when applied to the epidermis or injected in dermis. Depending on the concentration of the allergens in the tattoo ink contact allergy may also be induced by tattooing. The substances in Table A should not be allowed in tattoo ink due to the risk of allergic reactions; neither should other substances with a harmonised classification, which may be associated with tattoo inks in the future.

Table A (identical to Table 4 in the main text of the report):
Non CMR substances with a harmonized classification H317 and related to tattoo inks

NAME	Application	CAS
4-chloro-3,5-xylenol*	Preservants	88-04-0
Methenamine*	Preservants	100-97-0
p-phenylenediamine***	Colorant	106-50-3
1,2-benzisothiazol-3(2H)-one**	Preservants	2634-33-5
Rosin	Viscosity regulator	8050-09-7
Cobalt	Colorant	7440-48-4
2-octyl-2H-isothiazol-3-one**	Preservants	26530-20-1
3-iodo-2-propynyl butylcarbamate*	Preservants	55406-53-6
Mixture, 3(2H)-isothiazolone, 5-chloro-2- methyl- with 2-methyl-3(2H)-isothiazolone. 3:1*	Preservants	55965-84-9

*Preservatives that are allowed in cosmetic products with a maximum concentration limit (listed in Annex V of the Cosmetics Products Regulation).
**Substances with an antimicrobial effect, which are not allowed as preservatives in cosmetic products.
***The colorant p-phenylenediamine is also restricted in cosmetics and its use is confined to hair.

A second list of substances was also identified (n=49 given in Table B), which have a self-classification as skin sensitizers and identified in tattoo inks.

Four substances on this list were identified as very well-known allergens*: 2-methyl-2H-isothiazol-3-one (MI)*, which has been responsible for a recent epidemic of contact allergy in Europe and *5-chloro-2-methyl-2H-isothiazol-3-one; chromium salts* , which are also a well-established causes of contact allergy and *aluminium salts*, which are used in vaccines as an adjuvant and known to cause contact allergy by this route of exposure. Aluminium salts would also be able to cause allergic contact dermatitis in tattoos. These 4 substances should not be allowed in tattoo ink due to the risk of allergic reactions.

Table B (identical to Table 5 in the main text of the rapport):
List of H317 notified substances related to tattoo inks

NAME	CAS
Benzoic acid	65-85-0
Zinc oxide	1314-13-2
Aluminium	7429-90-5
Propane-1,2-diol	57-55-6
Salicylic acid	69-72-7
1,4-dihydroxyanthraquinone	81-64-1
9-(2-carboxyphenyl)-3,6-bis(diethylamino)xanthylium chloride	81-88-9
Phenanthrene	85-01-8
Hexachlorobuta-1,3-diene	87-68-3
Propyl 4-hydroxybenzoate	94-13-3
Butyl 4-hydroxybenzoate	94-26-8
Methyl 4-hydroxybenzoate	99-76-3
Melamine	108-78-1
Hexa-2,4-dienoic acid	110-44-1
Ethyl 4-hydroxybenzoate	120-47-8
2,4,7,9-tetramethyldec-5-yne-4,7-diol	126-86-3
29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32 copper	147-14-8
Copper oxide	1317-38-0, 1344-70-3
Disodium [29H,31H-phthalocyaninedisulphonato(4-)-N29,N30,N31,N32]cuprate(2-)	1330-38-7
Trisodium 5-hydroxy-1-(4-sulphophenyl)-4-(4-sulphophenylazo)pyrazole-3-carboxylate	1934-21-0
6-chloro-2-(6-chloro-4-methyl-3-oxobenzo[b]thien-2(3H)-ylidene)-4-methylbenzo[b]thiophene-3(2H)-one	2379-74-0
2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-N-phenylbutyramide	2512-29-0
2-methyl-2H-isothiazol-3-one	2682-20-4
4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide	2786-76-7
1-[(2,4-dinitrophenyl)azo]-2-naphthol	3468-63-1
4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one]	3520-72-7
Disodium 2,2'-(9,10-dioxoanthracene-1,4-diyldiimino)bis(5-methylsulphonate)	4403-90-1
Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]	5160-02-1
N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	5280-68-2
Calcium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	5281-04-9

2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-methylphenyl)-3-oxobutyramide]	5468-75-7
4-[(2,5-dichlorophenyl)azo]-3-hydroxy-N-phenylnaphthalene-2-carboxamide	6041-94-7
3-hydroxy-4-[(2-methyl-4-nitrophenyl)azo]-N-(o-tolyl)naphthalene-2-carboxamide	6410-32-8
N-(5-chloro-2,4-dimethoxyphenyl)-4-[[5-[(diethylamino)sulphonyl]-2-methoxyphenyl]azo]-3-hydroxynaphthalene-2-carboxamide	6410-41-9
1,3-bis(hydroxymethyl)-5,5-dimethylimidazolidine-2,4-dione	6440-58-0
3-hydroxy-4-[(2-methyl-5-nitrophenyl)azo]-N-phenylnaphthalene-2-carboxamide	6448-95-9
4-[(4-chloro-2-nitrophenyl)azo]-3-hydroxy-N-(2-methylphenyl)naphthalene-2-carboxamide	6471-50-7
3-hydroxy-N-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide	6535-46-2
Chromium	7440-47-3
Copper	7440-50-8
Xanthan gum	11138-66-2
5-chloro-2-methyl-2H-isothiazol-3-one	26172-55-4
2-bromo-2-(bromomethyl)pentanedinitrile	35691-65-7
N-(5-chloro-2-methoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	67990-05-0
Rose, Rosa centifolia, ext.	84604-12-6
Calendula officinalis, ext.	84776-23-8
Rose, Rosa damascena, ext.	90106-38-0
Sorbitan monolaurate, ethoxylated	9005-64-5
C.I. Pigment Yellow 36	37300-23-5

Further 3 substances were identified where a few or some cases have been reported of suspected allergic reactions: *4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide; Rose, Rosa centifolia, ext.;* and *Rose, Rosa damascena, ext.* An allergic reaction to colour *Red 210, CI 12477* was identified when reviewing the cases. This substance is not in Table B. All these 4 substances should be scrutinized concerning the quality of the data and if additional data exist to make a decision on their continued use in tattoo inks or not.

The reason for notification of the remaining substances in Table B is unknown. In case evidence exists, which fulfil the criteria for classification e.g. in animals (see Annex 2), they also pose a risk of sensitization in man.

Several substances of Table B are regulated by the Cosmetic Regulation.

Induction as well as elicitation of contact allergy is dose-dependent. The threshold dose of a number of sensitizers has been investigated in human and animal test systems as well as in clinical studies of sensitized individuals. In these studies, the allergens are applied on the skin. It is known that if allergens are deposited under the skin then stronger reactions will occur and to lower levels, however only few substances have been investigated in this way. The limits established based on exposures on the skin cannot be used to set limit values for tattoo inks, as even very small levels of allergens injected into the skin may pose a problem.

Recent reviews describe the size of the problem, the complex nature of the clinical presentations, the limited knowledge of the basic disease mechanisms, the nearly complete lack of diagnostic tools and safe treatments and finally the grim perspective of possible unknown multi-organ side effects coming up decennia after making of the tattoo.

General conclusions

Many persons with a tattoo experience adverse skin reactions as transient, intermittent or long term problems. The nature of these reactions has not been investigated except for isolated cases.

The number of persons with allergic reactions in their tattoos is unknown. Rarely cases have been thoroughly investigated and only in a limited number of cases it has been possible to definitely prove contact allergy. This may be due to limitations in the patch test methodology, the lack of information concerning ingredients in the specific inks having caused an adverse reaction as well as other mechanisms than allergy may play a role

Classic contact allergens (metals, colourants and preservatives) have been identified in tattoo inks. Some of these substances are strong or extreme allergens.

Contact allergy can both be introduced and elicited from epidermis and dermis.

Allergens deposited in dermis may elicit stronger reactions and at lower doses.

It is not possible to determine limit values in tattoo inks. The dose needed to provoke an allergic reaction is expected to be very low for most allergens.

It is also possible that some substances, which will normally not penetrate the skin due to substance properties (size or physio-chemical properties) and therefore are unreactive if applied on epidermis, can cause reactions when injected directly into dermis.

In case the allergen is a small molecular organic substance such as a preservative, it is expected that the substance will be cleared from the dermis after weeks and the reaction subside. Therefore some of these reactions may be over-looked.

If the allergen is part of the pigments, which are permanently deposited in the dermis, the allergic reaction may become chronic, due to the slow decomposition of the ink.

In this investigation 13 non-CMR substances associated with tattoo inks were identified, which are well known contact allergens. These should not be allowed in tattoo inks due to the risk of allergic reactions; neither should other substances with a harmonised classification, which may be used in tattoo inks in the future.

The lack of knowledge concerning the ingredients in the specific inks, which has caused adverse reactions hamper the possibility of diagnosis and prevention.

Apart from allergic reactions in tattoos other immune reactions have been described. The mechanisms are unknown.

Tattoo ink contains both large and small particles and will in theory be able to activate mechanisms of foreign body reaction.

A multi-pronged approach is needed, if possible yet unknown systemic side effects coming up decennia after making of the tattoo, should be prevented.

Recommendations

- Allergens -fulfilling the CLP criteria for classification- should not be present in tattoo ink due to the risk of allergic reactions.

  This applies to the identified substances mentioned in Table A with a harmonized classification as H137, and should also apply to all other substances with a harmonised classification, to prevent the use of these in tattoo inks in the future.

  This should also apply to the four substances indicated in Table B with a self-classification as H137, which are well known and clinical important skin sensitizers (contact allergens).

  Three substances from Table B (5) with few cases or suspected cases as well as one substance identified in reviewing the literature for this memo should be scrutinized concerning the quality of the data and if additional data exist prior to a decision.

  The reason for notification of the remaining substances in Table B is unknown. In case evidence exists, which fulfil the criteria for classification e.g. in animals (see Annex 2), these substances also pose a risk of allergic reactions in man.

- All ingredients in tattoo inks should be declared either on the container or in a SDS, regardless of if they are hazardous or not. This would mean full ingredient labelling. It will make targeted risk assessment (for all toxicological effects) and effective prevention possible. It will improve the possibilities of making a diagnosis of allergic contact dermatitis to tattoo inks.

- Research should be initiated to better understand, diagnose and prevent adverse tattoo reactions.

1. Introduction

Tattooing involves introduction of exogenous pigments into the dermis to create a permanent decorative design. According to a recent review 12 % of the European population has one tattoo or more, corresponding to more than 60 million people in the EU-28 (Piccinini P et al., 2016). It is expected that tattoos will continue to be trendy and the impact and prevalence will continue to increase (Piccinini P et al., 2016).

Tattoos involve injection of tattoo ink into the dermis to a depth of 1-2 mm using a tattoo machine oscillating at a rate of 50-3000 times per minute (Thum, CK, 2015). Temporary tattoos are applied on the skin like a drawing; it lasts two to four weeks and is also called black henna tattoos. The ink used for temporary tattoos may contain chemicals, which are strong allergens and are well-known causes of allergic reactions (Kind F et al, 2012). This memo concerns only the traditional tattoos injected into the dermis.

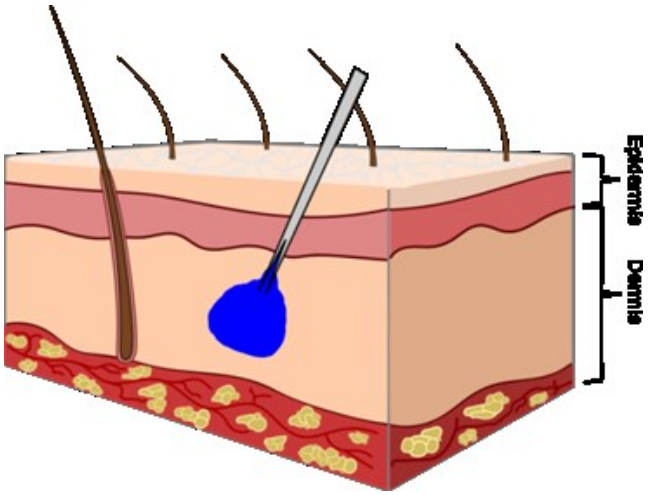

Figure 1 Diagram of the skin showing deposition of the tattoo ink (blue colour) in the dermis.

Tattoo inks are complex formulations containing several ingredients, both organic and inorganic, by-products and impurities. The inks are usually ready-to-use-products, which consist of insoluble pigments (responsible for the colour) in a liquid made of binder(s) and solvent(s) (Piccinini P et al., 2015; 2016). Preservatives are often added to the mixture to avoid microbiological contamination of the often water-based mixture (Piccinni P et al, 2016) and have been found in concentrations up to 1.5% by weight. Besides intentional ingredients other substances may be present as impurities such as metals from inorganic and organometallic pigments (Piccinni P et al., 2016). Colorants are by far the major ingredient of tattoo inks and may reach high concentrations. The colorants can be classified into dyes and pigments. Dyes are soluble in the vehicle, fast biodegradable and scarcely used, while pigments are insoluble, chemically resistant and the preferred choice of tattoo inks. Organic pigments represent the large majority of pigments used in tattoo ink today (Piccinini P et al., 2016). An important problem is that the pigments used in the formulation of tattoo inks are not produced for this purpose and do not undergo any risk assessment that takes into account their injection into the human body for long term permanence (Piccinini P et al., 2016). A number of recent papers (Islam PS et al., 2016; Laux P et al., 2015; Thum CK., 2015) and reports (Piccinni P et al. 2015; 2016; 2016)

have reviewed the potential risks associated with permanent tattooing. It is currently not elucidated to what extent tattoos cause contact allergic reactions.

The objective of this memo is to give an updated analysis of the current scientific knowledge concerning allergy and tattoos.

1.1 Introduction to contact allergy and allergic contact dermatitis

Skin symptoms are often reported in relation to tattoos, some of these may be caused by contact allergy induced by the tattoo inks. *Contact allergy* is a T cell mediated reaction induced by small soluble molecules (haptens) such as metal salts, dyes, preservatives and fragrances, which are able to bind to proteins in the skin and activate the immune system. This is the first part of an allergic reaction, it is called the induction or sensitization phase and is symptomless. In the sensitization process the individual develop life lasting specifically sensitized T-cells. The second phase is called the elicitation or provocation phase and occurs following induction in case exposure to the allergen in question continues or re-exposure to the allergen occurs. In the elicitation phase *allergic contact dermatitis* will occur, an inflammatory skin disease, which in the acute phase, is seen as redness, oedema and sometimes vesicles (Figure 2), whereas scaling and hyperkeratosis dominate in the chronic cases. A cardinal symptom is itch. Allergic contact dermatitis tends to spread locally beyond the area of exposure and even to unrelated skin areas. Contact allergy can both be induced and elicited by introduction of allergens in epidermis and/or dermis (Magnusson B, Kligman AM 1969; Warshaw EM et al. 2014; Möller H, 1989; Trollfors B et al., 2005). A description of the immunological mechanisms can be found in section 3 of this report.

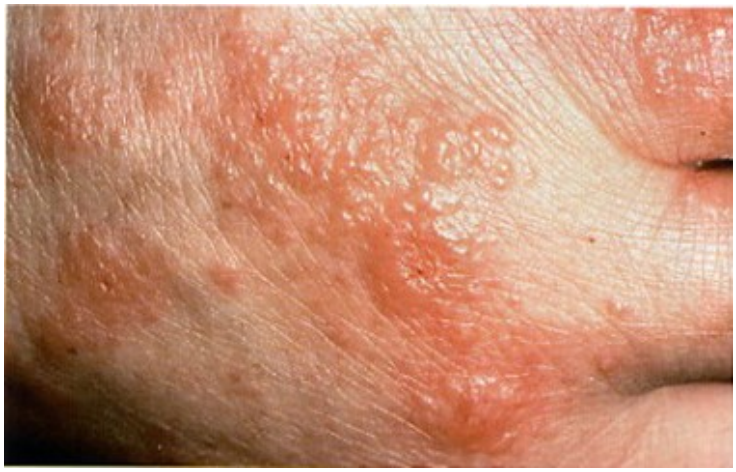

Figure 2 Allergic contact dermatitis

Contact allergy can be demonstrated by an allergy test, called patch test, which is an internationally accepted tool to diagnose contact allergy (Johansen JD, 2015). The methodology has been in use for over 100 years and is in use worldwide. At patch testing small amounts of the suspected allergens are applied in aluminium chambers to the upper back of the person under investigation (Figure 3). The patches are left in place for two days and then removed. The skin is inspected for allergic reactions several times over the following days. A positive test can be seen in Figure 3c. The diagnosis of *allergic contact dermatitis* requires typical clinical symptoms, as described, and positive results from patch testing to substances, which the person is exposed to.

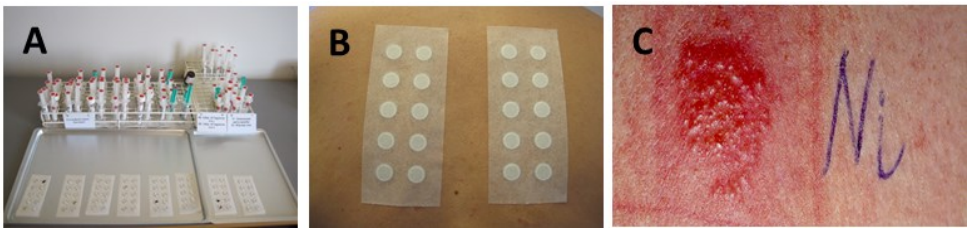

Figure 3 Patch test substances in syringes and filled chamber (A), patches applied to the upper back (B) a positive patch test reaction to nickel (C).

Contact allergy is frequent and affects 27% of the European population (Diepgen TL et al., 2016). The most common exposures causing allergic contact dermatitis are cosmetics, occupational epidermal exposures and skin piercing, where a dermal exposure also takes place (Schwensen J et al. 2016; Warshaw EM et al. 2014). If the culprit contact allergen is correctly diagnosed and contact abolished, allergic contact dermatitis will usually clear within 2-3 months. If future exposure to the chemical in question is avoided recurrences will not occur.

2. Overview of types and frequencies of adverse skin reactions

In this chapter an overview of the literature is given concerning information on frequencies and types of adverse skin reactions following tattooing based on population studies and published series of case reports.

2.1 Population/subpopulation studies

In total 7 epidemiological studies were identified, peer-reviewed and published in English, which concerned permanent tattoos and adverse skin reactions. The investigations were conducted between 2004 and 2013 in US (2), Germany (1), France (1) and Denmark (3). Two studies concerned samples of the general population in the US (2004) and Denmark (2011-13) (Laumann AE, Derick AJ, 2006; Dybboe R et al., 2016). Two other studies concerned intended random samples of a subset of the general population, and the remaining three studies included selected populations of tattooed people such as tattooist and sun-bathers (Hutton Carlsen K, Serup J, 2014; Klügl I et al., 2009). The studies are presented in brief in Table 1 and discussed below. More details are given in Annex 1.

Table 1 Overview of epidemiological studies: methodologies and main results

Country Year	Number of participants	Type of population	Age	Sex In %	Method	% tattooed persons	Types of adverse reactions	Frequencies	Ref.
General population studies, random samples									
United States 2004	500	National probability sample by random digit dialling	18-50	M:49% F:51%	Telephone survey	24% (120)	Medical problems within first 2 weeks such as bleeding, crusting, swelling etc.	15/120 (12.5%) of these 3 with sun sensitivity, otherwise not specified.	Laumann AE, Derick AJ, 2006
Denmark 2011-12	2308	General population. Random sample. 5-year follow-up study	23-74	M:44% F:56%	Questionnaire	14,2 % (313)	Adverse reactions defined by the following (below): Eczema/rash Infection Erosions All symptoms	18/306(5.9%) 9/306 (2.9%) 4/306 (1.3%) 3/306 (1.0%) 2/306 (0.7%)	Dybboe R et al., 2016
Random samples of tattooed persons									
United states (New York city central park)	300	Randomly selected tattooed people	18-69	M:50% F: 50%	Personal interview 17 questions	All (selection criteria)	Any skin sign or symptom which differs from normal part of tattooing or healing defined as: persistent redness, itching, rash, irritation, swelling,	10.3% (31/300) 13 (4.3%) acute reactions 18 (6.0%) chronic	Brady B et al., 2015

2013							scarring, infection, disfigurement, raising or photosensitivity. Chronic reactions defined as lasting for more than 4 months.	reactions	
German speaking countries (93% from Germany) 2007-8	3411	Internet survey. Recruitment of tattooed people by a variety of advertisements	29.3 ±8.6	M:41.1 % F:58.9 %	Internet survey 40 questions concerning the most recent tattoo	All (selection criteria)	Persistent skin problems.	6.0% (206/3411)	Klügl I et al.,2010
Selected groups of tattooed persons									
France 2013	448	Tattooists members of the French Tattoo Union		M:78.1 % K:21.9 %	Internet survey.	All (selection criteria)	A reaction on at least one tattoo Swelling during/after sun exposure Allergic reaction to a colour in at least 1 tattoo (self-reported and undefined) Permanent mild swelling	42.6% (180/420) 23% (91/392) 8.0% (34/402) 4% (17/397)	Kluger N, 2015
Denmark 2010-11	154 With 342 tattoos	Consecutive individuals, who spontaneously attended a clinic of venereology	27.5 ±7.5	M:51% F:49%	Questionnaire and examination	All (selection criteria)	Complaints beyond 3 months Elevation/ inflammation Sun-induced complaint	27% (41/154) 20% 15.6%	Høgsberg T et al., 2013
Denmark 2011 (summer)	144 with 301 tattoos	Sun bathers		M:48% F:52%	Personal structured interview	All Out of 467 sunbathers 36% were tattooed.	Complaints beyond 3 months Sun-induced: - Swelling - Itching, stinging, Pain - Readness	60/144 (41%) 31/144 (21%) 18/31 (58%) 16/31 (51%) 8 (25%)	Hutton Carlsen K, Serup J, 2014.

As can be seen from Table 1 two studies concerned samples from the general population; the sample size was 500 in the American study and 2308 in the Danish. A frequency of tattooing of 24% and 14.1% were found, respectively (Laumann AE, Derick AJ, 2006; Dybboe R et al., 2016).

Two other studies concerned random samples of tattooed persons and were performed in US New York City and in Germany speaking countries with sample sizes of 300 and 3411, respectively (Brady B et al., 2015; Klügl I et al.,2010).

The remaining studies concerned samples from selected groups of tattooed persons such as sunbathers or tattooists. The sample sizes ranged from 144 (Denmark) to 448 (France) (Hutton Carlsen K, Serup J, 2014; Høgsberg, 2013; Kluger N, 2015).

All studies used interview and/or questionnaires and contains no information concerning ingredients in the tattoo inks, which is reported to cause adverse reactions. A risk analysis concerning specific ingredients are therefore not possible based on these studies. They can be used to illustrate the general risk of adverse skin reactions in relation to tattooing.

The definition of adverse reactions differed between the studies, and several studies included also subjective symptoms e.g. stinging, pain or itch without distinguishing from clinically observed reactions such as swelling, redness and ulcerations. This makes a comparison of the results difficult.

In several studies acute symptoms occurring soon after the tattoo was made e.g. within first 14 days (Laumann AE, Derick AJ, 2006) are distinguished from later appearing reactions e.g. after 3 months (Høgsberg T et al., 2012; Hutton Carlsen K, Serup J, 2014), also persistent i.e. chronic problems (Klügl I, et al., 2009) are distinguished from transient (Laumann AE, Derick AJ, 2006). The challenge is that the distinction between acute, later onset and chronic reactions to tattoos is not done systematically across all studies. In spite of these differences in methodologies some common findings can be extracted. From a medical point of view all adverse reactions are of interest, however more severe and/or chronic/recurrent reactions will be of most impact and thus deserves special attention.

In the studies of random samples of the tattooed population chronic reactions were found in 6.0% (U.S) (Brady BG et al., 2015), age group 18-69 years, defined as a reaction involving a specific colour and lasting for more than 4 months, very similar to the finding in German speaking countries, where 6.0% reported persistent skin problems (Klügl I et al., 2010), also the Danish study of a random sample of the general population (age 23-74 years) showed that 5.9% had had adverse reactions defined as eczema/rash, infection and/or erosions (Dybboe R et al., 2016).

Transient/acute reactions were reported by 12.5% in one study from US (Laumann AE, Derick AJ, 2006) and 4.3% in another (Brady BG et al., 2015).

The frequency of adverse reactions would be expected to be higher if the group studied have many tattoos (Kluger N, 2015) or a risk-behaviour linked to adverse reactions such as sunbathing (Hutton Carlsen K, Serup J, 2014). Among French tattooists 42.6 % reported a reaction in at least one tattoo, 23% reported swelling after sunbathing and 4% reported permanent mild swelling (Kluger N, 2015). Among sunbathers 41% had complaints concerning one or more of their tattoos beyond 3 months and elevation/inflammation were reported by 21% (Hutton Carlsen K, Serup J, 2014) and in a total of 27% of tattooed young people (mean age 27.5 years) spontaneously attending a clinic of venereology (Høgsberg T et al.,2012).

Sun-induced reactions

In a German study 1.6% of women and 0.8% of men reported light sensitivity as the persistent problem, when asked to consider their most recent tattoo (Klügl I et al., 2010). Swelling during/after sun exposure was reported by 23% of French tattooists (Kluger N, 2015) and among 15.6% of tattooed individuals attending a clinic of venereology in Denmark (Høgsberg T et al., 2012). Among sunbathers on a Danish beach 21.5% reported sun-induced complaints beyond

3 months after tattooing (Hutton Carlsen K, Serup J, 2014). The precise pathophysiologic mechanism responsible for a sun-induced reaction is not known, but photochemical reactions with induction of reactive oxygen species has been suggested (Hutton Carlsen K, Serup J, 2014). It may also be due to a general sensitivity in the recently traumatized skin. Further it may be hypothesized that sun exposure in itself can break down the pigment, and in some cases a reaction may be due to these break-down products.

Allergic reactions in tattoos
Questions about allergic reactions in tattoos were formulated in several of the surveys, however in no case a definition was given, so it is difficult to know the background of the answers. Self-reported allergic reactions in a least one tattoo were reported by 8.0% of French tattooists (Kluger N, 2015), eczema/rash which may be compatible with an allergic reaction, but not necessarily are allergic reactions, were reported by 3.9% of a sample of the tattooed general Danish population (Dybboe R et al., 2016) and among sunbathers 3/144 (2.0%) reported an allergic reaction in a tattoo (Hutton Carlsen K, Serup J, 2014).

The diagnosis of an allergic reaction requires medical attention and allergy testing. Few who experience adverse reactions in tattoos reports to have consulted a doctor (Dybboe R et al., 2016; Brady BG et al., 2015). Some of the acute reactions in tattoos may be due to pre-existing contact allergy to ingredients of the tattoo ink. These reactions will be transient and clear over weeks to months in case of organic substances such as preservatives, which will disappear from the skin. Induction of contact allergy would also be possible depending on the concentration and would normally appear after at least 10 days. Some of the chronic reactions may be due to contact allergy to substances permanently deposited in the skin such as the pigments. However it is impossible to qualify more precisely based on self-reports from population studies alone and remains speculative.

2.1.1 Conclusion concerning population studies

There were major differences in sampling, definitions and analysis between the studies. With this reservation in mind some common findings can be extracted.
Chronic skin reactions in tattoos were reported by at least 5.9%-6.0% of tattooed persons if a random sampling technique is used and transient more acute reaction in 4.3% to 12.5%. Even higher numbers are obtained if special subgroups of tattooed persons are studies. The severity of reactions is in most cases unknown. Sun-induced complaints are reported with a high frequency of 15%-23% of investigated subgroups.
Self-reported allergic reactions in tattoos varied between 2.9%-8% of those with tattoos. Contact allergic reactions may be seen among both acute and more chronic adverse reactions, but cannot be more precisely estimated, as it requires a medical investigation to make the diagnosis.
Therefore an analysis of case-based information is necessary in order to further qualify allergic reactions in tattoos, see section 2.2.

2.2 Cases of allergic contact dermatitis in tattoos

Allergic contact dermatitis in tattoos has been reported regularly since the 1950ties. Historically the common etiological factors were contact allergy to mercury, chrome and cobalt representing the different dyes available at that time period (Snowdon, 1991). In more recent years organic colours have to a great extent replaced the metal derived colours (Gaudron, 2014). Different contact allergens have been identified in modern tattoo inks such as a variety of preservatives and metals (Hauri U, 2014; BVL, 2007; NVWA, 2008). In a Swiss investigation of 229 inks the preservatives benzisothiazolinone, methylisothiazolinone and formaldehyde were found respectively in 24%, 8% and 7% of inks (Hauri U, 2014). These are all potent skin sensitizers classified 1A according to CLP.

We have identified two case studies, where more than two patients with adverse skin reactions to tattoos were collected and where there has been a systematic approach to obtain exposure information and perform patch testing with the standard allergens supplemented with common inks and the patient specific related inks (Serup J et al., 2014; Gaudron s et al.,2014). The two studies provide useful information on reactions to metals and pigments.

Serup J et al., 2014 patch tested 89 consecutive patients with a history of having adverse reactions to tattoos lasting more than three month. Testing was performed with the European baseline patch test series. They found 19 (21%) patients reacting to nickel and five (5.6%) to cobalt. All were nickel sensitized before the tattooing. Among six patients with a similar history Gaudron S et al., 2014 found three sensitized to nickel and further two sensitized to both chrome and cobalt. The frequency of nickel allergy in these patients is higher than the frequency in the population if compared to the prevalence of nickel allergy in Sweden (Diepgen T et al., 2015). It cannot be excluded that tattooed individuals may have a high frequency of skin piercing, a known risk factor for nickel allergy. The Environmental Protection Agency in Denmark has recently investigated tattoo inks (based on the frequency of use) for their content of metals. Nickel was found in the samples in a range of 2-20µg/g (Danish EPA, 2012). During the process of making a tattoo an average of 1 mg ink is injected per cm^2 tattoo (Laux P et al., 2016). The EU regulation for nickel in items coming in direct contact with the skin is 0.5µg/cm^2/week (Ahlström M et al., 2016), however this is a limit based on investigations of nickel deposited on the skin and not in the skin. It can be noticed that in the purpose of risk characterisation, the absorbed fraction of nickel following dermal contact to several nickel compounds has been estimated to 2% (Danish EPA, 2008). So even though the amount of nickel is below the limit in the nickel regulation, it may still cause allergic reactions, if present as free ions or salts. For post assemblies a limit of 0.2µg/cm^2/week exist in the nickel regulation. This limit is however – in contrast to the limit for epi-cutaneous exposure mentioned above- not scientifically well supported and cannot be used as a safe limit for soluble nickel in tattoo ink.

Nickel is not part of the organic dyes, but may be present in the ink as an impurity from the production. It has never been investigated if nickel, cobalt and chromium are released from the needles during tattooing, where a metal needle is forced up to 3000 times a minute through the epidermis, penetrating the basal membrane and into the dermis.

Serup and Carlsen, 2014 tested 74 of their patients with selected textile azo dyes and found 4 (5.4%) positive reactions. They further investigated 79 of their patients with expected “problematic inks”, selected based on experience from patients having had a tattoo reaction. These are mixtures of substances, where there is no prior patch test experience, so irritation is not excluded. Here they found a total of 7 (8.8%) positive patch test reactions.

Gaudron S et al., 2014 provided more information on the dyes in their study on 6 patients with tattoo reactions induced by red ink. One patient with a reaction starting 2 weeks after tattooing reacted with a positive patch reaction to the colour Red 210, CI 12477. Among the 7 dyes was Red 170, CI 12475, which is on the list of H317 notified substances (Table 5). It gave no positive patch test reactions, but was suspected to be the cause of 2 reactions based on the clinical information. Based on these two studies patch testing may be a useful tool in the diagnostic evaluation of some adverse tattoo reactions, however in most cases patch testing remains negative. There are a number of reasons for this, which is listed in Table 2.

Table 2 Theoretical reasons for negative patch tests in patients with tattoo reactions

Reasons	Type
The reaction is not due to contact allergy	True negative
Patch testing has not been performed with the right substances, - due to lack of ingredient information - as allergens are formed in the skin	False negative
The substances do not penetrate the skin in sufficient amounts.	False negative
The reaction is due to photosensitivity, which is rarely tested.	False negative

Anaphylactic reactions caused by tattoos

An immunological anaphylactic reaction has been described to a tattoo ink (Lee Wong et al., 2009). The specific offending substance was not identified.

2.2.1 Conclusion concerning cases

Historically contact allergy has been seen in tattoo reactions caused by mercury, cobalt and chrome containing dyes. These types of dyes are rarely used now.

Two case studies were identified, where more than two patients with adverse skin reactions to tattoos were collected and where there has been a systematic approach to obtain exposure information and perform patch testing (Serup J et al., 2014; Gaudron s et al., 2014). These recent studies testing with the actually used azo dyes and pigments suggest that contact allergic reactions may be present in some patients, but the majority has a negative outcome of patch testing. Nickel allergy seems to be more frequent in those with tattoo reaction than in the general population however it is not possible to make a formal comparison. No investigations have been identified examining if tattoo needles can be a source of nickel release.

Published individual cases are often persons with very severe reactions, while the population data is based on self-reports and will cover very mild and transient symptoms, too. There is no way to link results from the population studies with the information from these individual cases.

3. Mechanisms of allergic contact dermatitis in relation to tattoos

The skin provides an important physical, chemical and immunological barrier protecting the human body from the environment. The skin consists of two major compartments: the epidermis and the dermis. The epidermis is the outer layer and the dermis the inner layer of the skin (figure 4). The keratinocytes are the most numerous cell type present in the epidermis but also Langerhans cells and T cells are present (Pasparakis M et al., 2014). A more diverse cellular composition is found in the dermis, which consists of both different subtypes of dermal dendritic cells, macrophages, mast cells, T cells and different stromal cells such as fibroblasts (Pasparakis M et al., 2014).

The immune system is very complex, but is generally dived into the innate and the adaptive immune system. The innate immune system is also called the in-borne immune system and provides immediate defence in a generic way. The adaptive immune system is highly specific in its response to a pathogen or allergen and provides long-term and often life-long immunological memory. This means that when a person has become sensitized to a contact allergen, the adaptive immune system will form memory cells which will be able to recognize the offending allergen. If sufficient exposure occurs to the allergen in question, the memory cells will initiate an immune response, which leads to inflammation also called allergic contact dermatitis. This may happen even many years after sensitization occurred.

Certain cell types are required for induction of contact allergy, these cell types are Langerhans cells and dermal dendritic cells, which are present in epidermis and dermis, respectively. During the last years, by studying different mice models, it has become clear that different skin antigen presenting cell subsets can induce different types of immune responses even though there exits some redundancy between the different antigen presenting cell subsets (Kaplan DH et al., 2012).

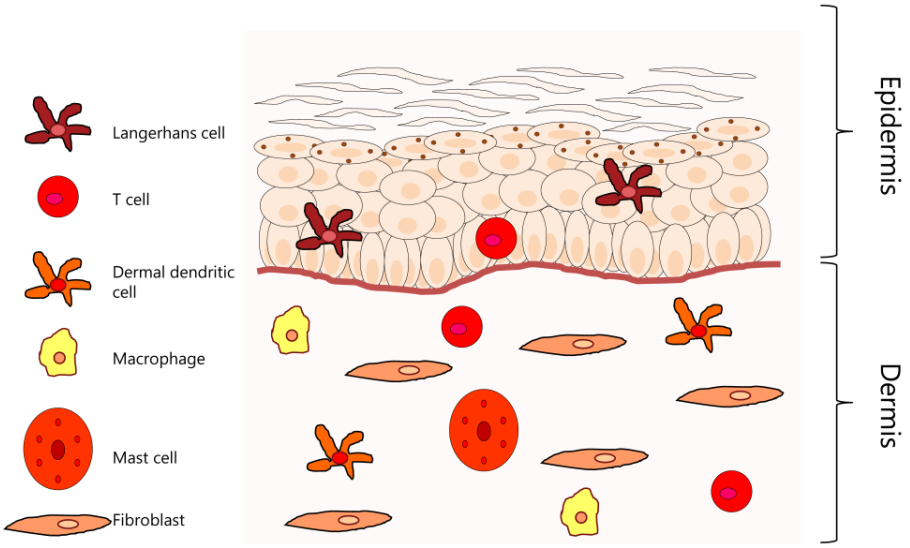

Figure 4 Schematic of cellular components of the skin

Allergic contact dermatitis is one of the most frequent forms of immune reactions and is induced after skin exposure to sensitizing chemicals called contact allergens. Allergic contact dermatitis is a T cell mediated reaction. T cells recognize antigens in the form of major histocompatibility complex-peptide complexes via their T cell receptor. Contact allergens can be of various sizes, but are generally smaller molecules (Fitzpatrick JM et al. 2016), also called haptens, that cannot induce T cell activation by themselves. Instead contact allergens have to bind to our own proteins and thereby modify these so that they appear foreign to the immune system and thereby can induce T cell activation (Kaplan DH et al., 2012). However, it is not enough that a contact allergen can induce modification of own proteins; unspecific inflammation needs to be induced in order to trigger a specific immune response. This unspecific immune stimulation can be triggered by the contact allergen itself, but can also be achieved by other allergens or irritants in a solution such as in a tattoo ink. Probably also the significant trauma to the basal membrane, dividing the epidermis from the dermis, caused by the tattoo needles can act as such unspecific immune stimulation.

Following skin exposure to a contact allergen, the keratinocytes and the Langerhans cells will sense the allergen and start to produce various pro-inflammatory mediators leading to local inflammation. In addition the Langerhans cells will become activated and will migrate to the draining lymph nodes where they will present the allergen to naïve T cells leading to the activation of these (Kaplan DH et al., 2012).

Often the allergen will not only stay in the epidermis but will penetrate further into the dermis leading to activation of dermal dendritic cells. Like for the Langerhans cells, the dermal dendritic cells will be activated and migrate to the draining lymph nodes and present the allergen for naïve T cells (Kaplan DH et al., 2012). However, as mentioned above the role of the different antigen presenting cells for the response is still not clear (Kaplan DH et al., 2012).

Intradermal injections of suspected allergens were for many years recommended for routine screening of substances in the guinea pig and this methodology was quite successful in detecting contact allergens (Magnusson B, Kligman AM, 1969). The guinea pig studies have now been replaced with mice, the Local Lymph Node Assay, where only epidermal exposures are performed.

Interestingly, studies of nickel allergy in mice suggest that different types of immune responses are induced dependent on if the skin is exposed to nickel via the epidermis or via injections of nickel into the dermis (Schmidt M et al., 2010; Vennegaard MT et al. 2014). Exposure via the epidermis induced nickel-allergy without the need of addition of adjuvants whereas injections of nickel into the dermis required addition of an adjuvant to induce an immune response (Schmidt M et al., 2010; Vennegaard MT et al. 2014). Thus, it seems that different immune responses are induced dependent on if the primary exposure to the allergen is epidermis or dermis. The difference lies in the mechanism, while the endpoint contact allergy and allergic contact dermatitis will be the same following the two routes of exposure.

In man the intradermal route of sensitization is sparsely researched compared to the epidermal route (Kligman AM, 1966). It is undoubtly possible to introduce contact allergy in man by the intradermal route, as evidenced by many cases of contact allergy to aluminium following childhood vaccinations (Trollfors B et al., 2005). Evidence also exist that it may be easier to elicit allergic contact dermatitis in patients sensitized to nickel by intradermal injections compared to epidermal applications (Möller H, 1989). However, no systematic comparisons between the epidermal and intradermal route concerning hapten, concentrations and number of applications have been carried out.

4. Non-allergic immune reactions in tattoos

According to current knowledge allergic contact dermatitis only covers a minority of the severe adverse reactions observed in relation to tattoos (Serup J, Hutton Carlsen K, 2014; Gaudron S et al., 2014; Islam PS et al., 2016). It may be that allergic reactions are over-looked to some extent due to lack of medical attention and the diagnostic limitations of the patch test. However, many of the reported severe adverse skin reactions do not have the characteristics of an allergic reaction (Thum CK, 2015). The non-allergic immune reactions differ clinically from the allergic contact dermatitis reactions. The latter typically has onset hours to days after the exposure in a pre-sensitized person and typical have a limited time period. The non-allergic immunological reactions may start after 2 weeks but typically have an onset after month or years (Gaudron S, 2014). They are accompanied by itching, which may be severe. They can persist for many years. The basic biological mechanism may be an activation of the macrophages because of “an overstimulation” caused by accumulation of tattoo pigments in the cells. The most common type, particularly related to the red colours, is the lichenoid reaction. Clinically it is characterized by oedema and swelling. The reaction is sharply restricted to the area of exposure. This is in contrast to allergic contact dermatitis reactions, which often spread outside the area of exposure. The treatment options are currently surgery and laser treatment.

The mechanisms of these reactions have not been investigated, but one possible mechanism is the foreign body reaction, which is described below.

4.1 Mechanism of foreign body reaction

Tattoos are made by introducing tattoo pigments into the dermis. Fibroblasts, macrophages and mast cells have been shown to be capable of taking up the tattoo pigments in the dermis (Taylor CR et al., 1991). Of these cells, macrophage is the only cell type that in theory can function as an antigen presenting cell. Interestingly, it was shown in biopsies of tattoos that macrophages and not dermal dendritic cells contained the tattoo pigments (Zaba LC et al, 2007). Furthermore, by isolating these macrophages from healthy skin it was shown that they could not induce T cells activation not even upon cytokine activation (Zaba LC et al, 2007). Thus, this suggests that macrophages might induce an immune response independent of T cells following dermal exposure to tattoo pigments. Furthermore, as tattoo pigments are found in the skin as particles it seems unlikely that they are presented to naïve T cells. To our knowledge it has not been investigated how tattoo pigments activate macrophages but it is likely that tattoo pigments can activate macrophages in the same way as wear debris from prosthetic implants.

Two different mechanisms have been shown depending on the size of the particles (Figure 5). Large particles (20-100 µm) generate ‘frustrated phagocytosis’ leading to activation of reactive oxygen species and inflammasome activation. Smaller particles (< 10 µm) can easily be phagocytes but as these particles cannot be degraded, the particles accumulate and damage the endosome membrane which creates ‘endosomal destabilization’ which eventually leads to inflammasome activation (Cobelli N et al., 2011). Inflammasome activation results in cleavage of pro-IL-1β and pro-IL-18 to form the active IL-1β and IL-18 (Cobelli N et al., 2011). However, pro-IL-1β and pro-IL-18 are not pre-formed in the cells but are produced following stimulation of Toll-like receptors. These receptors can both be stimulated after recognition of pathogens but also by molecules released from our own cells upon stress which are likely to be induced by the tattooing procedure. However, whether tattoo particles can activate macrophages via

small or/and large particles are currently not known. It will be important to investigate this to get a better understanding of the immunological mechanisms mediating skin inflammation seen in some individuals with a tattoo.

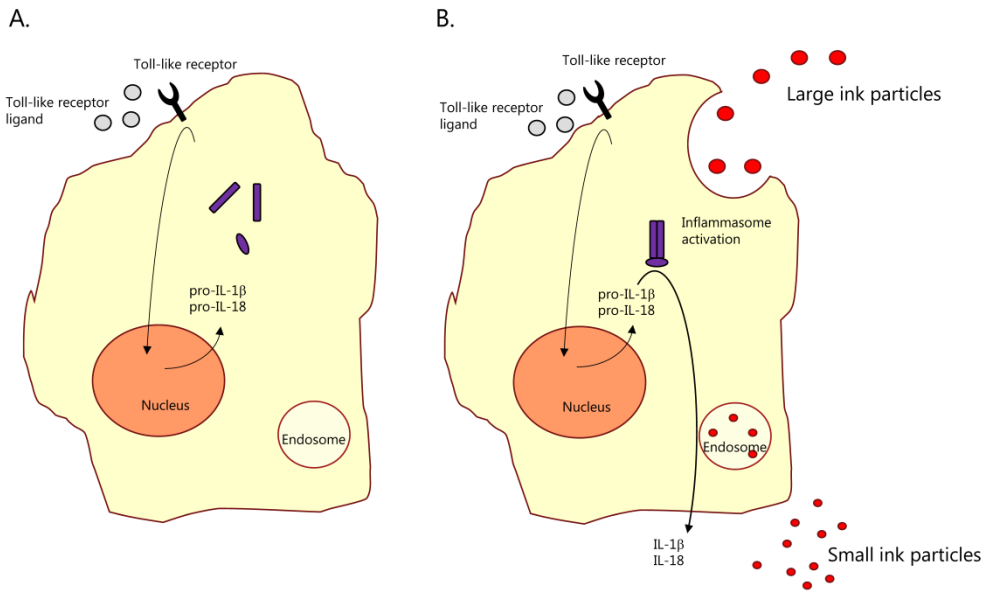

Figure 5 Theoretic schematic illustration of macrophage activation by tattoo ink particles. A. Shows production of pro-IL-1β and pro-IL-18 by macrophages upon stimulation of toll-like receptor B. Shows production of IL-1β and IL-18 by macrophages following stimulation of toll-like receptor as well as inflammasome activation by tattoo ink particles.

Particle size was measured in 58 typical tattoo inks with samples of red, blue, green, yellow, white and black purchased from 13 different manufacturers. The manufacturers were German, British, American, Dutch and Italian. The purchased inks were aqueous dispersions, ready-to-use inks with water as the main solvent. For size measurements, the dispersions were analysed by laser diffraction supplemented with TEM, scanning electron microscopy (SEM) and X-ray diffraction. Analysis of red, green, blue and yellow colours showed particle size (unfiltered) from 81-7074 nm, typically the size was around 150 nm. Mean diameter of black pigment (carbon black) was from 48-165 nm, and white from 317-738 nm (TiO_2). The vast majority of the tested tattoo inks contained significant amounts of nanoparticles (<100nm) except for white pigments (Høgsberg T et al., 2011). This means that both large and small particles can be found in tattoo ink and the mechanisms described above may potentially be relevant.

5. Clinical characterisation of adverse immune reactions in tattoos

The reactions can thus be divided into allergic and non-allergic immune reactions. The basic understanding of the non-allergic immune reactions to tattoos is at the research level and no diagnostic test is available for this type of immune reactions. In such a state of medical development for a new disease area the only available tool is descriptive pattern analysis based on clinical and histopathological observations, which has recently been suggested (Thum CK, 2015) shown in Table 3. All the different types of reactions have sophisticated names mainly of interest for the medical profession. It is important for lay people to know that tattoo reactions may look like cancer and other life threatening diseases. They all contain diagnostic and treatment challenges and need specialized medical attention.

Table 3 Patterns of immune reactions to tattoos (histologically) based on clinical series of severe cases (Thum CK, 2015)

Spongiotic reaction	**Typical for allergic contact dermatitis**
Psoriasisform reaction	Development of psoriasis in a tattoo, rarely reported. May appear de novo or be an activation of a pre-existing disease.
Interphase patterns	
- Lichenoid	Band-like inflammatory cell infiltrate at the junction between epidermis and dermis. Believed to be the most common inflammatory pattern encountered in tattoos. May have a positive patch test.
- Vacuolar	Patchy degeneration of the basal layer with patchy inflammation.
Nodular and diffuse patterns - Granulomatous - Pseudolymphomatous	Foreign body type reactions involving macrophages.
Vesiculobullous pattern	May be a sign of allergic contact dermatitis or autoimmune disease.
Vasculitis	Most develop shorty after tattooing.
Fibrosing pattern	Scarring or keloid formation.
Pseudopepitheliomatous pattern	Rapidly growing verrucous overgrowths developing between one week and a few months. Few cases reported.

The spongiotic, lichenoid (rarely), psoriasiform and vesiculobullous forms can all be seen as part of an allergic contact dermatitis reaction. In the remaining cases a non-allergic immune reaction, where the tattoo ink particles are presented to the immune system by the macrophages, may be the mechanism. The time factor is also important. Immediately after tattooing and during the following weeks the skin is read, oedematous and irritated in a varying degree caused by trauma by the repeated piercing process. Such skin inflammation may cover up symptoms of allergic contact dermatitis, if the person is already sensitized e.g. to nickel, fragrances or preservatives. Typically the person will not be seen by a dermatologist in this phase and the event of allergic contact dermatitis may pass undiagnosed. As the small chemicals, which cause allergic contact dermatitis, are soluble they will presumable disappear within short time (days to weeks). The types of immunological reactions mediated by the macrophages will typically appear later after months or years and can persist, if untreated, for years. It is possible that a patient can have more than one type of immunological reaction to a tattoo.

6. Selected substances classified as skin sensitizing 1A or 1B

6.1 Criteria (CLP)

The criteria for EU harmonized classification as skin sensitizing is given in Annex 2. Substances can be classified according to CLP criteria based on human data or non-human data (ECHA, June 2015). In total 1151 substances have an EU harmonized classification as skin sensitizer Cat. 1A or 1B. Of these only 22 have been found in association with tattoo inks (Piccinini P et al., 2015); these are listed in annex 3.

Relevant information with respect to skin sensitisation may be available from case reports, epidemiological studies, medical surveillance and reporting schemes based on human patch testing. Concerning non-human data there are three standard animal test methods used to evaluate skin sensitisation for substances: the mouse local lymph node assay (LLNA), the guinea pig maximisation test (GPMT) and the Buehler assay. Further data such as structural alert data (e.g. QSARs or expert systems) and *in vitro* assays may form part of the weight of evidence for classification.

Where data are sufficient a refined evaluation allows the allocation of skin sensitizers into sub-category 1A, strong sensitizers, or sub-category 1B for other skin sensitizers. The subcategorization is based on potency i.e. the induction thresholds, a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure, or other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure. The definitions of relatively high incidence and exposures can be found in annex 2. Similar substances can be subcategories according to non-human e.g. animal data. As an example an Effect Concentration EC3-value ≤ 2 categorize the substance as a strong or extreme sensitizer in group 1A.

6.2 ECHA lists of classified substances

The considerations presented in this note are relevant for all the substances that have an EU harmonized classification as skin sensitizer Cat. 1A or 1B (H317: may cause an allergic reaction) according to CLP and are applied in tattoo inks. Since CMR substances in tattoo inks will be addressed in parallel, of particular interest are the non CMR substances that have a harmonized classification as skin sensitizers. Among such substances 9 have been related to tattoo inks according to Piccinini P et al., 2015 (see Table 4).

These 9 substances are all well-known allergens based on clinical experience and problematic. Some are very strong allergens such as p-phenylenediamine, causing allergic contact dermatitis when applied in hair dyes and temporary tattoos (Thyssen JP et al., 2009), and the mixture of isothiazolinones used as preservative (Latheef F et al. 2015).

Table 4 Non CMR substances with a harmonized classification H317 and related to tattoo inks

Substance	Application	EC	CAS
4-chloro-3,5-xylenol*	Preservants	201-793-8	88-04-0
Methenamine*	Preservants	202-905-8	100-97-0
p-phenylenediamine***	Colorant	203-404-7	106-50-3
1,2-benzisothiazol-3(2H)-one**	Preservants	220-120-9	2634-33-5
Rosin	Viscosity regulator	232-475-7	8050-09-7
Cobalt	Colorant	231-158-0	7440-48-4
2-octyl-2H-isothiazol-3-one**	Preservants	247-761-7	26530-20-1
3-iodo-2-propynyl butylcarbamate*	Preservants	259-627-5	55406-53-6
Mixture, 3(2H)-isothiazolone, 5-chloro-2-methyl- with 2-methyl-3(2H)-isothiazolone. 3:1*	Preservants	611-341-5	55965-84-9

*Preservatives that are allowed in cosmetic products with a maximum concentration limit (listed in Annex V of the Cosmetics Products Regulation).
**Substances with an antimicrobial effect, which are not allowed as preservatives in cosmetic products (not included in Annex V).
***The colorant p-phenylenediamine is also restricted in cosmetics and its use is confined to hair.

All substances in Table 4 may cause allergic contact dermatitis when applied to the epidermis or injected in dermis. Depending on the concentration of the allergens in the tattoos ink contact allergy may also be induced by tattooing.

Table 5 lists the H317 self-classified substances related to tattoo inks according to Piccinini P et al., 2015. Some of these are all very well-known allergens: 2-methyl-2H-isothiazol-3-one(MI) has been responsible for an epidemic of contact allergy in Europe (SCCS opinion P94, 2015) and 5-chloro-2-methyl-2H-isothiazol-3-one and chromium salts are also well established causes of contact allergy (Diepgen T, 2016). Aluminium salts are used in vaccines as an adjuvant. Around 1% of children vaccinated with such vaccines develop contact allergy to aluminium which usually manifest as granulomas and rash (Trollfors B et al., 2005). Aluminium salts would also be able to cause allergic contact dermatitis in tattoos. Those substances which according to our knowledge have caused cases of allergic contact dermatitis in man are noted in the column “clinical evidence present”.

Further 3 substances were identified where a few or some cases have been reported of suspected allergic reactions: 4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide; Rose, Rosa centifolia, ext.; Rose, Rosa damascena, ext. Further an allergic reaction to colour Red 210, CI 12477 was identified when reviewing the cases. This substance is not in Table 5.

All these 4 substances should be scrutinized concerning the quality of the data and if additional data exist to make a decision on their continued use in tattoo inks or not.

Other substances in Table 5, where no clinical cases are reported, are probably classified based on evidence of sensitisation effects in unpublished animal studies. This poses a problem with quality assurance of the data. However, this is in general more seen as a problem in case of negative studies. In general, it is the experience that substances positive in predictive sensitization assays would in most cases cause sensitization if the individual is sufficiently exposed (Basketter D et al., 2007).

Table 5 List of H317 notified substances related to tattoo inks

Name of skin sensitiser	EC	CAS	Clinical evidence of allergy present*	Covered by the Cosmetic Products Regulation
Benzoic acid	200-618-2	65-85-0		Annex V
Zinc oxide	215-222-5	1314-13-2		Annex VI
Aluminium	231-072-3	7429-90-5	As a salt (many cases) e.g. Trollfors B, 2005	Annex IV (only CAS: 7429-90-5)
Propane-1,2-diol	200-338-0	57-55-6		-
Salicylic acid**	200-712-3	69-72-7		Annex III & Annex V
1,4-dihydroxyanthraquinone	201-368-7	81-64-1		-
9-(2-carboxyphenyl)-3,6-bis(diethylamino)xanthylium chloride	201-383-9	81-88-9		Annex II
Phenanthrene	201-581-5	85-01-8		-
Hexachlorobuta-1,3-diene	201-765-5	87-68-3		-
Propyl 4-hydroxybenzoate	202-307-7	94-13-3		Annex V
Butyl 4-hydroxybenzoate	202-318-7	94-26-8		Annex V
Methyl 4-hydroxybenzoate	202-785-7	99-76-3		Annex V
Melamine	203-615-4	108-78-1		-
Hexa-2,4-dienoic acid	203-768-7	110-44-1		Annex V
Ethyl 4-hydroxybenzoate	204-399-4	120-47-8		Annex V
2,4,7,9-tetramethyldec-5-yne-4,7-diol	204-809-1	126-86-3		-
29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32 copper	205-685-1	147-14-8		Annex II
Copper oxide	215-269-1	1317-38-0, 1344-70-3		-
Disodium [29H,31H-phthalocyaninedisulphonato(4-)-N29,N30,N31,N32]cuprate(2-)	215-537-8	1330-38-7		Annex II
Trisodium 5-hydroxy-1-(4-sulphophenyl)-4-(4-sulphophenylazo)pyrazole-3-carboxylate	217-699-5	1934-21-0		Annex III & Annex IV
6-chloro-2-(6-chloro-4-methyl-3-oxobenzo[b]thien-2(3H)-ylidene)-4-methylbenzo[b]thiophene-3(2H)-one	219-163-6	2379-74-0		Annex II
2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-N-phenylbutyramide	219-730-8	2512-29-0		-
2-methyl-2H-isothiazol-3-one	220-239-6	2682-20-4	Many cases eg. Latheef F 2015	Annex V
4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide	220-509-3	2786-76-7	2 cases reported suspected caused by Red 170, Gaudron, 2014	-
1-[(2,4-dinitrophenyl)azo]-2-naphthol	222-429-4	3468-63-1		Annex II
4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-	222-530-3	3520-72-7		-

5-methyl-2-phenyl-3H-pyrazol-3-one]				
Disodium 2,2'-(9,10-dioxoanthracene-1,4-diyldiimino)bis(5-methylsulphonate)	224-546-6	4403-90-1		-
Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]	225-935-3	5160-02-1		Annex II
N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	226-103-2	5280-68-2		-
Calcium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	226-109-5	5281-04-9		Annex IV
2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-methylphenyl)-3-oxobutyramide]	226-789-3	5468-75-7		-
4-[(2,5-dichlorophenyl)azo]-3-hydroxy-N-phenylnaphthalene-2-carboxamide	227-930-1	6041-94-7		-
3-hydroxy-4-[(2-methyl-4-nitrophenyl)azo]-N-(o-tolyl)naphthalene-2-carboxamide	229-102-5	6410-32-8		-
N-(5-chloro-2,4-dimethoxyphenyl)-4-[[5-[(diethylamino)sulphonyl]-2-methoxyphenyl]azo]-3-hydroxynaphthalene-2-carboxamide	229-107-2	6410-41-9		Annex II
1,3-bis(hydroxymethyl)-5,5-dimethylimidazolidine-2,4-dione	229-222-8	6440-58-0		Annex V
3-hydroxy-4-[(2-methyl-5-nitrophenyl)azo]-N-phenylnaphthalene-2-carboxamide	229-245-3	6448-95-9		-
4-[(4-chloro-2-nitrophenyl)azo]-3-hydroxy-N-(2-methylphenyl)naphthalene-2-carboxamide	229-314-8	6471-50-7		-
3-hydroxy-N-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide	229-440-3	6535-46-2		Annex II
Chromium***	231-157-5	7440-47-3	as a salt	Annex II & IV****
Copper*****	231-159-6	7440-50-8		-
Xanthan gum	234-394-2	11138-66-2		-
5-chloro-2-methyl-2H-isothiazol-3-one	247-500-7	26172-55-4	Many cases	Annex V
2-bromo-2-(bromomethyl)pentanedinitrile	252-681-0	35691-65-7		-
N-(5-chloro-2-methoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	268-028-8	67990-05-0		-
Rose, Rosa centifolia, ext.	283-289-8	84604-12-6	Cases exist	-
Calendula officinalis, ext.	283-949-5	84776-23-8		-
Rose, Rosa damascena, ext.	290-260-3	90106-38-0	Cases exist	-
Sorbitan monolaurate, ethoxylated	500-018-3	9005-64-5		-
C.I. Pigment Yellow 36	609-398-6	37300-23-5		-

*) no formal review was done of the evidence for each substance.
**RAC opinion from 2016 recommends harmonised classification as Rep 2 and Eye Dam
***Chromium (VI) impurities may be found in inorganic pigments based on chromium oxides
****Only chromic acid and its salts (Cas no 7440-47-3, EC no 231-157-5) are on Annex II, and only Chromium (III) oxide (CI no 77288) and Chromium (III) hydroxide (CI no 77289) are on Annex IV (positive list of colorants allowed in cosmetic products).
*****Substance on CoE, but not covered in the approach of the new REACH restriction dossier

Table 5 includes almost all preservatives that are known to be used in tattoo inks (Piccinini et al. 2015) without any relevant harmonised classification resulting in the substance being banned by the proposed REACH restriction (CMR, SS, SIr, SCr, EIr, ED). This is relevant information for the discussion on how to deal with substances on Annex V in the REACH restriction proposal for chemicals in tattoo inks and PMU.

In addition to these lists of substances (Table 4 and 5) an allergic reaction *to colour Red 210, CI 12477* was identified (Gaudron S et al., 2014) in reviewing the cases (section 2.2).

6.3 Conclusion

All the substances with a harmonized classification as H317 and the four substances indicated in Table 5 with many cases reported are proven skin sensitizers (contact allergens). It is recommended that these substances should not be allowed in tattoo inks due to the risk of allergic reactions.

The three substances with few cases or suspected cases and the additional substance identified by reviewing the case reports should be scrutinized concerning the quality of the data and if additional data exist prior to a decision.

The reason for notification of the remaining substances in Table 5 is unknown. In case evidence exists, which fulfil the criteria for classification e.g. in animals (see Annex 2), they also pose a risk of sensitization in man.

7. Establishing limit values for restriction

Induction as well as elicitation of contact allergy is dose-dependent. The threshold dose of a number of sensitizers has been investigated in human and animal test systems (Van Loveren H et al., 2008) as well as in clinical studies of sensitized individuals (Fisher LA et al., 2005). In these studies the allergens are applied on the skin (epicutaneously), like in normal use of consumer or work place products. Such studies have formed the basis of limit values in EU regulation. One example is the EU regulation for nickel items coming in direct contact with the skin where the limit value is set to 0.5µg nickel/cm^2/week.

It is known that if allergens are deposited in dermis (intradermally) then stronger reactions will occur and with lower doses (Möller H, 1989), however only few substances have been investigated in this way. The limits established based on epidermal exposures cannot be used to set limit values for tattoo inks, as even very small levels of allergens injected into the skin may pose a problem.

However, considering that all the substances in Table 4 and most of the substances in Annex 3 of this document are substances that have a specific function, such as preservative, colorant or viscosity regulator, it is possible to make a ban of these substances in practice applying the detection limits as a limit values in relation to a restriction. It is expected that the absence of use will also eliminate all trace amounts of the substances and thus the risk of elicitation of an allergy.

8. Gaps of knowledge

The increasing number of individuals in the population, who are getting a tattoo, makes it urgent to initiate research to better understand, diagnose and prevent adverse tattoo reactions. This is important to further advance the knowledge, so that new treatments can be found and safer products developed.

Research needs	Rationale
Clinical investigations into early and late reactions in prospective cohorts of tattooed people should be performed to characterise the reactions, determine their course and severity as well as the relationship to particular inks (brand and composition) and ingredients.	Knowledge is lacking concerning the epidemiology of adverse reaction to tattoos.
The mechanisms of non-allergic immune reactions need to be investigated to be able to diagnose, treat and prevent adverse reactions to tattoo inks. One such mechanism may be stimulation of macrophages by small or/and large particles in ink.	In many cases the mechanism and thus the type of adverse reaction is unknown. It is important to know the mechanisms to diagnose, treat and prevent adverse reaction in tattoos.
It should be investigated if it is possible to improved methodologies to detect allergic reactions to tattoo inks.	Only in relatively few cases it has been possible to definitively prove contact allergic reactions in tattoos.
Investigation of nickel release from tattoo needles and deposition of nickel in the skin should be investigated.	Nickel allergy is found in more persons who are tattooed than should be expected.
The adverse effects from the trauma caused by the tattoo process in itself (without ink).	The needle itself may cause severe damage to and necrosis of the basal membrane in the skin.
The mechanism of sun-induced adverse reactions	Many complain about sun-induced adverse reactions in their tattoo.

9. General conclusions

- Many persons with a tattoo experience adverse skin reactions as transient, intermittent or long term problems. The nature of these reactions has not been investigated except for isolated cases.

- The number of persons with allergic reactions in their tattoos is unknown. Rarely cases have been thoroughly investigated and only in a limited number of cases it has been possible to definitely prove contact allergy. This may be due to limitations in the patch test methodology as well as the lack of information concerning ingredients in the specific inks having caused an adverse reaction.

- Classic contact allergens (metals, colourants and preservatives) have been identified in tattoo inks. Some of these substances are strong or extreme allergens.

- Contact allergy can both be introduced and elicited from epidermis and dermis.

- Allergens deposited in dermis may elicit stronger reactions and at lower doses.

- It is not possible to determine limit values in tattoo inks. The dose needed to provoke an allergic reaction is expected to be very low for most allergens.

- It is also possible that some substances, which will normally not penetrate the skin due to substance properties (size or physio-chemical properties) and therefore are unreactive if applied on epidermis, can cause reactions when injected directly into dermis.

- In case the allergen is a small molecular organic substance such as a preservative, it is expected that the substance will be cleared from the dermis after weeks and the reaction subside. Therefore some of these reactions may be over-looked.

- If the allergen is part of the pigments, which are permanently deposited in the dermis, the allergic reaction may become chronic.

- In this investigation 13 non-CMR substances associated with tattoo inks were identified, which are well known contact allergens. These should not be allowed in tattoo inks due to the risk of allergic reactions; neither should other substances with a harmonised classification, which may be used in tattoo inks in the future.

- The lack of knowledge concerning the ingredients in the specific inks, which has caused adverse reactions hamper the possibility of diagnosis and prevention.

- Apart from allergic reactions in tattoos other immune reactions have been described. The mechanisms are unknown.

- Tattoo ink contains both large and small particles and will in theory be able to activate mechanisms of foreign body reaction.

- A multi-pronged approach is needed, if possible yet unknown systemic side effects coming up decennia after making of the tattoo, should be prevented.

10. Recommendations

- Allergens -fulfilling the CLP criteria for classification- should not be present in tattoo ink due to the risk of allergic reactions.

- This applies to all the identified substances mentioned in Table 4 with a harmonized classification as H137, and should also apply to all other substances with a harmonised classification, to prevent the used of these in tattoo inks in the future.

- This should also apply to the four substances indicated in Table 5 with a self-classification as H137, which are well known and clinical important skin sensitizers (contact allergens).

- Three substances from Table 5 with few cases or suspected cases as well as one substance identified in reviewing the literature for this memo should be scrutinized concerning the quality of the data and if additional data exist prior to a decision.

- The reason for notification of the remaining substances in Table 5 is unknown. In case evidence exists, which fulfil the criteria for classification e.g. in animals (see Annex 2), these substances also pose a risk of allergic reactions in man.

- All ingredients in tattoo inks should be declared either on the container or in a SDS, regardless of if they are hazardous or not. This would mean full ingredient labelling. It will make targeted risk assessment (for all toxicological effects) and effective prevention possible. It will improve the possibilities of making a diagnosis of allergic contact dermatitis to tattoo inks.

- Research should be initiated to better understand, diagnose and prevent adverse tattoo reactions.

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Appendix 1. Population studies short summaries

Brady B et al., 2015 performed a survey in June 2013 in Central Park among 300 randomly selected tattooed people, aged 18-69 years, with an equal distribution of women and men, 31 (10.3%) of the participants reported adverse reactions defined by any skin sign or symptom, which differed from normal part of tattooing or healing. Data was collected by personal interview (Brady B et al., 2015). In 13 (4.3%) participants the reaction was defined as acute by having occurred a few days to weeks after tattooing. The acute reactions lasted from a few days to 4 months and included pain at the tattooed area, infections requiring antibiotics, itching, swelling and prolonged scabbing. In 18 (6%) participants the reactions were defined as chronic colour-associated by involving a specific colour and lasting for more than 4 months. These reactions were described as itchy, scaly, raised, oedematous or a combination. Two chronic cases were described as scarring. One participant described a reaction in the red ink of the tattoo developing two weeks after tattooing and with subsequent development of a similar response in the red ink portion of an 8-year-old tattoo. About two-thirds of the participants with chronic reactions reported immediate on-set and the majority of these experienced ongoing symptoms. Only 29% (9/31) had obtained medical care for their symptoms and only five persons (all with chronic symptoms) had been seen by a dermatologist. Persons with chronic reactions had significantly more colours in their tattoos than other participants. The two ink colours most commonly involved in chronic colour-associated reactions were red (8/18) and black (6/18), although other colours were also reported (Brady B et al., 2015).

Laumann AE, Derick AJ, 2006 performed a study in US as a national probability sample. The sample was obtained by random digit dialling and consisted of 253 women and 247 men aged 18 to 50 years. The study was conducted as a telephone survey. In all 19.313 calls were made to complete 500 interviews. In total 120 (24%) reported to have at least one tattoo and 15 of those (12.5%) reported a medical problem with the tattoo, such as bleeding, crusting, swelling etc., within the first 2 weeks after having the tattoo done. Of these 3 has sun sensitivity. In all cases but 2 the reaction was limited to the first two weeks (Laumann AE, Derick AJ, 2006).

Klügl I et al.,2010 performed an internet-based survey of 3411 German-speaking tattooed persons. The participants were asked to consider their most recent tattoo. In total 2.302 (67.5%) reported a reaction directly after tattooing such as bleeding, oedema, burning or pain, while 264 (7.7%) reported continuing reactions 4 weeks after tattooing and 6.0% (206) reported persistent problems, which was seen more frequently in participants with coloured tattoos than with black tattoos (Klügl I et al.,2010). The persistent problems were most often reported as scars (2.0-1.6%), followed by intermittent oedema (1.2%-0.4%), itching (0.9%-0.4%), elevated skin (0.9%-0.4%), acne, papules and numbness. In total 1.6% of the participating women and 0.8% of the men reported light sensitivity as the persistent problem (Klügl I et al.,2010).

Kluger N, 2015 studied tattooists, members of the French Tattoo Union. The reasoning behind the study was that tattooists are usually heavily tattooed and constitute a special group of interest. The study was conducted as an internet survey in 2013. 448 out of 1000 (44.8%) responded to the questionnaire. In total 42.6% reported a tattoo reaction on at least one of their tattoos (including itch). Swelling during/after sun exposure was reported by 23% and permanent mild swelling by 4%. A previous self-reported tattoo allergy to one colour on at

least one of their tattoos was reported by 8% of the tattooists. No statistical relationship between colours and reactions could be found (Kluger N, 2015).

Dybboe R et al., 2016 report the results form a questionnaire study in a sample of the general population from the south-western part of the greater Copenhagen area in Denmark, which was conducted in 2011-12. As part of a 5-year follow-up of a population based cohort study, participants answered questions regarding permanent tattoos. Of 3471 participants at baseline, 2308 participated in the follow-up (66.5%); 2212 answered the questions regarding tattoos. 313 (14.2%) had one or more tattoos. 18 (5.9%) reported adverse reactions: 9 (2.9%) eczema/rash, 4 (1.3%) infection, 3 (1.0%) erosions, and 2 (0.7%) all symptoms. Red tattoo ink was involved in most reactions. Two (11.8%) had had their tattoo removed due to adverse reactions (Dybboe R et al., 2016). In 12 cases (70.6%) it disappeared by itself, 1 (5.9%) disappeared after medical treatment and 2 (11.8%) had the tattoo removed and 1 did not answer the question (Dybboe R et al., 2016).

Høgsberg T et al., 2013 collected data through personal interview and examinations of 154 tattooed consecutive individuals who spontaneously attended a clinic of venerology in Copenhagen, Denmark. The participants had in total 342 tattoos. In total 15% (23/154) reported complaints up to 3 months after tattooing, whereas 27% (41/154) reported complaints beyond 3 months after tattooing corresponding to 16% of the tattoos (55/342). Skin elevation and itching were most frequent complaints. The complaints varied in intensity, but were mainly minor. Most complaints were related to black and red ink. 58% of all tattoo complaints (32/55) were sun-induced corresponding to sun-sensitivity in 9% of all tattoos (32/342). The complaints were mainly minor, skin elevation and itching were most frequent (Høgsberg T et al., 2013). The complaints lasted minutes to days uncommonly they lasted weeks or months.

Hutton Carlsen K, Serup J, 2014 performed a study in the summer 2011 concerning photosensitivity of tattoos by interviewing sunbathers with tattoos on the beach. A total of 146 persons with 301 tattoos accepted to participate. In total 60 (41%) participants experienced complaints beyond 3 months after getting a tattoo and of these 31 (52%) complaints were sun-related, such as swelling (58%), itching, stinging, pain (52%) and redness (26%). Most participants had black tattoos (n=133) and 24 (21%) of these had sun-induced complaints related to this colour; 17/45 (37%) had suninduced complaints in the red colours, 10/25 (40%) in blue and 5/25 (20%) in yellow colours. Problems in the tattoos independent of the sun was seen in 29 (19%) of the 146 tattooed persons. Most reactions were due to heat (n=12) and described as swelling and/or itching. 9 reported constant swelling and 3 ‘allergic reactions’, these were in 2 cases in red colour and 1 in black and yellow (Hutton Carlsen K, Serup J, 2014).

Appendix 2. Criteria for skin sensitizers and their sub-categorization

Tables with the criteria for skin sensitizers and their sub-categorization from ECHAs “Guidance on the Application of the CLP Criteria (ECHA, June 2015)

Table 3.4.2 **Hazard category and sub-categories for skin sensitisers**	
Category	Criteria
Category 1	Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria: (a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or (b) if there are positive results from an appropriate animal test (see specific criteria in paragraph 3.4.2.2.4.1).
Sub-category 1A:	Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.
Sub-category 1B:	Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered.

Table 3.4.2—b Relatively high or low frequency of occurrence of skin sensitisation*

Human diagnostic patch test data	High frequency	Low/moderate frequency
General population studies	≥ 0.2 %	< 0.2 %
Dermatitis patients (unselected, consecutive)	≥ 1.0 %	< 1.0 %
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0 %	< 2.0 %
Work place studies:		
1: all or randomly selected workers	≥ 0.4 %	< 0.4 %
2: selected workers with known exposure or dermatitis	≥ 1.0 %	< 1.0 %
Number of published cases	≥ 100 cases	< 100 cases

Table 3.4.2—c Relatively high or low exposure *

Exposure data	Relatively low exposure (weighting)	Relatively high exposure (weighting)
Concentration / dose	< 1.0% < 500µg/cm^2 (score 0)	≥ 1.0% ≥ 500µg/cm^2 (score 2)
Repeated exposure	< once/daily (score 1)	≥ once/daily (score 2)
Number of exposures (irrespective of concentration of sensitizer)	<100 exposures (score 0)	≥100 exposures (score 2)

Table 3.4.2—d Sub-categorisation decision table

	Relatively low frequency of occurrence of skin sensitisation	Relatively high frequency of occurrence of skin sensitisation
Relatively high exposure (score 5-6)	Sub-category 1B	Category 1 or case by case evaluation
Relatively low exposure (score 1-4)	Category 1 or case by case evaluation	Sub-category 1A

Appendix 3. Substances with a harmonised classification as skin sensiticer and associated with tattoo ink in the JRC report, 2015.

NAME	EC nr.	CAS nr.
Formaldehyde	200-001-8	50-00-0
Benzo[def]chrysene	200-028-5	50-32-8
Aniline	200-539-3	62-53-3
4-chloro-3,5-xylenol	201-793-8	88-04-0
3,3'-dichlorobenzidine	202-109-0	91-94-1
4-methyl-m-phenylenediamine	202-453-1	95-80-7
4-o-tolylazo-o-toluidine	202-591-2	97-56-3
Methenamine	202-905-8	100-97-0
4,4'-methylenedianiline	202-974-4	101-77-9
4-chloroaniline	203-401-0	106-47-8
p-phenylenediamine	203-404-7	106-50-3
Glyoxal	203-474-9	107-22-2
4,4'-methylenedi-o-toluidine	212-658-8	838-88-0
Chromium trioxide	215-607-8	1333-82-0
1,2-benzisothiazol-3(2H)-one	220-120-9	2634-33-5
Nickel	231-111-4	7440-02-0
Cobalt	231-158-0	7440-48-4
Rosin	232-475-7	8050-09-7
2-octyl-2H-isothiazol-3-one	247-761-7	26530-20-1
3-iodo-2-propynyl butylcarbamate	259-627-5	55406-53-6
Mixture, 3(2H)-isothiazolone, 5-chloro-2- methyl- with 2-methyl-3(2H)-isothiazolone. 3:1 [EC no. 220-239-6]	611-341-5	55965-84-9
polyhexamethylene; biguanide hydrochloride	608-723-9	32289-58-0

Allergy and Tattoos
With the increasing popularity of tattooing the immunological reactions from modern organic inks has become a new disease entity representing a public health issue. It has been suggested that some of these reactions may be due to contact allergy toward ingredients in the inks. It is known from several investigations that tattoo inks may contain contact allergens as metals, colorants and preservatives.

In this report, the National Allergy Research Centre, Denmark, review the present literature concerning adverse skin reactions in relation to tattoos.

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