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Chemical Substances in Tattoo Ink

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Chemical Substances in Tattoo Ink
Kemiske stoffer i tatoveringfarver

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Preface

The project ”Chemical Substances in Tattoo Ink” was carried out from September 2010 till December 2011.

This report describes the project results and also includes a survey, legislation, exposure scenarios, chemical analyses of selected tattoo inks and a health and risk assessment of selected compounds in tattoo ink as well as patient reactions.

The project was carried out by Danish Technological Institute in co-operation with Bispebjerg Hospital and the National Food Institute, Technical University of Denmark.

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The project was financed by the Danish Environmental Protection Agency.

The report reflects the author’s views and opinions, but not necessarily the views of the EPA

Summary and conclusions

In Denmark, the number of professional tattooists has increased and it is estimated that 13 % of the adult population, i.e. app. 600.000 persons, have a tattoo.

In 2002, the Danish Environmental Protection Agency carried out a project on the investigation of colorants in tattoo inks. The objective of this project is to illustrate which tattoo inks were used by professional Danish tattooists in 2010, analyse the content of selected substances and estimate the possible health-related risks connected with tattooing.

The survey of tattoo inks on the Danish market appears from Chapter 1. The objective of the survey was through interviews to identify which tattoo inks are used on the Danish market and to select products for analysis.

A total of nine tattooists were interviewed. The contacted tattooists informed which tattoo inks they use and specified the proportional distribution among their consumption of six main colours – black, red, blue, green, yellow and white. In addition, the tattooists described the distribution according to age and sex.

Apart from the contacted tattooists, Danish Tattooist Guild (Dansk Tatovør Laug[1]) informed which colour series/brands the members use.
Table 0.1 gives an outline of which colour series appeared during the survey. It also shows how many tattooists informed that they use the individual series. A tattooist typically uses colours from more than one series.

However, the information from the members of the Danish Tattooist Guild (from a total of 34 tattooists) only comprises one series per tattooist as they alone have informed about their preferred series, see Table 0-2.

Table 0.1 Outline of applied tattoo inks/series used by the interviewed tattooists.

Colour series	Number of tattooists
Starbrite	6
Micky Sharpz	4
Eternal	4
Classic	2
Alla prima	2
MOM's	2
Dermaglo	2
Dannys tattoo supplies	1
Silverback	1
Intenze	1
ONE	1
Dynamic	1

[1] Danish Tattooist Guild (Dansk Tatovør Laug (DTL)) has just under 50 members

Table 0.2 Outline of preferred tattoo ink series of members of the Danish Tattooist Guild

Colour series	Number of tattooists
Intenze	26
Micky Sharpz	6
Starbrite	1
MOM's	1

All interviewed tattooists informed that black is the predominant ink. Black is used to draw up the tattoo, for black/white tattoos and to make shadows. Most of the interviewed tattooists stated that they use red tattoo ink second-most.

Many people between 18 and 30 years of age get a tattoo; however, the tattooists stated that the customers represent all age groups – still, there are less customers in the age group over 50 years than under 50 years.

More and more women get a tattoo. Several tattooists informed that they have a small majority of female customers.

A total of 65 tattoo inks were purchased from 10 different colour series. Among them, the following number of tattoo inks were selected for the various analyses:

- 61 tattoo inks to be analysed for metals and other elements
- 5 tattoo inks to be analysed for carbon black
- 6 tattoo inks to be analysed for content of phthalocyanines
- 19 tattoo inks to be analysed for selected aromatic polycyclic aromatic hydrocarbons (PAH)
- 19 tattoo inks to be analysed for selected primary aromatic amines (PAA) liberated from azo colorants
- 30 tattoo inks to be analysed for p-phenylendiamin (PPD).

Danish legislation and European steps in the field appear from Chapter 2.

Today, no special regulation exists concerning which chemical substances may be used for tattoos. Tattoo inks are chemical products that are covered by the Product Safety Act as well as by REACH and the related restrictions on a number of chemical substances. In addition, the rules and regulations of the Executive Order on lead apply to chemical lead. Tattoo inks are not comprised by legislation on cosmetic products or on medicine.

Likewise, tattoo inks have to meet the regulations of labelling and classification that apply to all chemical products, including in relation to the content of CMR compound. That e.g. means that if a tattoo ink contains more than 0.1 % of a carcinogenic substance, then it must not be used for tattooing unless it has been reported to the the Danish Working Environment Service.

It was not possible to procure safety data sheets for 19 of the 65 purchased inks. In 10 cases there was not agreement between the pigments stated on the label and the pigments stated in the safety data sheet.

The Council of Europe is not part of the EU and their resolutions should be regarded as proposals. In resolution ResAP (2008)1 the Council of Europe stated specifications for tattoo inks, their acceptable composition, packaging, labelling, sterility etc. In addition, the resolution has a negative list of aromatic

amines that could be carcinogenic or mutagenic and a list of permitted metals and elements with stated max. permitted concentrations.

The exposure scenarios appear from Chapter 3, including how tattoo ink is entered into the skin.

In average, the tattoos cover 2.5 % of the skin surface corresponding to an area of 2½ palms per person.

When making a tattoo, the tattoo ink is transferred via an electric vibrating device from the skin surface into the skin where the pigment is deposited for a long time. After installation in the derma the pigment distributes itself as it partly trys to get out of the skin but is caught under epidermis, partly trys to move into the vessels especially into the lymphatics that drain to the lymph nodes. Tattoo pigment often exists in the lymph nodes and tattooing of the skin is indirectly a tattooing of the lymph node that drains the tattooed skin area. In the light of a study on mice, it is anticipated that a larger part of the tattoo ink in the initial phase after tattooing disappears out of the skin and is deposited in the lymph node or comes into systematic circulation and finally is deposited in other tissue, metabolised or released by the body. The initial phase is followed by several years of slow oozing of pigment out of the skin at the same time as the tattoo perhaps is visibly bleached. Distribution and elimination of tattoo pigment will differ according to which tattoo ink is used. Tests in rats have proven that small particles such as nanoparticles go directly into the bloodstream while larger particles are caught in the lymph nodes. On that basis, the systematic distribution of pigment and the exposure of other organs and thus the risk profile and elimination are dependent on the particle size of the pigment in addition to the chemical properties of the colorant. Many tattoo inks are nano-particulate.

The frequency of adverse events due to tattooing and actual skin complications has not been assessed. According to the medical literature, reports on dermatological complications are rather rare. Cases of transfer of infections, including HIV and hepatitis and special complications such as lichenoid reactions, pseudolymphoma and granulomatous and sarcoid reactions have been reported. Skin cancer has been reported in the form of basal cell carcinoma and malignant melanoma, but compared to the high frequency of skin cancer in the background population, cancer in a tattoo can be a simple statistic coincidence without the tattoo pigments being the reason why clinical skin cancer has developed. The effect of pigment being deposited in the lymph glands in close contact to the immune system and the blood-forming tissue has not been examined.

Chemical analyses, including method descriptions and results appear in chapter 4.

A wide range of different metals and other elements were demonstrated in 61 tattoo inks. No link was demonstrated between tattoo inks (colour) and content of certain elements. The results were compared with the recommendations of ResAP(2008)1[2] of the Council of Europe for max. content of As, Ba, Cd, Co, Hg, Ni, Pb, Se, Sb, Sn and Zn. Eight colours exceed the limit for barium, one colour exceeds the limit for cadmium, four

[2] Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up

colours exceed the limit for lead and one colour exceeds the limit for zinc. All colours contain nickel.

The carbon black content was determined in one grey and four black inks at 5.500 µg/g, 334.000 µg/g, 316.000 µg/g, 108.000 µg/g and 332.000 µg/g, respectively.

Phthalocyanines were demonstrated in blue, green and violet inks. The four colours with the highest copper content were used to calculate phthalocyanine Blue 15:3. The highest content of phthalocyanines was demonstrated in the blue colours followed by the green colours. The highest content was demonstrated in a blue colour, where the content of phthalocyanine Blue 15:3 was estimated to 189.000 µg/g.

Quantitative analyses for selected polycyclic aromatic hydrocarbons (PAH) demonstrated a content of PAH exceeding 0.5 µg/g in 14 of the 19 investigated tattoo inks. ResAP(2008)1 of the Council of Europe recommends a concentration of <0.5 µg/g (<0.5 ppm). The content of PAH varies with regard to concentration as well as to which PAH's are present. The highest concentrations were found in some black colours. Black colours with no content of PAH's were also found on the market.

p-Phenylendiamine (PPD) was not demonstrated in the 30 investigated tattoo inks.

A content of primary aromatic amines (PAA) was demonstrated in 20 of the 30 investigated tattoo inks. ResAP(2008)1 of the Council of Europe recommends no content of the demonstrated PAA. Five of the inks demonstrated a content of PAA that was so high that it might indicate that the five colours contain an azo pigment that can be decomposed to PAA. o-Anisidine was demonstrated in three of the inks. In the two other inks, aniline and 4-Methyl-m-phenylendiamine were demonstrated, but it was not possible within the framework of this project to identify the azo colorants that might give rise to these break-down products. Skin reactions were registered in connection with tattoo inks containing the three mentioned PAA. In the light of the results of the analysis of PAA it was not possible to conclude that certain colours contain specific PAA as the content in the colours differs a lot with regard to concentration as well as to which PAA have been demonstrated.

The health effect assessment (risk assessment) consists of a hazard assessment, an exposure assessment and a risk characterisation. In relation to tattooing where chemical substances in the tattoo inks are deposited in the skin, the health effect assessment is an assessment of whether a given chemical substance deposited in the skin is associated with a health risk. The health effect assessment includes local effects in the skin as well as systemic effects, i.e. effects that occur in tissues and organs in the body after absorption of the substance from the tattooed skin area.

Twenty-one substances / substance groups were selected for the health effect assessment: Eight elements, ten aromatic amines, carbon black, PAH and phthalocyanines.

The hazard assessment included an identification of the critical effect(s) in relation to tattooing and establishment of DNEL (Derived No Effect Level)

or DMEL (Derived Minimal Effect level) for the critical effect(s) if possible. The identification of the critical effect(s) was based on the EU classification of the selected substances according to Annex I of the Council Directive 1967/548/EEC and the IARC classification for carcinogenic effects when available, as well as on the critical effects identified in selected expert opinions from national and international bodies. The NOAELs / LOAELs were generally those presented in the selected expert opinions from national and international bodies.

Carcinogenicicity was considered as the critical effect in relation to tattooing for PAH and the ten selected PAA. For these two groups of substances (PAH and PAA) it is considered that there is no lower limit (threshold) for the carcinogenic effects and therefore, a DNEL cannot be established. For one of the PAH (benzo(a)pyrene) and for two of the PAA (aniline and o-anisidine), a DMEL has been established. For the remaining substances in these two groups, a DMEL could not be established based on the available data.

Sensitisation was considered as a critical effect in relation to tattooing for a number of the selected substances (aluminium, chromium, nickel, aniline, p-chloroaniline, 3,3'-dichlorobenzidine and 4-methyl-m-phenylenediamine). In the EU, these substances are, with the exception of aluminium, classified 'R43 - may cause sensitisation by skin contact' according to Annex I of the 67-Directive. For the selected substances, the available data are not sufficient for an evaluation of neither the potency nor the threshold value for sensitisation and therefore, a DNEL for sensitisation could not be established for these substances.

For a number of the selected substances, other effects than carcinogenicity and sensitisation were considered as the critical effect in relation to tattooing: Barium (effects on the cardiovascular system), lead (effects on the developing nervous system), cadmium (effects on bones and kidney) and phthalocyanines (decreased number of red blood cells). For barium, cadmium and phthalocyanines, a DNEL was established for the critical effect. For lead, a DNEL could not be established for the critical effect as a threshold for the critical effect (effects on the developing nervous system) has not been identified.

For some substances, (copper, titanium (titanium dioxide) and carbon black), a critical effect in relation to tattooing could not be identified.

In the risk characterisation, the outcome of the exposure assessment (exposure estimate) is compared with the outcome of the hazard assessment (DNEL/DMEL for the critical effect) and the so-called risk characterisation ratio (RCR) is calculated. RCR is thus the ratio between the exposure estimate and the DNEL/DMEL (RCR = exposure/DN(M)EL). If the exposure estimate is lower than the DNEL/DMEL, i.e. the RCR <1, the exposure is not considered to pose a risk for the given application. The DNEL / DMEL established for the critical (systemic) effects for the selected chemical substances in the analysed tattoo inks are generally expressed in the unit 'mg/kg bw per day'. Therefore, the exposure estimates also have to be expressed in the same unit for an RCR to be calculated.

In order to express the exposure in the form of a systemic dose expressed in the unit 'mg/kg bw per day', it is necessary to know how much of the substance deposited in the tattooed skin area following tattooing is

subsequently absorbed, i.e. to know the percentage of the deposited substance that is transported from the tattooed skin area to the tissues and organs in the body via the blood circulation and /or lymphatic system.

This project has revealed a number of limitations as well as lack of knowledge in order to evaluate the uptake and transport of substances from a tattooed skin area to the tissues and organs in the body. Consequently, it is not possible with currently available knowledge to perform a valid quantitative exposure assessment, i.e. to estimate the systemic dose of the selected chemical substances in the analysed tattoo inks. In addition, the project has also revealed limitations and lack of knowledge in relation to the hazard assessment (identification of critical effect in relation to tattooing and establishment of DNEL/DMEL for the critical effects) for a number of the selected chemical substances.

The limitations and the lack of knowledge in relation to the exposure assessment for the selected chemical substances / substance groups in the analysed tattoo inks, as well as in relation to the hazard assessment for a number of the selected substances, imply that a valid risk characterisation according to the REACH guidance documents (calculation of RCR) could not be performed.

Overall, based on the current knowledge, it could not be evaluated whether the selected chemical substances / substance groups would pose a health risk following tattooing with tattoo inks containing the selected chemical substances / substance groups as such, or containing other chemical substances (pigments, coformulants, chemical impurities) from which the selected chemical substances / substance groups might be released in the skin following tattooing.

It should be noted that several case studies have described adverse reactions among tattooed individuals who have been tattooed with several of the tattoo inks analysed in this project.

Reactions to tattoos comprise immediate reactions and delayed reactions and the clinical picture is rather manifold and not mono-morph. Therefore, the reactions cannot be related to one single chemical substance or type of substance, one single physical property or one single release mechanism.

That is confirmed by observing 8 cases. The pigments in the inks varied a lot – only one pigment, CI 77891, recurred in 2 cases, possibly by pure chance. The tattoo reaction could not be related to a certain pigment as characterised by the CI number from the manufacturer.

The allergy test carried out on persons with a general allergy test panel and with the tattoo ink that gave the particulate reaction had a negative result also with regard to nickel and chrome. That indicates that allergic mechanism is ordinary especially as the colours were concentrated, however, with the reservation that tattoo inks are particulate and possibly coated and therefore probably not suited for patch tests. Nickel or chrome allergy does not seem to have any importance.

However, the person with a very severe reaction in the form of wounds with necrosis in the skin in a red tattoo, had a serious reaction (3+ reaction) to the

patch test and analyses of PAA in the tattoo ink indicated that the red colorant was of the azo type. This case should be examined more closely.

The cases confirm that reactions in red ink or red ink mixtures are frequent and perhaps related to the content of azo colorant in some or other form that cannot be read from the stated CI number or the declaration of contents on the ink labels. The particulate form of the azo colorants, their possible coating and other systematic conditions concerning the pigments, currently unknown, can be especially important for the occurrence of a clinical reaction. Like local metablism of azo colorant in the skin and its elimination it could become the object of a future study.

These cases do not unambiguously point at one specific pigment that according to documentation can or should be eliminated from tattoo inks.

Sammenfatning og konklusioner

I Danmark er antallet af professionelle tatovører steget, og det anslås, at 13 % af den voksne befolkning, dvs. omkring 600.000 personer, er tatoverede.

Miljøstyrelsen gennemførte i 2002 et projekt om undersøgelse af farvestoffer i tatoveringsfarver. Nærværende projekt er igangsat med det formål at få belyst, hvilke tatoveringsfarver der i 2010 anvendtes af professionelle danske tatovører, analysere indholdet af udvalgte stoffer og vurdere den eventuelle sundhedsmæssige risiko ved tatovering.

Af kapitel 1 fremgår kortlægningen af tatoveringsfarver på det danske marked. Formålet med kortlægningen har været ved interviews at identificere, hvilke tatoveringsfarver der anvendes på det danske marked, og udvælge produkter til analyse.

I alt ni tatovører er interviewet. De kontaktede tatovører har oplyst, hvilke tatoveringsfarver de anvender og anslået den forholdsmæssige fordeling mellem deres forbrug af seks hovedfarver - sort, rød, blå, grøn, gul og hvid. Tatovørerne beskrev desuden fordeling af tatoverede på alder og køn.

Ud over de kontaktede tatovører er det via Dansk Tatovør Laug[3] oplyst, hvilke tatoveringsfarver/-serier medlemmerne anvender.

Af Table 0.1 fremgår en oversigt over, hvilke farveserier der er fremkommet ved kortlægningen. Endvidere fremgår, hvor mange tatovører der har oplyst, at de anvender de enkelte serier. En tatovør anvender typisk farver fra flere end en serie.

Oplysninger fra medlemmer af Dansk Tatovør Laug (fra i alt 34 tatovører) omfatter dog kun én serie pr. tatovør, idet disse alene har oplyst deres foretrukne serie, se Table 0.2.

Tabel 0.1 Oversigt over anvendte tatoveringsfarver/-serier anvendt af de interviewede tatovører

Farveserie	Antal tatovører
Starbrite	6
Micky Sharpz	4
Eternal	4
Classic	2
Alla prima	2
MOM's	2
Dermaglo	2
Dannys tattoo supplies	1
Silverback	1
Intenze	1
ONE	1
Dynamic	1

Tabel 0.2 Oversigt over foretrukne tatoveringsfarveserier for DTL-medlemmer

[3] Dansk Tatovør Laug (DTL) har knap 50 medlemmer

Farveserie	Antal tatovører
Intenze	26
Micky Sharpz	6
Starbrite	1
MOM's	1

Samtlige interviewede tatovører oplyser, at den farve, der langt overvejende anvendes, er sort. Sort anvendes til optegning af tatoveringen, til sort/hvid tatoveringer og til skygger. De fleste af de interviewede tatovører nævner rød som den næstmest anvendte tatoveringsfarve.

Der er mange mellem 18 og 30 år, der får foretaget tatoveringer, men tatovørerne oplyser, at kunderne repræsenterer alle aldersgrupper - dog færre i aldersgruppen over 50 år end under 50 år.

Flere og flere kvinder får udført tatoveringer. Flere tatovører oplyser, at de har en lille overvægt af kvindelige kunder.

Der blev indkøbt i alt 65 tatoveringsfarver fra 10 forskellige farveserier. Af disse blev der udvalgt følgende antal tatoveringsfarver til efterfølgende analyser:

- 61 tatoveringsfarver til analyse for metaller og andre grundstoffer
- 5 tatoveringsfarver til analyse for carbon black
- 6 tatoveringsfarver til analyse for indhold af phthalocyaniner
- 19 tatoveringsfarver til analyse for udvalgte polycykliske aromatiske hydrocarboner (PAH)
- 19 tatoveringsfarver til analyse for udvalgte primære aromatiske aminer (PAA) afspaltet fra azofarvestoffer
- 30 tatoveringsfarver til analyse for p-phenylendiamin (PPD).

Af kapitel 2 fremgår den danske lovgivning samt europæiske tiltag på området.

Der er i dag ingen særlig regulering af, hvilke kemiske stoffer der må anvendes til tatoveringer. Tatoveringsfarver er kemiske produkter, som er omfattet af både produktsikkerhedsloven og REACH og de dertil hørende begrænsninger på en række kemiske stoffer. Desuden gælder blybekendtgørelsens regler for kemisk bly. Tatoveringsfarver er ikke omfattet af lovgivningen om kosmetiske produkter eller om lægemidler.

Tatoveringsfarver skal ligeledes overholde reglerne om mærkning og klassificering, som gælder alle kemiske produkter, herunder i forhold til indholdet af CMR-stoffer. Det betyder fx, at hvis en tatoveringsfarve indeholder mere end 0,1 % af et kræftfremkaldende stof, må den ikke anvendes til tatovering, med mindre det er anmeldt til arbejdstilsynet.

For 19 ud af de 65 indkøbte farver kunne der ikke fremskaffes sikkerhedsdatatblade. I 10 tilfælde var der ikke overensstemmelse mellem de pigmenter, som var angivet på embalagen og de pigmenter, der var angivet i sikkerhedsdatabladet.

Europarådet, som ikke er en del af EU, og hvis resolutioner er at betragte som forslag, angav i resolutionen ResAp (2008)1 specifikationer for tatoveringsfarver, farvernes acceptable sammensætning, emballering, mærkning, sterilitet mv. Resolutionen indeholder desuden en negativliste over

aromatiske aminer, der kunne være carcinogene eller mutagene, samt en liste over tilladte metaller og sporstoffer med angiven maksimal tilladt koncentrationer.

Af kapitel 3 fremgår eksponeringsscenarier, herunder hvorledes tatoveringsfarven indføres i huden.

Tatoveringerne dækker i gennemsnit 2,5 % af hudoverfladen svarende til arealet af 2½ håndflade for en person.

Ved tatovering føres tatoveringsfarven med et elektrisk vibrerende apparat fra hudoverfladen ind i huden, hvor pigmentet langvarigt deponeres. Efter installation i læderhuden fordeler pigmentet sig, idet det dels søger ud af huden, men fanges under overhuden, dels søger ind i karrene og særligt lymfekarrene, der dræner til lymfeknuderne. I lymfeknuderne findes ofte tatoveringspigment, og tatovering af huden er indirekte en tatovering af lymfeknuden, der dræner det tatoverede hudområde. I initialfasen efter tatovering antages det ud fra studie i mus, at en større del af tatoveringsfarven forsvinder ud af huden og bliver deponeret i lymfeknuden eller kommer i systemisk cirkulation og sluttelig deponeres i andre væv, nedbrudt eller udskilt af kroppen. Initialfasen følges af en årelang fase med langsom siven af pigment ud af huden parallelt med, at tatoveringen evt. bleges synligt. Fordeling og elimination af tatoveringspigment vil være forskellig for de forskellige tatoveringsfarver. Forsøg i rotte har vist, at små partikler af typen nanopartikler går direkte over i blodstrømmen, mens større partikler fanges i lymfeknuder. Ud fra dette er den systemiske fordeling af pigment og eksponering af andre organer, og dermed risikoprofil og elimination, afhængig af partikelstørrelse af pigmentet ud over at være afhængig af de kemiske egenskaber af farvestoffet. Mange tatoveringsfarver er nanopartikulære.

Hyppigheden af gener ved tatovering og egentlig hud komplikationer er ikke opgjort. Ud fra den medicinske litteratur er raporter om dermatologiske komplikationer dog ret sjældne. Der er rapporteret overførsel af infektioner herunder HIV og hepatitis og specielle komplikcationer i form af lichenoide reaktioner, pseudolymfomer og granulomatøse og sarkoidale reaktioner. Der er rapporter hudcancer i form af basalcellecarcinomer og ondartet modermærke, men set i forhold til den høje hyppighed af hudcancer i baggrundsbefolkningen kan cancer i tatovering være et simplet statistisk sammenfald, uden at tatoveringspigmentet har forårsaget udvikling af klinisk hudcancer. Betydningen af pigment lejret i lymfekirtler i tæt kontakt med immunsystemet og det bloddannende væv er ikke undersøgt.

Af kapitel 4 fremgår kemiske analyser, herunder metodebeskrivelser og resultater.

Der er påvist en lang række forskellige metaller og andre grundstoffer i 61 tatoveringsfarver. Der er ikke påvist en sammenhæng mellem tatoveringsfarve (kulør) og indhold af bestemte grundstoffer. Der er foretaget en sammenligning af resultaterne med Europarådets ResAP(2008)1[4] anbefalinger for maksimale indhold af As, Ba, Cd, Co, Hg, Ni, Pb, Se, Sb, Sn og Zn. Otte farver overskrider grænsen for barium, en farve overskrider grænsen for cadmium, fire farver overskrider grænsen for bly, og en farve overskrider grænsen for zink. Alle farver indeholder nikkel.

[4] Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up

Carbon black-indholdet er bestemt i en grå og fire sorte farver til hhv. 5.500 µg/g, 334.000 µg/g, 316.000 µg/g, 108.000 µg/g og 332.000 µg/g.

Phtalocyaniner er påvist i blå, grønne og lilla farver. De fire farver med det højeste kobberindhold, blev brugt til beregning af phthalocyanine Blue 15:3. Der er påvist de højeste indhold af phthalocyaniner i de blå farver, efterfulgt af de grønne. Det højeste indhold er påvist i en blå farve, hvor indholdet af phthalocyanine Blue 15:3 er estimeret til 189.000 µg/g.

Ved kvantitativ analyse for udvalgte polycykliske aromatiske hydrocarboner (PAH) er der påvist indhold af PAH over 0,5 µg/g i 14 ud af de 19 undersøgte tatoveringsfarver, hvor Europarådets ResAP(2008)1 anbefaler koncentration på <0,5 µg/g (<0,5 ppm). Indholdet af PAH varierer både mht. koncentration og hvilke PAH'er, der er til stedet. De højeste koncentrationer er fundet i nogle sorte farver. Der er også fundet sorte farver på markedet, som ikke indeholder PAH'er.

Der er ikke påvist p-phenylendiamin (PPD) i de 30 tatoveringsfarver, der blev undersøgt herfor.

Der er påvist indhold af primære aromatiske aminer (PAA) i 20 ud af 30 undersøgte tatoveringsfarver. Europarådets ResAP(2008)1 anbefaler, at der ikke er indhold af de påviste PAA. For fem af farverne er der påvist et så højt indhold af PAA, at det kan tyde på, at disse fem farver indeholder et azofarvepigment, som kan nedbrydes til PAA. I tre af disse farver er der påvist o-anisidin. I de to andre farver er der påvist anilin og 4-Methyl-m-phenylendiamin, men det har ikke været muligt inden for dette projekts rammer at identificere de azofarver, som kan give anledning til disse nedbrydningsprodukter. Der er registreret reaktioner i huden ved tatoveringsfarver indeholdende de tre nævnte PAA. Det er ikke muligt ud fra resultaterne for analyse af PAA at konkludere, at bestemte farver indeholder specifikke PAA, da indholdet i farverne er meget forskellig både mht. koncentration og mht., hvilke PAA der er påvist.

Den sundhedsmæssige vurdering (risikovurdering) består af en farevurdering, en eksponeringsvurdering og en risikokarakterisering. I relation til tatovering, hvor kemiske stoffer i tatoveringsfarver deponeres i huden, er den sundhedsmæssige vurdering en vurdering af, hvorvidt et givent kemisk stof deponeret i huden er forbundet med en sundhedsmæssig risiko. Vurderingen omfatter lokale effekter i huden såvel som systemiske effekter, dvs. effekter, der opstår i kroppens væv og organer efter optagelse af stoffet fra det tatoverede hudområde.

Der er udvalgt 21 stoffer/stofgrupper til den sundhedsmæssige farevurdering: otte grundstoffer, 10 aromatiske aminer, carbon black, PAH og phthalocyaniner.

Farevurderingen dvs. identifikation af de(n) kritiske effekt(er) for de udvalgte stoffer er dels baseret på EU-klassificeringen af stofferne i henhold til Annex I i 67-Direktivet (Directive 67/548/EEC) såvel som IARC's klassificering for kræftfremkaldende effekt, dels på de kritiske effekter udpeget i udvalgte nationale og internationale ekspertvurderinger. NOAEL/LOAEL er generelt taget fra de udvalgte nationale og internationale ekspertvurderinger.

Kræftfremkaldende effekt er vurderet som den kritiske effekt i relation til tatovering for PAH samt de 10 udvalgte PAA. For disse to stofgrupper (PAH og PAA) er det vurderet, at der ikke er en nedre grænse (tærskel) for effekt, hvorfor der ikke kan fastlægges DNEL. For en enkelt PAH (benz(a)pyren) samt for to PAA (anilin og o-anisidin) er der angivet DMEL. For resten af stofferne har det ikke været muligt at angive eller fastlægge DMEL på baggrund af de tilgængelige data.

Sensibilisering er vurderet som en kritisk effekt i relation til tatovering for en række af de udvalgte stoffer (aluminium, chrom, nikkel, anilin, p-chloranilin, 3,3'-dichlorbenzidin og 4-methyl-m-phenylendiamin). Disse stoffer er, med undtagelse af aluminium, i EU klassificeret 'R43 – kan give overfølsomhed ved kontakt med huden'. For de udvalgte stoffer er datagrundlaget ikke tilstrækkeligt med henblik på vurdering af potens eller tærskelværdi, hvorfor der ikke kan fastsættes en DNEL for sensibilisering.

For flere af de udvalgte stoffer er andre effekter end kræftfremkaldende effekt og sensibilisering vurderet som den kritiske effekt i relation til tatovering: Barium (effekter på hjerte-karsystemet), bly (effekter på nervesystemet hos børn og det ufødte barn), cadmium (effekter på knogler og nyrer) og phthalocyaniner (effekt på røde blodlegemer). For barium, cadmium og phthalocyaner er der fastsat en DNEL. For bly kan der ikke fastsættes en DNEL, da en nedre tærskel for den kritiske effekt af bly (effekterne på nervesystemet hos børn og det ufødte barn) ikke er identificeret.

For enkelte stoffer (kobber, titanium (titandioxid) og carbon black) kunne eventuelle kritiske effekt(er) i relation til tatovering ikke identificeres, hvorfor der ikke kan fastsættes DNEL/DMEL for den eventuelle kritiske effekt.

Ved risikokarakteriseringen sammenholdes resultaterne af farevurderingen (DNEL eller DMEL) med resultaterne af eksponeringsvurderingen, idet den såkaldte risikokarakteriseringsratio (RCR) beregnes. RCR er således forholdet mellem den beregnede eksponering og den fastsatte DNEL eller DMEL. DNEL/DMEL fastsat for de kritiske (systemiske) effekter af de udvalgte kemiske stoffer i de analyserede tatoveringsfarver er udtrykt i enheden 'mg/kg legemsvægt per dag'. Den beregnede eksponering skal derfor udtrykkes i samme enhed, for at RCR kan beregnes.

For at kunne udtrykke eksponeringen i form af en systemisk dosis udtrykt i enheden 'mg/kg legemsvægt per dag' er det nødvendigt at vide, hvor meget af det stof, der deponeres i huden ved tatovering, som efterfølgende optages i kroppen. Viden om optagelsen af et givent stof fra det tatoverede hudområde er således en væsentlig del af den sundhedsmæssige vurdering af dette stof i relation til tatovering.

Dette projekt har afdækket, at der mangler viden med henblik på at kunne vurdere optagelse og transport af stoffer fra tatoverede hudområder til kroppens væv og organer. Dette betyder, at det ikke er muligt med den nuværende viden at foretage en regelret kvantitativ eksponeringsvurdering, dvs. beregne en systemisk eksponering for de udvalgte kemiske stoffer i de analyserede tatoveringsfarver. Hertil kommer, at der også mangler viden i relation til farevurderingen for en række af de udvalgte kemiske stoffer. Den manglende viden i relation til eksponeringsvurderingen for de udvalgte kemiske stoffer i de analyserede tatoveringsfarver såvel som i relation til farevurderingen for en række af de udvalgte stoffer betyder, at det ikke har

været muligt at foretage en regelret risikokarakterisering i henhold til REACH vejledningerne, dvs. beregning af RCR.

På baggrund af den nuværende viden kan det således ikke vurderes, hvorvidt de udvalgte kemiske stoffer /stofgrupper vil kunne udgøre en sundhedsmæssig risiko ved tatovering med tatoveringsfarver, der indeholder de udvalgte stoffer som sådan eller indeholder andre kemiske forbindelser, hvorfra de udvalgte stoffer kan frigives i huden efter tatovering.

Det skal dog bemærkes, at der er flere cases, der beskriver patientreaktioner efter tatovering med flere af de analyserede tatoveringsfarver.

Reaktioner på tatoveringer omfatter både straks-reaktioner og sen-reaktioner, og det kliniske billede er relativt mangfoldigt og altså ikke mono-morft. Dette taler for, at reaktionerne ikke kan relateres til et enkelt kemisk stof eller type af stof, en enkelt fysisk egenskab eller en enkelt udløsningsmekanisme.

Dette bekræftes af observationer i otte cases. Pigmenterne i farverne udviste stor variation – kun et pigment, CI 77891, gik igen i 2 cases, muligvis en tilfældighed. Tatoveringsreaktion kunne ikke relateres til et bestemt pigment som karakteriseret ved det af producenten angivne CI-nummer.

Allergitest (laptest) af personerne med et generelt allergitestpanel og med tatoveringsfarven, der gav den enkelte reaktion, faldt negativt ud, også med hensyn til nikkel og krom. Dette taler mod, at allergisk mekanisme er almindelig, navnlig idet farverne blev anlagt koncentreret, dog med den reservation, at tatoveringsfarver er partikulære og evt. coatede og dermed ikke sikkert egnede til laptest. Nikkel eller kromallergi synes ikke at have betydning.

Personen med særlig kraftig reaktion i form af sår med nekrose i huden i rød tatovering reagerede derimod kraftigt (3+ reaktion) ved laptest, og analyser af PAA i tatoveringsfarven indikerede, at det røde farvestof var af typen azofarvestof. Dette casus er et index casus egnet til dybere studium.

Cases bekræfter, at reaktion i rød farve eller røde blandingsfarver er hyppig og muligvis relateret til indhold af azofarvestoffer under en eller anden form, der ikke kan aflæses ud fra det angivne CI-nummer eller indholdsdeklaration på farvernes emballage. Azofarvernes partikulære form, deres evt. coating og andre systematiske forhold omkring disse pigmenter, p.t. ukendte, kan være særligt betydningsfulde for, at klinisk reaktion opstår. Det kan ligesom den lokale omsætning af azofarvestof i huden og eliminationen gøres til genstand for fremtidigt studie.

Casene peger ikke entydigt på et bestemt pigment, der på et dokumenteret grundlag kan eller bør søges elimineret fra tatoveringsfarver.

1 Survey

An increasing number of people get a tattoo[5]. All of the tattooists who were contacted in connection with this project concordantly informed that recent years have demonstrated an increase in the number of tattoos that have been made as well as in the number of tattooists. According to the tattooists who were contacted, not only the number of professional tattooists has increased. The number of amateur tattooists, the so-called "kitchen table tattooists", has also increased. In 2010, it was estimated that 13% of the Danish adult population, i.e. around 600,000 people, has one or more permanent tattoos (also refer to chapter 3 under exposure scenarios).

1.1 Objective of the survey

The objective of the survey was to:

- Identify which tattoo inks are used on the Danish market
- Select and purchase products for chemical analyses.

A short description of the related legislation appears in chapter 2, and section 1.5 gives an outline of previously completed literature surveys (with focus on Germany, Switzerland and Sweden).

Finally, an assessment has been carried out of the labelling of the products on the basis of whether or not the compounds in each purchased product are recorded in Regulation no. 1272/2008 on Classification, Enclosure 6, part 3. The results of the assessment appear in section 1.8.

1.2 Delimitation and definitions

1.2.1 Delimitation

The survey only covers tattoo inks used by professional tattooists. The tattoos are made according to traditional methods where ink is injected into the skin by means of a needle.

The survey does not cover ink used for semi-cosmetic tattoos.

Ink for non-permanent tattoos that are painted on the skin and that usually disappear after a short period of time ("henna tattoos") do not form part of the investigation and neither do transferable "tattoos", (frequently used by children) where a picture is transferred to the skin.

[5] www.berlingske.dk/danmark/tatoverings-boom-blandt-unge-danskere
www.politiken.dk/tjek/sundhedogmotion/levevis/561318/tatoveringer-er-blevet-allemandseje/Lokalavisen

1.2.2 Definitions

There are different types of tattoos:

- ***Traditional, permanent tattoos*** made as self-imposed writing, symbol or motif or made as permanent make-up where e.g. the eyebrows or lip contours are permanently tattooed by injecting tattoo ink intradermally (please also refer to the construction of the skin in section 3.3.). Made by traditional tattooists.
- ***Semi-permanent tattoos***, also called cosmetic tattoos where e.g. eyebrows or lip contours are tattooed with ink that is intended to disappear after some months. The ink is installed in the derma by means of the same technique. Most frequently carried out by a cosmetologist.
- ***"Henna" tattoos.*** A traditional tattoo motif is painted with a brush on the surface of the skin and it simulates a permanent tattoo. The ink is exfoliated together with the spontaneous renewal of epidermis in a few weeks and the ink does not go as far down as the derma. Most frequently carried out at tourist resorts.
- ***Medical tattoos*** made within the framework of the health service, e.g. as marker tattoo of a radiation field or as recreation of pigmentation around the nipple after cancer surgery. Made in the same way as a permanent tattoo by using the same technique and the same type of ink as for a permanent tattoo. Made by doctors or professional tattooists at the doctor's risk.
- ***Tattoo stickers*** are tattoo images that stick on to the skin as a transfer picture. Typically used by children and purchased in toyshops.

Tattoo ink is a product that is ready for use and used to make a tattoo, be it a commercial product or a primitive ink.

Pigments are micro or nano particulate elements (crystals or particles), typically constructed from a chemical single substance (colorant) that in molecular form can be either dyed or undyed. It is the pigment that gives a certain colour to the products sold as tattoo inks and it makes the tattoo permanent.

Dissolvent, the so-called dispergent, is the medium in which tattoo inks or pigments are dissolved or suspended and they condition the liquid consistency of the tattoo ink.

Additives are chemical substances added to the tattoo ink and their specific purpose is e.g. to preserve or adjust the consistency or tixotropy.

Coating agents are chemical substances used for surface treatment of the pigments that form part of the tattoo ink.

Contaminants are chemical subtances of any kind or micro organisms that unintentionally appear in the finished tattoo ink.

This report solely deals with permanent tattoos made by professional tattooists and commercial tattoo inks purchased as finished products.

1.3 Procedure

The survey was carried out in September 2010.

In order to outline which tattoo inks currently are used by tattooists in Denmark a number of professional tattooists were contacted – either by telephone or at a personal meeting. The tattooists were asked which colour series they use and how the consumption is distributed on each individual colour (with weight on the main colours black, red, blue, green, yellow and white).

A survey of tattoo colours has previously carried out: "Danish Environmental Protection Agency – Survey no. 2, 2002, Investigation of colorants in tattoo inks". A brief description of the results of the report appears in section 1.4.

In addition, the results from other studies appear in section 1.5. Information from the studies serve as background for the choice of products that were analysed in this report and for the choice of analysis programme.

In connection with all substances mentioned in the procured safety data sheets, a search was carried out on the CAS no. stated in the list of hazardous substances that can be found on the homepage of the Danish Environmental Protection Agency and the result of the search was compared with the label on the packaging and/or with the data safety sheet[6].

1.4 Results of survey no. 2, 2002

The objective of the investigation behind report no. 2, 2002 from the Danish Environmental Protection Agency – "Investigation of colorants in tattoo inks" - was to identify the pigments that at that time were used in tattoo inks on the Danish market. The below is based on the content of that report.

It was the objective of the investigation to clarify which trades use "pigment colours" and to investigate which of the colours already had been assessed in other connections.

During the investigation, traditional tattooists as well as cosmetic tattooists were contacted in order to identify suppliers of tattoo inks that are used on the Danish market. Subsequently, the suppliers were contacted for identification of the pigments found in the tattoo inks.

Concerning the contact with traditional tattooists, the investigation showed that all contacted tattooists (apart from one who made his own tattoo inks) used tattoo inks that were made abroad.

The investigation showed a high degree of uniformity in the selection of suppliers as the majority of the contacted tattooists mainly used tattoo inks from one and the same supplier.

[6] http://www.mst.dk/Virksomhed_og_myndighed/Kemikalier/Stoflister+og+databaser/Listen+over+farlige+stoffer/Søgning+i+farlige+stoffer.htm

A total of 17 pigments were identified in the products about which information had been obtained during the investigation. The pigments were generally used, industrial pigments and they did not differ from what was used in other trades. Inorganic pigments in the form of iron oxide or titanium dioxide as well as organic pigments such as azo, phtalocyanine, acridine and naphthol, besides carbon black were used. An outline of the identified pigments appears in Enclosure A.

In the investigation from 2002, no assessment was carried out of the health related risks of using the found pigments for tattooing purposes.

During the investigation, 53 tattooists were registered in Denmark. Through interviews, the following suppliers of tattoo inks were identified:

- Custom Tattoo Suppliers
- Davis's Tattooing Supplies
- Dermagraphics manufacturing & Supply Inc.
- Dynamic Color Co.
- Huck Spaulding Enterprice Inc.
- Mickey Sharpz Supplies Limitid
- National Tattoo Supply Inc.
- Robinson & Dixon
- Skin & Colors tattoo product
- Tattoo InKorporated Ltd.
- Tattoo-Shop.

In addition, it appears from the report that a widespread use of black ink took place, though black ink is intended for and marketed for drawing and writing purposes and though the suppliers specifically stress that the products are not recommended for tattooing.

1.5 Results from other studies

1.5.1 General observations

Commercial tattoo inks consist of dry matter in the form of one or more pigments that are crystals or grains in the size of 20-900 nanometer and of a liquid carrier fluid that consists of i.a. water, alcohol and glycerine. In addition, they consist of various additives such as preservatives and viscosity creating substances.

Most frequently, the pigments are coloured because of their crystal or grain structure. If they are broken down to chemical molecules they become colourless and if they are metabolised in the skin, e.g. under the influence of light, the tattoo will fade.

The pigments are industrial pigments from international suppliers and their main sales are to the lacquer industry, leather industry, textile industry and others.

As the tattoo inks are supposed to be lasting, i.e. permanent in the user situation, they are in general hardly soluable and as mentioned in crystalline form.

One study demonstrated that the colorant in a red tattoo ink had a low chemical degree of purity, app. 80 %. That might be typical for tattoo inks, as the raw material is industrially produced and the purity control has not been documented. Therefore, an unknown amount of related or unrelated chemical compounds might exist in a tattoo ink in addition to the declared colorant stated with a CAS number or a Colour index number[7].

A number of tattoo inks are secondary colours with several pigments and titanium dioxide is often used as lightener. It is visible that secondary colours can segment on standing. The dry matter content in tattoo ink is in average 47 % calculated as the weight percentage (span 31-62 %)[8].

The carrier fluid can contain preservatives and substances that contribute with viscosity and tixotropy as well as inorganic and organic residue from production.

Substances apart from the pigment are normally not stated on the label or in the data sheet of the product and therefore they are unknown although they can form a substantial part of the dry matter content of a colour.

In addition to the chemical substance, the way crystallization takes place is vital to the shape and size of the crystals and to a great extent also to the colour. The crystal shapes can vary for a given chemical substance. It is unknown to which degree the crystals/particles have been treated with a coating agent.

It has not been possible to find out which additives are included in the production of tattoo inks and which contaminents might be provided through the production process. Finally, it is unknown under which sterility conditions the production and further processing of commercial tattoo inks take place.

In recent decades, the composition of tattoo inks has undergone considerable changes and is now dominated by synthetic, chemical substances such as azo colorants and thalocyanines[9].

A widespread use of titanium dioxyde and coal still takes place in the shape of carbon black, while colours such as ochre (iron oxides mixed with clay), mercury, chrome, cadmium and manganese are suppressed.

1.5.2 Specific investigations

A German study from 1988 defined 41 commercial tattoo inks[10] and demonstated a content of substances such as cadmium, chrome, cobalt, mercury and aluminium besides titanium dioxide (anastase and rutile form) and organic colorants.

Another German study from 2000 of 41 tattoo inks demonstrated a content of 16 different synthetic substances within the groups of azo colorants,

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[7] Engel, modern tat cause, contact dermatitis 2007
[8] Bispebjerg Hospital, unpublished data
[9] Schmidt H. Tatoveringer. Kulturhistoriske, kunstneriske og medicinske aspekter. Løvens Kemiske Fabrik 1967; Nordstrøm J. Dansk Tatovering. Nordstrom, 2009. ISBN 978-87-993150-0-0
[10] Lehmann G et Pierchalla P. Tätovierungsfarbstoffe. Derm Beruf Umvelt 1988;36:152-56

thalocyanines and others, and titanium dioxide was found as lightener, both in anastase and rutile crystaline form[11]. The chemical analysis was difficult due to the poor solubility of the substances. The crystal size varied from between 20 and 900 nanometers, i.e. with content of nanoparticles. The investigation did not comprise black pigments.

An American study from 2001 of 29 tattoo inks from the supplier Huck Spaulding Enterprises Inc. and of China ink (carbon black) especially investigated the content of inorganic elements by means of the radio diffraction method that determines all substances in the sample with an atomic weight above 11[12].

The following content was found in the samples: aluminium 87 %, oxygen/oxides 73 %, titanium 67 %, chlorine 29 %, iron 15 %, silicium 12 %, copper 12 % and substances such as magnesium and sulphur in less than 10 % of the samples. Chrome was found in one single sample. Cadmium, cobalt, mercury and lead were ***not*** found during the analysis.

In another American study carried out by FDA in 2004, 7 yellow tattoo inks from commercial suppliers were analysed. The monoazo pigment Yellow 74 appeared frequently. The pigment was subject to a light chemical change when exposed to light, including sunlight of photodecomposition with the creation of a number of new chemical substances[13].

In an Italian study from 2009, 56 samples of tattoo ink from 4 suppliers, including the colours called Starbrite, were analysed for content of metals[14].

The dominating metals were aluminium, barium, copper and iron. Metals such as cadmium, manganese, lead, antimony (Sb) and vanadium exceeded 1 µg/g. Metals that can cause allergy at a defined concentration exceeding 1 µg/g were found: chrome, nickel and cobalt in 63, 16 and 2 %, respectively, of the samples - chrome having the highest concentration.

In a German study from 2009, carried out by Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (carried out in the light of the implementation of the regulation in the field of tattooing in Germany that came into effect on 1 May 2009), the metal content and preservative content was investigated in 148 commercial tattoo inks. The samples showed a large content of copper, iron, chrome and zinc and a smaller amount of tin, lead, manganese, selenium, arsenic, thallium, mercury and uranium[15].

6.7 % of the samples showed a content of preservative (23 substances were tested). The dominating preservatives were benzoic acid, benzoisothiazolone, metylisothiazoline and octylthiazolone.

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[11] Baumler W et al. Q-switch laser and tattoo pigments: first results of the chemical and photophysical analysis of 41 compounds. Lasers in Surgery and Medicine 2000;26:13-21

[12] Anthony L et al. In vitro quantitative chemical analysis of tattoo pigments. Arch Dermatol 2001;137:143-47

[13] Cui Y et al. Photodecomposition of pigment yellow 74, a pigment used in tattoo inks. Photochemistry and Photobiology 2004;80:175-84

[14] Forte G et al. Market survey on toxic metals contained in tattoo inks. Science of the Total Environment 2009; 407:5997-6002

[15] www.aktionsplan-allergien.de

In a report from the Swiss Federal Office of Public Health (FOPH) from 2009, 152 tattoo ink samples were collected by inspectors from 55 tattooists and they were analysed and assessed with regard to compounds, preservation and microbiology, and only 36 % of the samples were acceptable according to the Swiss standard for compounds in tattoo inks, while a large number were criticizable or subject to bans, most frequently due to content of non-permitted type or amount of pigment or preservative[16].
As of 1 January 2006, with a transition period of 2 years, Switzerland introduced the rules for tattoo inks according to the RESAP 2003 recommendation of the Council of Europe in a modified version.

Literature after year 2000 demonstrates the following:

In the chemical determination of the content of tattoo inks, synthetic pigments such as azo colorants and pthalocyanines are in the lead together with carbon black and titanium dioxide, both in anastase and rutile form, the two known crystals of titanium dioxide.

Ferrous pigment still plays a part, but certain older types of pigment such as cadmium and mercury seem displaced. Aluminium exists in many colours for tattooing and many tracers also exist.

Potentially sensitizing metals, especially chrome, still form part of the colours.

Few tattoo inks contain preservatives.

Due to physical and chemical complexity, including crystalline form and poor solubility of the chemical compounds, the tattoo inks are difficult to define analytically.

1.6 Result of survey – interviews 2010

A total of nine tattooists were contacted and interviewed. Some at a personal meeting and others by telephone.

All of the contacted tattooists were helpful and favourably disposed towards the actual investigation and in addition they were interested in getting the applied tattoo inks analysed. The contacted tattooists were all preoccupied with ensuring that the colours they use do not cause allergy, allergic reactions or any other adverse events to their customers.

The contacted tattooists have informed which colour series/brands/suppliers they use and they have estimated the proportional distribution between their consumption of 6 main colours – black, red, blue, green, yellow and white. They also informed the distribution by age and sex of the tattooed persons.

In addition to the contacted tattooists, Danish Tattooist Guild (***Dansk Tatovør Laug***)[17] has informed which colour series/brands the members use.

The contacted tattooists informed that they had very few or no cases of allergy/ allergic reactions among their customers. One of the interviewed

[16] www.foph-report_tattoo-colours_control-campain
[17] Danish Tattooist Guild, (Dansk Tatovør Laug, DTL) has almost 50 members

tattooists informed that he had experienced a total of six allergic reactions in the course of 20 years and all cases were connected with the use of red ink.

Another tattooist informed that he had seen some cases of severe allergic reactions when a specific red colour had been used.

1.6.1 Applied tattoo inks

Table 1.1 gives an outline of which colour series appeared from the survey. In addition, it appears how many tattooists use the relevant series. A tattoist typically uses ink from more than one series.

However, information from the members of the Danish Tattooist Guild, DTL, (a total of 34 tattooists) only comprises one series per tattooist as they solely have informed their preferred series, see Table 1.2.

Table 1.1 Outline of applied tattoo ink series used by the interviewed tattooists

Colour series	Number of tattooists
Starbrite	6
Micky Sharpz	4
Eternal	4
Classic	2
Alla Prima	2
MOM's	2
Dermaglo	2
Dannys Tattoo Supplies	1
Silverback	1
Intenze	1
ONE	1
Dynamic	1

Table 1.2 Outline of tattoo ink series preferred by DTL members

Colour series	Number of tattooists
Intenze	26
Micky Sharpz	6
Starbrite	1
MOM's	1

Some of the tattooe inks identified in this investigation were also registered in the investigation from 2002 (see section 1.4).

The tattoo inks that are used in Denmark are predominantly imported, finished products that are manufactured abroad. The tattooists often purchase the inks directly from a foreign supplier via the internet.

An attempt was made to localise the manufacturers of tattoo inks through searches on the suppliers' homepages and through direct contact to the suppliers. An attempt was made to localise the manufacturers in order to procure safety data sheets when that had not been possible during contact with the suppliers.

1.6.2 Distribution of applied inks

All interviewed tattooists informed that black is the predominant ink. Black is used to draw up the tattoo, for black/white tattoos and to make shadows. Some tattooists informed that they use between two and four times as much black tattoo ink as the other colours and other tattooists inform that they use up to 10 times as much black as the other colours.

Most of the interviewed tattooists stated red to be the tattoo ink they use second-most. Subsequently, it is individual if blue or green is used the most. Yellow was not mentioned at all by the interviewed tattooists, but white is used to a certain degree i.a. to mix shades of the other applied colours.

1.6.3 Tattoos – age and sex

Several of the interviewed tattooists informed that quite a few young people want a tattoo as soon as they turn 18. Many people between 18 and 30 get a tattoo; however, the tattooists state that the customers represent all age groups – however, there are less customers in the age group exceeding 50 years than under 50 years.

More and more women get a tattoo. Several tattooists informed that they have a small majority of female customers. Women often choose tattoos that are smaller and they are made in places that easily can be covered up. However, an increasing amount of women get large tattoos – e.g. covering the entire back.

The first tattoo is often limited in size and located somewhere that is not too visible.

Currently, sleeve tattoos are very popular among men. It takes between 25 and 100 hours to get a tattoo that covers most of the arm depending on how detailed it is.

1.7 Purchased products

Table 1.3 shows the products that were selected for purchase.

The tattoo inks were purchased from European suppliers. At first, some foreign suppliers refused to sell their products to customers abroad, which caused a long time of delivery because the suppliers first of all had to obtain permission to sell the products. Other suppliers refused to sell products to non-professional tattooists.

When purchasing the inks, safety data sheets concerning the purchased inks were requested. However, not all foreign suppliers and manufacturers responded to the inquiry for safety data sheets though a reminder had been sent. Therefore, it has not been possible to procure safety data sheets for all tattoo inks. That is why we additionally have looked for safety data sheets on the internet, e.g. on www.painfulpleasures.com.

Some safety data sheets do not supply all the desired information, e.g. the tattoo colorant or there is no information about the concentration of the pigments. In the light of our contact with tattooists it is our impression that tattooists normally are not acquainted with or do not ask for safety data sheets.

A total of 65 tattoo inks were purchased from 10 different colour series distributed as follows:

- 11 black
- 12 red
- 10 green
- 8 blue

- 6 white
- 8 yellow
- 3 orange
- 3 peach
- 3 violet
- 1 brown

The purchased colour series and inks were selected according to the following:

- The four series that most tattooists use, see Table 1.1 and Table 1.2.
- A majority of black and red inks were purchased as they according to the interviews that were carried out are the inks that are used most frequently.
- During the interviews, the tattooists mentioned some specific shades within the colours black, red, blue, green, yellow, white, peach and orange which they use a lot. A number of those colours were purchased.
- A black colour was chosen as it according to a supplier is sold to tattooists though it is specified on the internet that it is not tattoo ink.
- Bispebjerg Hospital, Department of Dermatology, has registered a number of tattoo inks that are related to reactions in the skin. In addition, descriptions have been found on the internet concerning reactions in the skin from two specific colours and one tattooist has also mentioned one specific colour. It has been possible to identify nine of the colours and to purchase them from suppliers of tattoo ink. The nine purchased colours are marked with ”*” at ”Colour no.” in Table 1.3. See detailed description of tattoo inks registered in connection with skin reactions in Chapter 5.

In Enclosure B, there is a table showing the purchased tattoo inks organised according to colour, stating which pigments they contain. The information about the pigments are copies of the information on the label and on the safety data sheets. Concordance does not exist in all cases between the pigments stated on the label and the pigments stated in the safety data sheets.

Most pigments in Enclosure B belong to one of the following pigment groups: azo colorants, phthalocyanines, acridines, carbon black or inorganic pigment (titanium dioxide).

The reason why certain colours were selected for analysis appears from section 1.10.

Table 1.3 Purchased tattoo inks

Ink no.	Colour	Safety data sheet	Caption on label
1	Red	No	Synthetic pigment, anionic surfactant, non-ionic sufatant, humectants, preservatives
2	Black	No	Synthetic pigment, anionic surfactant, non-ionic sufatant, humectant, preservatives
3	Black	General safety data sheet for the series	CI# 77266, Pigment, glycerine, alcohol, preservative
4	White	**	CI# 77891, Pigment, glycerine, alcohol, preservative
5	Red	**	CI# 12475, Pigment, glycerine, alcohol, Isopropanol, polyether, anti foam

Ink no.	Colour	Safety data sheet	Caption on label
6	Pale green	**	CI# 77891 CI# 74260 Pigment, glycerine, alcohol, preservative
7	Dark green	**	CI# 77891 CI# 74160 CI# 13980 CI# 21108, Pigment, glycerine, alcohol, preservative
8	Blue	**	CI# 77891 CI# 74160, Pigment, glycerine, alcohol, preservative
9	Yellow	**	CI# 21160 CI# 21108, CI#13980 Pigment, glycerine, alcohol, preservative
10	Grey	**	Pigment number: 77266 Pigment, glycerine, alcohol, preservative
11	Black	Yes	CI# 77226, Alcohol, glycerine
12	Black	No	CI# 77226, Alcohol, glycerine
13	Dark green	Yes	CI# 74260, Alcohol, glycerine
14	White	Yes	CI# 77891, Alcohol, glycerine
15	Blue	Yes	CI# 77891 CI# 74160, Alcohol, glycerine
16	Pale green	Yes	CI# 11741 CI# 74260, Alcohol, glycerine
17	Red	Yes	CI# 12477, Alcohol, glycerine
18*	Red	Yes	CI# 12390, Alcohol, glycerine
19	Yellow	Yes	CI# 11741, Alcohol, glycerine
20	Orange	No	CI# 12477 CI# 77491, Glycol, rubbing alcohol 99 %
21	Peach	No	CI# 12477 CI# 77891, CI# 11741 Glycol, rubbing alcohol 99 %
22	White	Yes	CI# 77891, glycerin, isopropanol
23	Black	Yes	CI# 77226, glycerin, isopropanol
24*	Red	Yes	CI# 73915 CI# 21110, CI# 77891 CI# 12477, glycerin, isopropanol
25	Blue	Yes	CI# 77891 CI# 74160, Glycerine, witch hazel
26	Pale green	Yes	CI# 11740 CI# 74160, CI#11740 CI# 77891, glycerin, isopropanol
27	Yellow	Yes	CI# 11740 CI# 77891 Proprietary, glycerin, isopropanol
28	Orange	Yes	CI# 11740 CI# 77891, CI#21110 glycerin, isopropanol
29	Peach	Yes	CI# 77891 CI# 73915, CI# 21110 Glycerine, witch hazel / isopropanol, glycerine
30	Black	No	
31	Dark green	No	CI# 77266 CI# 12490, Glycerin, isopropyl
32	Blue	No	CI# 77266 CI# 77891, CI#12485 CI# 74260, Glycerin, isopropyl
33	Red	No	CI# 12485 CI# 12490, CI# 77266 Glycerin, isopropyl
34	Red	Yes	CI# 12477 CI# 11740, CI#21110 glycerin, isopropanol
35*	Violet	Yes	CI# 73900, glycerin, isopropanol
36*	Yellow	Yes	CI# 21108 CI# 77891 Alcohol, glycerine
37*	Violet	No	CI# 15880 CI# 74160, CI# 77891 CI#74260, Alcohol, glycerine
38	Blue	No	CI# 74160 CI# 77891 Isopropyl, alcohol
39	Red	No	CI# 12475 CI# 77891 Isopropyl, alcohol
40	Yellow	No	CI# 11741 CI# 77891 Isopropyl, alcohol
41	Green	No	CI# 74260 CI# 21110 CI# 77891 Isopropyl, alcohol
42	Black	No	CI# 77266, Isopropyl, alcohol

Ink no.	Colour	Safety data sheet	Caption on label
43	Black	General safety data sheet for series with no specification of content	
44	Pale green	***	
45	Blue	***	
46	White	***	
47	Yellow	***	
48*	Red	***	
49*	Red	***	
50	Violet	***	
51	Black	No	
52	White	No	
53*	Red	No	
54	Yellow	No	
55	Green	No	
56	Blue	No	
57*	Brown	No	
58	Black	Yes	Glycerine CI# 77226
59	White	Yes	Glyserine CI# 77891
60	Green	Yes	Glycerine CI# 12075, 77891, 77226, 21095
61	Yellow	Yes	Glycerine CI# 21095, 12075, 77891
62	Blue	Yes	Glycerine CI# 74260, 77891, 74160
63	Red	Yes	Glycerine CI# 12475
64	Peach	Yes	Glycerine, alcohol CI# 77891, 12075,
65	Orange	Yes	Glycerin, alcohol CH# 21160, 20195, 12475

* Tattoo ink that is registered in connection with skin reactions
** See text for ink no. 3
*** See text for ink no. 43

1.8 Executive Order on cosmetics

On the basis of the information about the CAS no. from the data sheets or the text on the labels it has been investigated if the tattoo colorants (pigments) are stated as being permitted or not permitted in the Executive Order on Cosmetics, Executive Order no. 422 of 4 May 2006, see Table 1.4.

Pigment Orange 5 is not permitted and is stated in the safety data sheet of three tattoo inks from the same colour series (colour no. 60, 61 and 64). Pigments belonging to the application area 4 are colorants that solely are permitted in cosmetic products intended for brief skin contact only. The matter concerns Pigment Yellow 83 (ink no. 7, 26 and 36), Pigment Red 122 (ink no. 24, 29 and 35) and Pigment Violet 19 (ink no. 35) – the stated ink numbers belong to three different colour series. Ink no. 24, 35, 36 are registered in connection with skin reactions, see chapter 6.

No information exists about the concentration of these pigments in the safety data sheets or in the text on the label. Determination of the concentration of these pigments does not form part of this project.

Table 1.4 Pigments found in the executive order on cosmetics

Pigment name	CAS no.	Used in colour	Permitted/not permitted	Areas of application	Type of pigment
Tianium dioxide	13463-67-7	White, green, red, blue, yellow, orange, violet	Permitted	1	Inorganic pigment
Pigment Green 7 / Phthalocyanine Green 7	1328-53-6	Green, blue, violet	Permitted	2	Phthalocyanine
Phthalocyanine Blue 15:3 / Pigment Blue 15	147-14-8	Green, blue, violet	Permitted	1	Phthalocyanine
Pigment Yellow 83	5567-15-7	Green, yellow	Permitted	4	Azo colorant
Pigment black 7	1333-86-4	Green, red, blue, black	Permitted	1	Carbon black
Pigment Red 5	6410-41-9	Green, red	Allowed	1	Azo colorant
Pigment Orange 5	3468-63-1	Green, yellow, peach	Not permitted		Azo colorant
Pigment red 122	980-26-7	Red, peach, violet	Permitted	4	Acridine
Pigment Red 101	CI# 77491	Orange	Permitted	1	Inorganic pigment
Pigment Violet 19	1047-16-1	Violet	Permitted	4	Acridine
Pigment Red 63:1	6417-83-0	Violet	Permitted	1	Azo colorant

Explanation of Table 1.4, Areas of Application:

1. Colorants permitted in all cosmetic products.
2. Colorants permitted in all cosmetic products with the exception of cosmetic products to be used around the eyes, especially eye makeup and cleansers for that purpose.
3. Colorants solely permitted in cosmetic products that are not intended for contact with the mucous membranes.
4. Colorants solely permitted in cosmetic products intended for brief skin contact.

1.9 Classification Order no. 1272/2008

A search was carried out on the CAS no. referred to in the list of dangerous substances on all substances stated in the safety data sheets or on the labels.[18] All pigments with a CAS no. are shown in Enclosure B.
All in all, only one substance, classified in the list of dangerous substances, was found. The matter concerns isopropyl alcohol F;R11 XI;R36 R67.

That substance typically forms part of most products for which it has been possible to procure safety data sheets. The concentration of the substance is below the limit of max. 20 % with the requirement to mark the product as causing local irritation.

[18] Executive Order no. 329, 2002 on classification, packagaing, labelling, sale and storage of chemical substances and products.
http://www.mst.dk/Virksomhed_og_myndighed/Kemikalier/Stoflister+og+databaser/Listen+over+farlige+stoffer/Søgning+i+farlige+stoffer.htm

Please note that Danish safety data sheets ought to have been prepared for the products that are imported or resold to the tattoo industry (tattooists). It has not been possible to procure such data sheets.

1.10 Choice of chemical analyses and tattoo inks for analysis

This section describes the criterion for choice of chemical analyses and criterion for which tattoo inks have been chosen for the various analyses. Chapter 4 describes the chemical analyses and results.

1.10.1 Metals and other elements

There is no or only limited information in the safety data sheets of tattoo inks concerning the content of metals and other elements.

In the safety data sheets of three of the purchased tattoo inks (ink no. 38, 39 and 42, blue, red and black, respectively) belonging to the same colour series a content of the following elements and metals is specified: Ba, Cu, Ni, Pb, Sn, Zn and Cr. A general safety data sheet for another colour series (ink no. 43-50, black, green, blue, white, yellow, two red and violet, respectively) specifies that the tattoo inks have been tested for selected heavy metals (<1 ppm).

On the homepage http://ctl-tattoo.eu there is a data base of tattoo inks and permanent makeup products, which have been tested according to ResAP (2008)1[19] of the Council of Europe, and a number of recommended, maxium, permitted element concentrations in tattoo inks are stated, see Table 1.5.

In a Swedish report called "Farliga ämnen i tatueringsfärger"[20] various tattoo inks were tested for i.a elements. Some of the elements were demonstrated in concentrations exceeding the values that also are stated in Table 1.5. The colours were black, red, orange, violet, blue, green and yellow.

Table 1.5 Max. permitted concentrations of elements according to the ResAP (2008)1 of the Council of Europe

Element	µg/g (ppm)
Arsenic (As)	2
Barium (Ba)	50
Cadmium (Cd)	0.2
Cobalt (Co)	25
Chromium (Cr) (VI)	0.2
Copper (Cu), dissolvable	25
Mercury (Hg)	0.2
Nickel (Ni)[1]	As low as technically possible
Lead (Pb)	2
Selenium (Se)	2
Antimony (Sb)	2

[19] Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up (superseding Resolution ResAS(2003)2 on tattoos and permanent make-up)

[20] Farliga ämnen I tatueringfärger. Utredning av tellsynsansvar samt behov av ytterligare reglering – rapport från ett regeringsupdrag som utförts i samråd med Läkemedelsverket, Socialstyrelsen och Konsumentverket. Kemikalieinspektionen, juni 2010.

Element	µg/g (ppm)
Tin (Sn)	50
Zinc (Zn)	50

[1]The resolution recommends that it must be stated on the label if the tattoo ink contains nickel.

In this project, 61 tattoo inks were selected among the 65 purchased inks for analysis of metals and selected elements in order to investigate if there is a correlation between the colours and the content of elements and to compare them with the limits stated in Table 1.5. All of the purchased colour shades and colour series are represented.

Colour no. 52, 54, 55 and 56 were not chosen as they belong to a colour series that the interviewed tattooists do not use. The colours were solely purchased because they are sold as a package deal with two tattoo inks that are registered in connection with skin reactions (colour no. 53 and 57).

1.10.2 Carbon black

The safety data sheets and the labels of the black tattoo colours state that carbon black is used as pigment in seven of the 11 purchased tattoo inks, see Enclosure B. There is no indication of how much carbon black exists in each tattoo ink. This project analysed the content in five black tattoo inks (ink no. 10, 12, 23, 30 and 43).

Among the five selected inks, three were chosen because they form part of the most frequently used colour series (ink no. 12, 23 and 43). One ink was chosen because it is a brighter shade of black (grey, ink no. 10) and it is used by one of the interviewed tattooists. It is expected that the grey tattoo colour will demonstrate a lower content of carbon black. The final colour is a black ink that is sold to tattooists, but the supplier's internet page states that it is not a tattoo ink (colour no. 30). In connection with the grey (ink no. 10) and two of the black inks (ink no. 12 and 23) it is stated on the label of the tattoo inks that they contain carbon black.

1.10.3 Phthalocyanines

The labels or safety data sheets of 18 inks state that they contain phthalocyanines (Phthalocyanine Blue 15:3 and Phthalocyanine Green 7), see Enclosure B. Seven green (ink no. 6, 7, 13, 16, 26, 41, 60), six blue (ink no. 8, 15, 25, 32, 38, 62) and one violet ink (ink no. 37) are in question.

Six tattoo inks (ink no. 31, 35, 44, 45, 50, 60) were selected for analysis, and neither the labels nor the safety data sheets state whether or not they contain phthalocyanines. Green, blue or violet colours were selected because as previously mentioned similar tattoo colours contain phthalocyanines. The colours were selected among the most frequently used tattoo series. One colour (ink no. 35) was registered in connection with skin reactions, see section 1.7.
4 tattoo colours were selected as the analyses for elements demonstrated a high content of copper and according to the safety data sheets there is a content of phthalocyanine Blue 15:3 and no other phthalocyanines (selected among ink no. 7, 8, 15, 25, 38, 62, see Enclosure B). An estimate of the content of phthalocyanine Blue 15:3 was carried out on these tattoo inks in the light of the measured copper concentration.

1.10.4 Polycyclic aromatic hydrocarbons (PAH)

The ResAP (2008)1 of the Council of Europe recommends a maximum permitted concentration of polycyclic aromatic hydrocarbons (PAH) in tattoo inks of 0.5 ppm, however, 5 ppb for benzene-a-pyrene (BaP). In a recently published investigation[21], a content of PAH was demonstrated in a number of black tattoo inks that contain carbon black.

Carbon black can be used to darken other colours and therefore a content of carbon black and related PAH in other shades than black can be expected. According to the safety data sheets, ink no. 31, 32, 33 and 60 (in the colours green, red and blue) contain carbon black. The Swedish report ”Farliga ämnen i tatueringsfärger” has reported the discovery of PAH in orange, violet and blue colours.

A total of 19 tattoo inks were selected for analysis of PAH (ink no. 2, 3, 10, 11, 12, 15, 17, 18, 20, 23, 30, 42, 43, 45, 48, 49, 50, 51, 58). The selected tattoo colours are black, orange, violet, blue and red, as the most frequently used colour series are represented.

Analyses were carried out for the following PAH, which form part of ResAP (2008)1 of the Council of Europe: naphthalene, acenaphthylene, acenaphtene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benz(b)fluorathene, benz(k)fluoroanthene, benz(a)pryene, indeno(1,2,3)pyrene, dibenz(ah)anthracene and benz(ghi)perylene.

1.10.5 Primary aromatic amines (PAA) liberated from azo colorants

In ResAP (2008)1 of the Council of Europe a number of primary aromatic amines (PAA) are stated. They should not exist in tattoo inks and should not emerge when liberated from azo colorants.

The Swedish report ”Farliga ämnen i tatueringsfärger” has reported the discovery of PAA in orange, red, yellow, green and brown colours.
19 tattoo colours were selected for analysis of PAA liberated from azo colorants (ink no. 1, 5, 7, 18, 20, 24, 26, 27, 35, 36, 37, 44, 45, 48, 49, 53, 57, 60, 65). The colours are red, green, blue, yellow, violet, orange and brown. Emphasis was placed on selecting tattoo colours where it is unknown which pigments the tattoo inks contain and on selecting the most frequently used tattoo colours (red, green and blue) among the most frequently used colour series, see table 1-1 and table 1-2. In addition, tattoo inks connected with skin reactions were selected, see section 1.7 (ink no. 18, 24, 35, 36, 37, 48, 49, 53, 57).

Analyses were carried out on the following PAA that form part of ResAP (2008)1 of the Council of Europe and DS/EN 14362-1[22]: aniline, 4-aminobiphenyl, benzidine, 4-chlor-o-toluidine, 2-naphthylamine, 5-nitro-o-toluidine, p-chloraniline, 4-methoxy-m-phenylendiamine, 4,4'-methylendianiline, 3,3'-dichlorbenzidine, 3,3'-dimethoxybenzidine, 3,3'-dimethylbenzidine, 3,3'-dimethylendianiline, 6-methoxy-m-toluidine, 4,4,'-

[21] Tattoo inks contain polycyclic aromatic hydrocabons that additionally generate deleteriuous singlet oxygen, Experimental Dermatology 2010;19:e275-e281
[22] DS/EN 14362-1 Methods for determination of certain aromatic amines liberated from azo colorants and pigments

methylenbis(2-chloraniline), 4,4'-oxydianiline, 4,4'-thiodianiline, o-toluidine, 4-methyl-m-phenylendiamine, 2,4-5-Trimethylaniline, o-anisidine and 2,4-xylidine/2,6-xylidine. In addition, content of p-phenylendiamine was determined, see section 1.10.6.

Black is the most frequently used tattoo colour, but was not chosen as literature, see section 1.5, and the safety data sheets state that carbon black and not azo colorant is used. The black inks were analysed for content of free PAA in connection with the analysis for PPD, see section 1.10.6.

The PAA analysis determines the total content of PAA which was liberated from azo colorants during the analysis, residue from the production of azo colorant as well as PAA added to the product to give colour (the two last-mentioned are in the report described as ”free PAA”). That means that the result will be a total of residues/added PAA (”free PAA”) and PAA liberated from azo colorants.

1.10.6 p-Phenylendiamine (PPD)

p-Phenylendiamine (PPD) is stated in ResAP (2008)1 of the Council of Europe concerning substances that are undesirable in tattoo inks because they might be carcinogenic. PPD is a primary aromatic amine (PAA) that i.a. is used in black henna tattoos and in hair dyes to darken the colour and it can give allergic reactions [23, 24].
24 tattoo colours were selected for analysis of PPD (ink no. 5, 7, 8, 10, 12, 13, 15, 18, 20, 23, 24, 25, 30, 34, 35, 36, 37, 43, 45, 48, 49, 50, 53, 57). Mainly dark colours were selected (black, red, blue, green, orange, violet), colours where pigments are not informed and colours from the most frequently used colour series, see table 1-1 and table 1-2. In addition, tattoo inks were chosen that had been registered in connection with skin reactions, see section 1.7 (ink no. 18, 24, 35, 36, 37, 48, 49, 53, 57).

As PPD is a primary aromatic amine (PAA), the analysis for PAA liberated from azo colorants, see section 1.10.5, will also determine possible content of PPD. Therefore, six other tattoo colours will be examined for PDD (ink no. 1, 8, 26, 27, 44, 60, 65).

A total of 30 tattoo colours were examined for content of PPD.

[23] ”Hair dye, including bleeching agents, the Danish Environmental Protection Agency, Cosmetics guide” http://www.mst.dk/Borger/Kemikalier/Kosmetikguiden/V%C3%A6lg+et+produkt/02010700.htm

[24] Kommissionens direktiv 2009/130/EF af 12. oktober 2009 om ændring af Rådets direktiv 76/768/EØF om kosmetiske midler med henblik på tilpasning af bilag III til den tekniske udvikling (EØS-relevant tekst). EU-Tidende nr. L 268 af 13/10/2009 s. 0005 - 0008

This analysis for PPD also determines the content of PAA, which can be added to the product to give a colour or be residue from the production of azo colorants. In the report, this group is described as “free PAA”. The analysis cannot quantify the content of PAA liberated from azo colorant apart from break-down products.

1.10.7 Summary of analysis program

The following number of tattoo inks were selected for the various analyses:

- 61 tattoo inks to be analysed for metals and other elements
- 5 tattoo inks to be analysed for carbon black
- 6 tattoo inks to be analysed for content of phthalocyanines
- 19 tattoo inks to be analysed for selected aromatic polycyclic aromatic hydrocarbons (PAH)
- 19 tattoo inks to be analysed for selected primary aromatic amines (PAA) liberated from azo colorants
- 30 tattoo inks to be analysed for p-phenylendiamine (PPD).

The method descriptions of the chemical analyses and results are described in Chapter 4. The results are applied in Chapter 5.

2 Legislation

2.1 Danish legislation

Today, no special regulation exists as to which chemical substances may be used for tattoos.

Tattoo inks are chemical products that are covered by the Product Safety Act as well as REACH and the related restrictions on a number of chemical substances.
Tattoo inks also have to comply with the rules concerning labelling and classification that apply to all chemical products, including the content of CMR substances. That e.g. means that tattoo inks containing more than 0.1 % of a carcinogenic substance must not be used to make a tattoo.

The Executive Order on Cosmetics and the EU cosmetics directive that apply to products intended for improving the appearance, cleaning and care of the skin cannot be used within the field of tattoo inks as cosmetic products are applied to the surface of the skin and do not perforate the skin barrier, which is the case with tattoos.

According to the Danish law on tattooing dated 8.6.1966 with effect from 15.6.1966, a person who tattoos somebody under the age of 18 will be punished with a penalty or imprisonment unless higher punishment is deserved according to other legislation. A person who tattoos somebody on the head, on the neck or on the hands will be punished in the same way.

No other legal provision exists on tattooing. The law does not apply to Greenland or the Faroe islands.

The law does not consider who is allowed to make a tattoo, if the person is an amateur, a semi-amateur or a professional tattooist with considerable experience.

In a case, where a professional tattooist from Vesterbro (a district in Copenhagen) tattooed a text in large types across a the face of a customer, the tattooist was fined DKK 1.000[25] by the Copenhagen City Court.

In a current investigation carried out at Bispebjerg Hospital[26], see the chapter on exposure, it was noted that among 72 people who had been tattooed and who belonged to the age group of 20-25-year-olds, 19.4 % had been tattooed before they turned 18. 10 % had been tattooed in the face/on the neck and 7 % on their hands. The law is often violated and the violation is rarely protested against.

On 20.11.2009, the Danish Parliament[27] unanimously passed a proposal, V18, concerning the tattoo trade and it imposes the Danish Government to submit a proposal concerning the authorisation of tattooists before the end of 2010.

[25] Ekstra Bladet (Danish newspaper) 28.4.2010
[26] Bispebjerg Hospital, Department of Dermatology, 2010, J. Serup, unpublished data
[27] www.ft.dk/dokumenter/tingdok

In Denmark, tattoo inks are regarded as chemical products. They are not covered by legislation on cosmetic products or on medicine although the ink is injected into the skin and is subject to some degree of systematic absorption.

2.2 The Council of Europe

The Council of Europe is not part of the EU and their resolutions should be regarded as proposals that do not have to be implemented nationally. In resolution ResAP (2003)2 the Council of Europe stated specifications for tattoo inks, their acceptable composition, labelling, marking, sterility etc. with a negative list of aromatic amines that could be carcinogenic or mutagenic.

The resolution prohibited preservatives and introduced sealed, sterile, single use packaging. The resolution introduced consumer information, but it did not consider which level of education or which qualifications a tattooist should have. However, the updated resolution ResAP (2008)1 permits the use of preservatives and subsequent tattoos may be made of tattoo ink taken from larger multi-use containers that have been opened. The requirement to sterile single use packaging has been omitted and limited use of preservatives is permitted.

A type of positive list covering permitted metals and tracers with stated maximum approved concentration has been introduced. Switzerland and the Netherlands have contributed to the development of the resolutions of the Council of Europe with background information on tattoo inks on the market and on the microbiology in the products. The resolutions have not been implemented in Denmark. The resolutions have been implemented in original or modified form in Switzerland, Germany, France and the Netherlands and it is being considered if the resolutions should be introduced in Sweden[28].

The resolutions of the Council of Europe are recommodations of intervention based on theoretical considerations. They lack concrete clinical-epidemiological validation or closer specific recommendations regarding how tattoos are implemented in practice.

2.3 Medical tattoos

In Denmark, doctors make medical tattoos such as marker tattoos during radiation treatment to ensure the consistancy of a radiation field. Such work comes within health legislation. Doctors can also carry out tattoos with a cosmetic aim in connection with corrective breast surgery after breast cancer with restoration of the colour of the nipple and the pigmentation of the areola around it or a professional tattooist can carry out the treatment. In both cases, the activity comes within health legislation as the tattooist acts as the doctor's assistant.

[28] Kemikalieinspektionen, Farliga ämnen i tatueringsfärger, rapport 3/10 af juni 2010, www.kemi.se

2.4 Removal of tattoos

Removing tattoos by means of laser treatment is not covered by the "Executive Order on Cosmetic Treatment" of the Danish National Board of Health dated 1 December 2007 and can be carried out by anybody without the requirements to information, record keeping etc. being applicable, which doctors are subject to when carrying out laser treatment for cosmetic treatment. Today, tattoos are removed on the free market without involving doctors and without any type of control. When removing a tattoo there is no complaints board, supervision or sanctions.

In the instructions of the Danish National Board of Health concerning Executive Order no. 64 dated 24.10.2007 it is directly stated that tattooing, piercing and scarification and treatment hereof are covered by the tattooing law dated 1966, which, however, has no decisions that regulate the mentioned conditions.

3 Exposure scenarios

3.1 Frequency of tattoos

Historically, tattoo shops were located near ports and the customers were predominantly sailors and people from the marine environment[29]. Today, commercial tattoo shops exist all over Denmark and tattooing is popular among a broad section of the population and is not linked to particular professions.

From August – September 2010, a survey including interviews and examinations of 140 adults was carried out with an accidental segment of skin diseases and it showed that 18 had a tattoo ((13 %), seven women and 11 men, and the average age was 40.8 years (span of 19-72)[30]. The persons came from Greater Copenhagen and Sealand.

The Danish public opinion poll, Gallup, carried out an interview of 1.112 young Danes in the age-group of 15-25 for the Danish newspaper ***Berlingske Tidende.*** It was informed that every eight – in the investigation it was 13 % - had one or more tattoos and 43 % stated that they definitely or perhaps would like another tattoo at a later date. The investigation also revealed that out of the 143, who said they had a tattoo, 41 % had more than one tattoo[31].

A telephone interview of 1.007 representative Danes in the age-group 18-74 indicated that 12 % were tattooed[32].

Therefore, the frequency of tattooing in an age-related broad segment of the Danish population is around 13 %. 13 % tattooed people means that app. 600.000 adult Danes had a tattoo in 2010.

Evidently, tattooing is very widespread among some segments of the population -especially among biker gang members such as e.g. Hells Angels, where the tattoos resemble tribal signs that are carried by all approved members.

3.2 Exposed parts of the body

A clinical investigation of 72 tattooed persons, 42 women and 30 men from Greater Copenhagen belonging to the youth segment in the period from August – September 2010 revealed 171 tattos, that is 2.4 tattoos per person[33]. In average, the tattoos covered 2.5 % of the surface area of the skin calculated according to the ”palm of the hand” method. As the palm of a human corresponds to 1 % of the total skin surface of a human, the discovery implies that an average tattoo covers an area corresponding to 2½ palms.

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[29] Nordstrøm J. Dansk Tatovering. Nordstrom, 2009. ISBN 978-87-993150-0-0
[30] Bispebjerg Hospital, Department of Dermatology, 2010, J. Serup, unpublished data
[31] Berlingske Tidende, Danish newspaper, 9.7.2010
[32] MetroExpress, Danish newspaper, 16.9.2009 with reference to YouGov Zaperas Danmarkspanel
[33] Bispebjerg Hospital, Department of Dermatology, 2010, J. Serup, unpublished data

According to REACH, the surface area of the skin is standard 1.69 m^2 for women and 1.94 m^2 for men and if 2.5 % of the skin is tattooed, then the tattooed area is in average 423 cm^2 for women and 485 cm^2 for men. In average 454 cm^2 for both sexes – corresponding to a square area of 21.3 x 21.3 cm.

9 persons had larger tattoos that covered 3-12 % of the skin surface, in average 6.4 % of the skin surface. So there was a sub-group of particularly exposed where the tattooed average area was 1,090 cm^2 for one person, corresponding to a square area of 33.0 x 33.0 cm.

In connection with the 171 tattoos, the tattooed body regions were as follows: arms including wrist area 59 (35 %), legs including ankle joint and genital area 15 (9 %), body 71 (42 %), hands 7 (4 %), feet 10 (6 %) and face and neck 10 (6 %).

17 (10 %) of the tattoos were made on the face, neck or hands, which according to the "Danish law on tattooing" is forbidden.

3.3 Exposure of the body

The skin consists of epidermis, dermis and subcutis, see Figure 1.

Epidermis consists of a thin layer, Stratum Corneum, on the outer side and of a hypercellular layer down towards dermis, which is rich in collagen fibres.

Tattoo pigment gathers in the outer 1/3 of dermis and centres just under epidermis where the so-called basement membrane does not allow further passage of pigment to epidermis and thus out of the skin.

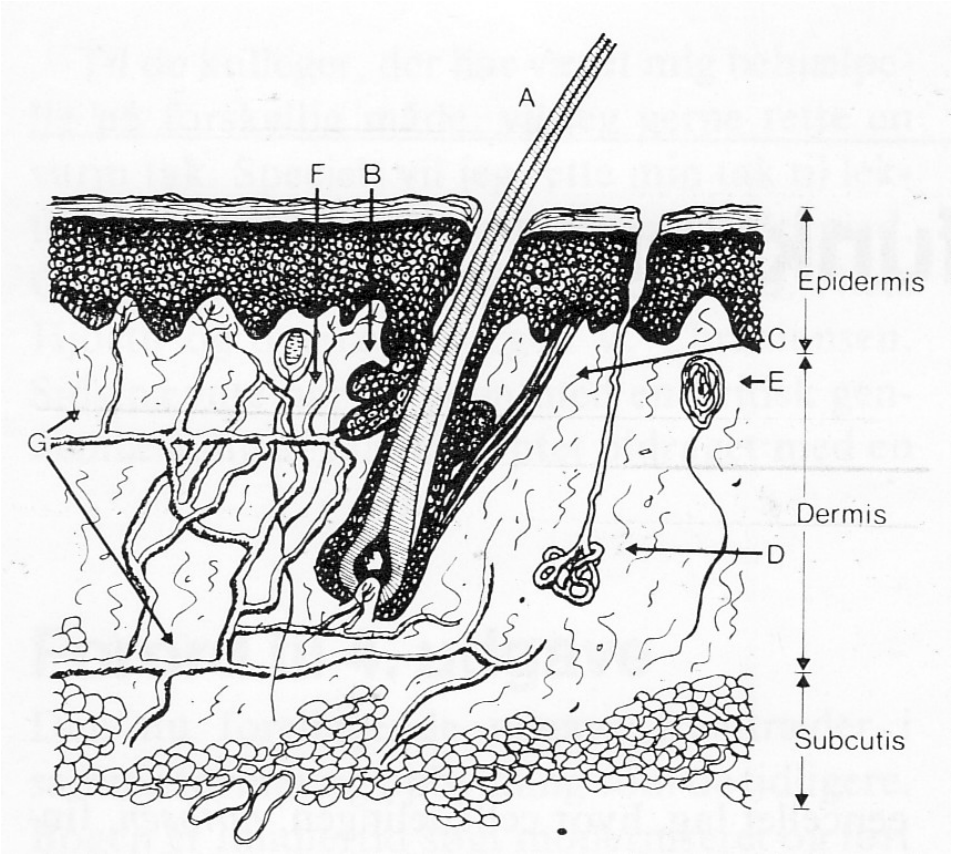
In a few weeks, epidermis is renewed and is then devoid of pigment in a tattoo that has healed. Dermis consists of vessels and vascular loops, especially up towards epidermis. The blood vessels drain to the venous system and the large vessels of the body. The lymphatics drain via larger vessels in the subcutis to the sentinel lymph nodes that function as a filter. The lymph then passes on to the central vessels and to the bloodstream. The sentinel lymph nodes can be found in the flexure of the knees and the cubital fossa, in the groin of the arms, in the groin and armpits, on the neck and inside the body along the vertebral column and along the vessel supply to the large organs. That means that there are two drainage paths from a tattoo: one directly to the venous system, the other through the lymph tracts and lymph nodes to the bloodstream. It is unclear what determines the tissues' path away from the skin – presumably, it is a question of molecular size, probably so the macro molecules and particles mainly travel via the lymph and the filtration in the lymph nodes.

The skin has special structures such as hair (A), sebaceous glands (B), muscle fibres attached to hair follicles (C), sweat glands (D), sensory bodies attached to nerve fibres (E) and free nerve ends (F). The thicker subcutis is located under dermis and it constitutes a layer of fat against underlying structures.

In the areas that are usually tattooed (arms, body, legs) epidermis is 0.1-0.2 mm thick and the total thickness of the skin is 0.6-1.5 mm, on the body it is

up to 2.5 mm[34]. The female skin is app. 0.2 mm thinner than the skin of men, but the echodensity is geater (expression of density of collagen fibres in the skin, which is measured by ultrasound examination). When tattooing, the tattoo ink is pricked or injected around 0.1-0.5 mm into the skin and then the pigments are distributed.

Figure 1 Structure of the skin



The skin barrier against penetration of chemicals lies in the outer 3-5 cell layers that constitute the Stratum Corneum of the epidermis that mainly consists of dead epidermis cells that normally are exfoliated as scale. Penetration is difficult – only small fat-soluble molecules can pass and only in small amounts. For instance, with regard to locally effective medicine only 1-5% penetrates through the barrier. Most of it remains unused on the surface and falls off with scale or is washed off.

When tattooing, the colorant is particles that mechanically are led through the barrier by pricking with a tattoo needle in the upper part of the underlying dermis, meaning below epidermis. Normally, the skin does not bleed when tattooing as the tattoo needles are blunt needles compared to injection needles for medical use, where the needle point is cutting and typically sharpened with faceting. In case of unintentional vascular lesion during tattooing, with visible blood that most often appears on the skin surface, the pigment is washed out of the skin and it is the general experience that in case of bleeding there is a risk that the tattoo might get an uneven colour and some areas will not receive enough colour. Vascular lesion by bleeding disrupts the continuity of the

[34] Olsen L, Takiwaki H, Serup J. High-frequency ultrasound characterization of normal skin. Skin thickness and echographic density of 22 anatomical sites. Skin Res Technol 1995;1:74-84

vessel and the risk of installing ink directly in the vessel system after vascular lesion from needles is, however, estimated to be very small.

Tattoo needles consist of a number of very small needles collected as a sort of brush in the tattoo needle that is mounted in the electric tattooing equipment. Needles for the tattooing of points, typically consist of 3-5 small individual needles, and needles for tinting of larger areas and for shading typically consist of a larger amount of needles, if necessary mounted as a fan.

Due to the quickly vibrating movement of the needle and as the needle regularly is dipped in the tattoo ink, ink with a content of particles is led through epidermis and down into dermis. The applied amount of ink depends on the tattooist, his trade skills and his intention with regard to dosing the colour more or less intensively.

In connection with drawing op of a tattoo, where an old tattoo will be covered by another colour or refreshed, the colour will be dosed so it becomes particularly vivid. Therefore, there will be great variation in the local dosage of tattoo ink merely conditioned by the technique and purpose, including the selected motif.

The chairman of the Danish Tattooist Guild gives a practicle example where 1 ml tattoo ink covers an area of 11 x 11 cm corresponding to a dose of 8.3 mg/cm^2. However, during tattooing surplus ink is regularly wiped away with a serviette and the amount pricked into the skin is a bit smaller. A more precise determination of the effective dose requires measurement of the amount deposited in the skin.

In a study with chemical extraction and analysis of azo pigment (Pigment Red 22) from tattoos made by an experienced tattooist on humans and on pigs, extraction showed that the deposit of pigment in the skin varied from 0.6 to 9.4 mg/cm^2, mean value 2.5 mg/cm^2, depending on the tattooing technique[35].

As the dry matter content (meaning the pigment) of tattoo ink is 30-60 % and considering that some of the ink is dried away with a serviette there is good agreement between the pigment dose in the skin stated in literature and the tattooist's estimation of the tattoo ink dosage.

Assuming that the average tattooed area of a person is 454 cm^2 and that the average dose is 2.53 mg/cm^2, then the average exposure, understood as the amount of pigment in chemical extractable form deposited in the skin, amounts to 1,148 mg for one person. For the group with tattoos that cover a large area, i.e. 1,090 cm^2, and that have the highest dosage, i.e. 9.42 mg/cm^2, exposure will amount to 10,268 mg pigment for one person.

Some of the pigment that is deposited in the skin - irrespective of the substance being particulate or dissolved – will pass directly on to the lymphatics and vascular system (especially nanoparticles) and under the influence of light some might be decomposed to other chemical substances or be metabolised locally in the skin.

As a starting point, commercial tattoo inks are not chemically pure, which the various findings from the chemical analyses in this project also indicate.

[35] Engel E et al. Modern tattoos cause high concentrations of hazardous pigments in skin. Contact Dermatitis 2008;58:228-233

Therefore, there will in the early phase be a wide range of chemical substances with different metabolism in the skin, different solubility properties and different kinetics, penetration into the body and local metabolism.

During the first weeks, the pigment in the verticle pricks will physically move locally in the skin and gather as a rather even layer in the outer part of dermis just below epidermis. Through a magnifying glass, the ink in new tattoos appears to be evenly distributed without visible prick traces corresponding to the original needle pricks, by means of which the colour was introduced into the skin. In old tattoos, the colour can spread to the surrounding skin and appear vague and bleached due to further transfer of the pigment inside dermis.

Pigment, which is a foreign body, trys to get out of the skin in every thinkable way – for years. Kinetics has been studied in mice - also when using Pigment Red 22[36].

42 days after tattooing, the pigment in the skin was reduced to 32 % of the initial dose. During systematical exposure to sunlight the reduction was larger. During the course of events and especially during light-affection including laser, new chemical substances were created and they are classified as carcinogenic chemical substances and light and laser substantially reduced the amount of the substance.

That means that there is an initial phase in the weeks after tattooing with physical redistribution of the pigment locally in the skin, washing out to the body and local metabolism i.a. under the influence of light, by means of which a substantial part of the colorant leaves the skin or is transformed to a substance of similar chemical composition. There is great uncertainty connected to stating the size of distribution, transformation and elimination that must be assumed to vary for the different pigments as they chemically and structurally are very different.

During the initial phase, 2/3 of the primarily deposited pigment disappears or is metabolised, estimated in the light of the determination of the azo pigment Pigment Red 22 in mice as mentioned above. However, the histological structure of the skin of mice is very different from the structure of human skin, as the skin of mice is thinner. Therefore, the result cannot uncritically be extrapolated to humans. Besides, the skin structure varies in the different body regions, e.g. the skin in the face/neck is thicker than the skin on the arms and legs, but the skin in the face/neck is nevertheless more penetrable e.g. for cortisone cream[37].

After the initial phase there is a phase with slow liberation and therefore kinetics is assumed to be two-phased as the tattoo in spite of the initial liberation is permanent.

It is the general experience that black ink is more lasting in a tattoo than other colours and the frequent practical experience is that the colours red, yellow and green loose intensity after some years and that tattoos with those colours

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[36] Engel E et al. Tattooing of skin results in transportation and light-induced decomposition of tattoo pigments – a first quantification ***in vivo*** using a mouse model. Exp Dermtol 2009;19:54-60

[37] Feldman RJ, Maibach HI. Regional variation in percutaneous penetration of C-14 cortisol in man. J Invest Dermatol 1967;48:181-183

in time completely can loose their colour as an expression of the colorant either disappearing from the skin or metabolising to colourless chemical substances.

As the passage of pigment from the skin to the sentinel lymph node is frequent with secondary deposit in the lymph node and visible colouring of the node, the tattooing of skin is indirectly the tattooing of the sentinel lymph node. On the basis of autopsies, it is well-known that sentinel lymph nodes to a tattoo in skin can carry visible colouring corresponding to the dominating colour of the tattoo, just as it is well-known that people who smoke tobacco often have black lymph nodes near the primary bronchus.

When removing lymph nodes as part of an operation for malignant melanoma, findings of visible dark or black lymph nodes can give rise to interpretation problems if the person has a black tattoo in the draining area of the lymph node.

The appearance of visible colouring of lymph nodes that drain the skin area with a tattoo indicates that a substantial part of the tattoo pigment is detected in sentinel lymph nodes. In the light of autopsies it is well-known among pathologists that the node behind a red tattoo is red, behind a green tattoo it is green etc. Animal studies have shown that app. ¼ of the administered amount of ink during tattooing can be recoverd in the lymph nodes. Pigment that is secondarily deposited in the lymph node forms a special exposure as that pigment is in direct contact with the blood-forming system and with the immune apparatus whose main organs are the lymph nodes and the bone marrow.

In the light of an experiment on animals, see 3.4, it must be assumed that the extent of pigment deposited in the lymph node (above estimated to ¼ of the administered pigment) depends on the size of the pigment particles, as small particles of nanosize according to the mentioned experiment and in the light of general knowledge of nanoparticle kinetics in the body must be assumed to a larger degree than nanoparticles exceeding nanosize to pass unfiltered through the lymph node and on to the systematic blood circulation with possible exposure of much body tissue.

Assuming that app. 1/3 of the pigment that is installed in the skin during tattooing and that the rest is not eliminated locally in the skin but solely is made up of nanoparticles, then the hypnotic systematic exposure in the initial phase could amount to up to 2/3 of the administered amount of tattoo pigment.

The description of exposure is further complicated by the pigments appearing in particle form. That means that slow liberation of chemical substances from the surface of the particles could take place (perhaps they could even be chemically coated).

During the chemical analysis of tattoo inks it was observed that there is a considerable variation in the chemical composition of tattoo inks. In addition, it was not possible during the investigation to account for all chemical substances that exist in the colours. The chemical substances in the inks might influence pigment kinetics and in that way the local, regional and systematic exposure.

3.4 Nanomaterials in tattoo inks

As mentioned, pigment in the form of nanoparticles down to a size of 20nm was found in tattoo ink after analysis of random colours.

Under an electron microscope, skin tattoos disclosed skin cells in dermis with particles of black, red, yellow and green colours, with sizes in the nanoarea[38]. These particles had also accumulated around vessels and their localisation indicates that they in the redistribution phase after tattooing seek towards the vascular bed.
As mentioned, it is well-known that pigment grains of tattoo ink exist in lymph nodes that drain the tattooed skin area. Tests in rats with injection of silver nanoparticles and larger particles in subcutis have shown that nanoparticles also reach the blood circulation and distribute themselves to the kidneys, liver and spleen, while larger particles above nanosize are not transferred to the blood[39]. The fact that nanoparticles can distribute themselves differently in the body than soluble substances and larger particles is utilised as "drug targeting" in connection with development of vaccines[40]. The content of nanoparticles in tattoo ink especially creates uncertainty as to which organs actually are exposed in addition to skin and lymph nodes, which drain the tattooed area.

Nanoparticles have special biological effects that are conditioned by their small size and chemical reactions on the surface of the particles. The effects exceed the effect of the chemical substance, which the particles consist of. For instance, titanium dioxide nanoparticles in mice can induce harm and instability in the genetic code of the cells, DNA, whereas titanium dioxide normally is assumed to be intert[41]. There is an increasing development and appreciation of the field of nanotoxicology[42].

The particle distribution in a representative selection of different tattoo inks has been systematically investigated and is being published[43].

3.5 Definition of dermatological terms

Lichenoide reaction, meaning benign tumours in the skin above its level, possibly with dryness and scale on the surface, often pruritic and troublesome.

Pseudolymphoma, meaning roundish tumours in the skin with content of structures and cells corresponding to leukaemia according to microscopy, but most often with a clinically benign course.

Granulomatous reaction, meaning roundish tumours that swell as a dome above the skin and have a special microscopic structure with content of so-called epitheloid cells and perhaps sarcodial structure, i.e. a structure with giant cells

[38] T. Kobayasi, Bispebjerg Hospital, Department of Dermatology, unpublished data
[39] Tang J et al. Distribution, translocation and accumulation of silver nanoparticles in rats. J Nanosci Nanotechnol 2009;8:4924
[40] Nasir A. Nanoparticles in vaccine development: a step forward. J Invest Dermatol 2009;129:1055-1059
[41] Trouiller B et al. Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice. Cancer Res 2009;69, 8784-9
[42] Oberdörster G. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environmental Health Perspectives 2005;113:823-39
[43] Bispebjerg Hospital, Department of Dermatology, 2010, J. Serup, unpublished data

in special arrangement, a structure that could resemble tuberculosis and is seen in that connection liberated by the tubercle bacillus. Small foreign bodies in the skin such as metal particles, especially aluminium, can include the formation of foreign body granuloma.

3.6 Known dermatological and other side-effects from tattoos

In 2003, the EU made an outline of the side-effects from tattoos based on an investigation of scientific literature[44]. A Danish key article has just been published in the journal of The Danish Medical Association and it gives a current status of the situation[45].

Transfer of infections in connection with tattooing such as hepatitis, HIV, tetanus, syphilis, tuberculosis and leprosy are known and are ascribed to insufficient hygiene.

Local infections in the skin with staphylococci, streptococci and bacteria of the species pseudomonas and fungi exist and are also ascribed to insufficient hygiene or contamination of the tattoo ink. Among the non-infectuous side-effects reference is made to allergic, granulomous/lichenoide, pseudo-lymphomatoud, sarcoidal and keloid reactions and skin cancer including malignant melanoma.

Compared to the spreading of tattoos, reports on side-effects in the medical literature are rare and they mostly appear as a description of isolated cases. There is a high degree of uncertainty on how allergic reactions appear clinically and how the appearance of allergic mediator mechanism is documented.

Contact allergy tests in the form of patch tests are not suited for testing particulate provocations and were not developed or validated for this purpose as exposure takes place down in the skin and not above the skin. It is assumed that allergy against permanently deposited substances in dermis is clinically different from ordinary contact eczema, where the eczema reaction is initiated by the contact of the allergen with the surface of the skin. It is often assumed that cronical, scaling reactions with lichenoid or granulmatous appearance can have allergic reasons. But other mechanisms such as chemical irritation reactions, biological reactions of non-allergic nature and foreign body reactions are also possible.

The development of nodular elements in the skin has been described. Under the microscope it is a leucemic condition, diagnosed as pseudolymphoma or as B-cell lymphoma that has arisen in a pseudolymphoma[46] [47] [48]. Skin cancer in the form of basal cell carcinoma, squamous cell carcinoma and malignant melanoma originating in a tattoo have been described, but rarely and only in

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[44] EC. Risks and Health Effects from Tattoos, Body Piercing and Related Practices, Ispra, 05 May, 2003

[45] Hoegsberg T, Serup J. Tattoos in dermatologic perspective. Journal of The Danish Medical Association, 2011;173:34-39

[46] Gutermuth J. et al. Cutaneous pseudolymphoma arising after tattoo placement. J Eur Acad Dermatol 2007;21:566-67

[47] Arminger WG, Caldwell EH. Primary lesion of a non-Hodgkin's lymphoma occurring in a skin tattoo: case report. Plast Reconstr Surgery 1978;62:125-27

[48] Sangueza OP et al. Evolution of B-cell lymphoma from pseuolymphoma. Am J Dermtopahtol 1992;14:408-13

individual cases in the form of case reports. Skin cancer in the form of carcinoma originating in the epidermis is very frequent (the most frequent type of cancer among humans) and therefore it is irrespective of the tattoo likely that skin cancer in a tattoo merely is an accidental finding, a coincidence[49]. The Swedish chemical inspection has in a report in detail reviewed the literature with the same conclusion; that a connection between skin cancer and tattoos has not been proven and is not actually probable[50].

As mentioned earlier, tattoo pigment is transported to the sentinel lymph node that can become swollen with microscopic and macroscopic content of tattoo pigment, just as more widespread lymph node tumour can arise[51] [52] [53]. In addition to skin and lymph nodes, certain side-effects from tattoos have not been reported in other organs. There is no awareness of the situation, no studies aim at detecting such distant complications and such risks have not been systematically clarified.

A number of cases of eye complications have been reported in the form of iritis that have arisen in connection with tattooing carried out on an occasional place on the body without necesarrily having been localised near the eye region.[54] Iritis is most often an expression of an immunological reaction and iritis is typically seen in connection with rheumatism i.a. articular rheumatism.

[49] Kluger et al. Skincancers Arising in Tattoos: Coincidental or not? Dermatology 2008;217:219-221
[50] Kemikalieinspektionen rapport 3/10, Farliga ämnen i tatueringfärger, 2010, www.kemi.se
[51] Goldstein N. Complications from tattoos. J Dermatol Surg Oncol 1979;5:869-878
[52] Friedman T. et al. Tattoo pigment in lymph nodes mimicking metastatic malignant melanoma. Plast Reconstr Surgery 2003;111:2120-22
[53] Moehrie M. et al. Tattoo pigment mimics positive sentinel lymph node in melanoma. Dermatology 2001;203:342-44
[54] Rorsman H et al. Tattoo granuloma and uveitis. Lancet 1969;2:27; Saliba N. et al. Tattoo-asociated uveitis. Eye (London) 2010;24:1406

4 Chemical analyses

4.1 Objective of the analyses

The chemical analyses shall illustrate to which extent a number of selected tattoo inks contain one or more of the following substances and the amount/concentration of the substances:

- Metals and other elements
- Carbon black
- Phthalocyanines
- Polycyclic aromatic hydrocarbons (PAH)
- Primary aromatic amines (PAA) that have appeared through the liberation of azo colorants.
- p-Phenyldiamines (PPD).

In connection with the analyses for PAA and PPD the content of PAA will also be determined. It might have been added to the product to give colour, be residue from the production of azo colorants or perhaps break-down products. In the report, these PAA in free form are called ”free PAA”.

The reason why certain tattoo inks were chosen for each analysis is described in section 1.10.

In this chapter on chemical analyses the applied methods are described in section 4.2 and the analysis results are described in section 4.3. A summary of the results in section 4.4 concludes the chapter.

The analysis results were used to implement the Health and Risk Assessment in chapter 5.

4.1.1 Outline of analyses and tattoo inks

The analysis programme is shown in Table 4.1. X means that the tattoo ink was analysed according to the stated method. Ink no. marked with * indicates that the tattoo ink is registered in connection with skin reactions, please also refer to Survey, section 1.7 and chapter 6.

Table 4.1 Outline of analyses of tattoo colours

Ink no.	Colour	ICP/MS (metals and elements)	TGA (carbon black)	Colour test (phthalo-cyanin)	GC/MS A (PAH)	GC/MS B (PAA+ PPD) [1)]	GC/MS C (PAA+ PPD) [2)]
1	Red	X				X	
2	Black	X			X		
3	Black	X			X		
4	White	X					
5	Red	X				X	X
6	Pale green	X					
7	Dark green	X				X	X
8	Blue	X					X
9	Yellow	X					
10	Grey	X	X		X		X
11	Black	X			X		

Ink no.	Colour	ICP/MS (metals and elements)	TGA (carbon black)	Colour test (phthalo-cyanin)	GC/MS A (PAH)	GC/MS B (PAA+ PPD) [1)]	GC/MS C (PAA+ PPD) [2)]
12	Black	X	X		X		X
13	Dark green	X					X
14	White	X					
15	Blue	X			X		X
16	Pale green	X					
17	Red	X			X		
18 *	Red	X			X	X	X
19	Yellow	X					
20	Orange	X			X	X	X
21	Peach	X					
22	White	X					
23	Black	X	X		X		X
24 *	Red	X				X	X
25	Blue	X					X
26	Pale green	X				X	
27	Yellow	X				X	
28	Orange	X					
29	Peach	X					
30	Black	X	X		X		X
31	Dark green	X		X			
32	Blue	X					
33	Red	X					
34	Red	X					X
35 *	Violet	X		X		X	X
36 *	Yellow	X				X	X
37 *	Violet	X				X	X
38	Blue	X					
39	Red	X					
40	Yellow	X					
41	Green	X					
42	Black	X			X		
43	Black	X	X		X		X
44	Pale green	X		X		X	
45	Blue	X		X	X	X	X
46	White	X					
47	Yellow	X					
48 *	Red	X			X	X	X
49 *	Red	X			X	X	X
50	Violet	X		X	X		X
51	Black	X			X		
53 *	Red	X				X	X
57 *	Brown	X				X	X
58	Black	X			X		
59	White	X					
60	Green	X		X		X	
61	Yellow	X					
62	Blue	X					
63	Red	X					
64	Peach	X					
65	Orange	X				X	

[1)] Method GC/MS B determines PAA liberated from azo colorants (p-phenylendiamine (PPD) is a PAA). The result is a sum of the liberated PAA and PAA that appear in free form in the tattoo ink.

[2)] Method GC/MS C determines p-phenylendiamine (PPD) and other primary aromatic amines (PAA) that appear in free form in tattoo ink.

* Indicates tattoo inks registered in connection with skin reactions.

4.2 Method descriptions

The following outlines how subsamples are extracted. The applied analysis methods are also described.

4.2.1 Extraction of subsamples

The tattoo inks are supplied in different types of plastic containers. Visually, the tattoo inks are very different as some seem homogeneous while others form sediment and the colour is not homogeneously distributed in the container. Some tattoo inks are easy-flowing while others are thick-flowing.

All tattoo inks were shaken thoroughly immediately before extraction of subsample for analysis in order to obtain a sample that was as homogeneous as possible. In connection with sampling, weight was used rather than volume because in connection with some tattoo inks it was impossible to remove a known volume due to high viscosity (thick-flowing).

4.2.2 ICP/MS screening analysis for metals and other elements

The analysis is quantitative. For determination of metals and other elements a sample preparation was carried out with acid and subsequent ICP/MS screening analysis. The expert programme TotalQuantIII was applied. It quantifies the content on the basis of an instrument response curve of the elements from mass 6 (Li) to mass 238 (U).

The weighed subsamples are heated with concentrated nitric acid (Subboiling Quality) by means of microwaves in a quartz autoclave. Subsequently, the sample is filtered and diluted. Blank specimens are made in the same way.

Ge, Rh and Re are added to the prepared samples as internal standards online and they are screened for content of elements by inductive-coupled-plasma-mass-spectrometry (ICP/MS) during the application of the expert programme TotalQuantIII. The instrument response curve is updated before and after the sample measurements by means of multielement standard containing elements that cover the entire mass area. Elements such as Br, C, Cl, F, I, N, O and S are not quantified because of interferences.

The recommended detection limit in the measurement solution is 0.5-50 ng/ml. Detection limits in µg/g are stated in result charts.

A number of substances can influence the analysis result of other substances, so they are estimated to be larger than the correct content in the sample.

A high content of chlorine can influence the result of vanadium and arsenic. A high content of carbon can influence the result of chromium. A high content of calcium can influence the result of nickel. Very high concentrations can be underestimated because of deviation from linearity as seen in some of the samples for copper.

The concentration of elements such as Al, Ti, Zr, Hf and Th can be underestimated as it probably will be difficult to dissolve them with the applied method. Correspondingly, the concentrations of Ba and Sr can be underestimated depending on which salts they appear in, in the samples, e.g. the sulphate salts will result in an underestimation.

In addition, selected samples with a high content of copper are diluted and analysed by means of ICP-AES.

4.2.3 TGA analysis for carbon black

The analysis is quantitative. For determination of carbon black in tattoo inks a TGA analysis was carried out. During the analysis, the samples are gasified in nitrogen and the weight loss is weighed. Subsequently, the sample is burned in oxygen (carbon black) by means of which the content of carbon black is determined.

The analysis was carrried out with a starting point in ASTM D 1603-06, Standard Test Method for Carbon Black Content in Olefin Plastics, with the following conditions:

50 °C-60 °C in nitrogen with 1 °C/min.
60 °C-600 °C in nitrogen with 20 °C/min.
600 °C-200 °C in nitrogen with 100 °C/min.
200 °C-900 °C in oxygen with 30 °C/min.

The result of carbon black can be overestimated if the sample has a content of other non-volatile organic substances or coked organic substances.

4.2.4 Colour test for phthalocyanines

The analysis is qualitative. This analysis determines the content of blue or green phthalocyanines that contain copper. The test consists of a colour reaction and a flocculation, respectively.

The analysis was carried out according to ASTM D 3256-86, Chemical Analysis of Phthalocyanine Blue and Green Pigments. A sample of app. 0.05 g is extracted in a 50 ml cup and 30 ml sulphuric acid is added. Stir for 15 min. and if necessary heat the sample to dissolve the pigment. The creation of a dark green/yellow colour indicates a content of phthalocyanine blue and the creation of a reddish colour indicates a content of phthalocyanine green. The solution is poured into 250 ml water and stirred. If the sample contains the phthalocyanine pigment it will immediately be precipitated as fluff balls.

4.2.5 GC/MS analysis (A) for PAH

The analysis is quantitative. This analysis determines the content of polycyclic aromatic hydrocarbons (PAH) in tattoo inks. The method is based on the article: ”Tattoo inks contain polycyclic aromatic hydrocabons that additionally generate deleteriuous singlet oxygen”, Experimental Dermatology 2010;19:e275-e281.

A subsample of the tattoo ink (app. 1 g accurately weighed) is mixed with 1 ml acetone on a whirley mixer. 100 µl internal standard (naphthalene-d8, antracene-d10, pyrene-d10 and benz(a)pyrene-d12) and 2 ml benzene is added, which are mixed on the whirley mixer. It is heated in an ultrasound bath for 60 min. at 60 °C and centrifuged at 3000 rpm for 10 min. The supernatant is transferred to a new glass and saved. Extraction is repeated another 2 times with 1 ml acetone and 2 ml benzene. The supernatant is pooled with the previous supernatants. Evaporation takes place till app. 1 ml and filtration takes place if the sample is unclear. The sample is then diluted 1:10 with dichlormethane.

Analysis by means of capillary gas chromatography combined with mass spectrometry (GC/MS):
Large volume injection: 25 µl
Column: Phenomenex ZB-1MS 20 m x 0.18 mm x 0.18 µm.
Temperature program: 40 °C (1 min.) to 320 °C (5 min.), rate 20 °C/min.
He: 13 psi
Scan: 45-350 amu

Calibration standards were prepared in benzene:acetone (2:1) added internal standards (naphthalene-d8, antracene-d10, pyrene-d10 and benz(a)pyrene-d12) and diluted in dichlormethane (1:10). The detection limits are 0.15-0.5 µg/g if nothing else is stated in the result charts.

4.2.6 GC/MS analysis (B) for primary aromatic amines (PAA) liberated from azo colorants and free PAA

The analyses are quantitative. This analysis determines the primary aromatic amines (PAA) as the sum of PAA liberated from azo colorants and content of PAA from other sources e.g. residue or PAA added as colour (called ”free PAA”). The method is based on methods described in Resolution ”ResAp(2008)1 of the Council of Europe on requirements and criteria for the safety of tattoos and permanent make-up”, which is a modified method of ”DS/EN 14362-1, Methods for determination of certain aromatic amines liberated from azo colorants and pigments”.

5 ml 5 % dithionite solution in citrate buffer is added to a subsample of the tattoo ink (app. 0.5 g accurately weighed), it is shaken mechanically for 30 min. and heated to 70 °C for 90 min. during regular shaking. The solution is extracted with 2 x 5 ml MTBE added internal standards of aniline-d_5 and naphthalene-d8 during mechanical shaking for 10 min. Analysis in duplicate was carried out.

The extracts were analysed by means of capillary gas chromatography combined with mass spectrometry (GC/MS):
Column: Varian CP Sil 8 MS 30 m x 0.25 mm x 0.5 µm.
Temperature program: 45 °C (0.5 min.) to 320 °C (5 min.), rate 15 °C/min.
He: 15 psi
Scan: 50-275 amu

Calibration standards were prepared in MTBE added internal standards of aniline-d5 and naphthalene-d8.

Recovery was determined by preparation of control standards in 5 % dithionite solution in citrate buffer with subsequent preparation corresponding to the tattoo inks. The detection limits are stated in the result charts.

4.2.7 GC/MS (C) analysis for p-phenylendiamine (PPD) and free PAA

The analyses are quantitative. This analysis determines p-phenylendiamine and primary aromatic amines that are not liberated from azo colorants, but added by other means e.g. as part of the colour, as residue or a break-down product (called ”free PAA”). The method differs from the method GC/MS (B) as dithionite solution is not added and therefore the azo colorants are not decomposed.

5 ml citrate buffer is added to the subsample of the tattoo ink (app. 0.5 g accurately weighed). Ultrasound extraction for 60 min. The solution is extracted with 2 x 5 ml MTBE added internal standards of aniline-d_5 and naphthalene-d8 during mechanical shaking for 10 min. Analysis in duplicate was carried out.

The extracts were analysed by means of capillary gas chromatography combined with mass spectrometry (GC/MS):
Column: Varian CP Sil 8 MS 30 m x 0.25 mm x 0.5 µm.
Temp. program: 45 °C (0.5 min.) to 320 °C (5 min.), rate 15 °C/min.
He: 15 psi
Scan: 50-275 amu

Calibration standards were prepared in MTBE added internal standards of aniline-d5 and naphthalene-d8. Recovery was determined by preparation of control standards in citrate buffer with subsequent preparation corresponding to the tattoo inks. The detection limits are stated in the result charts.

4.3 Results of chemical analyses

4.3.1 Results for metals and other elements

ICP/MS screening analysis was carried out for metals and other elements in 61 tattoo inks. The results appear in Enclosure C. Table 4.2 states the highest concentrations found in the tattoo inks.

The results of the analyses for metals and other elements have been compared with the recommendations in ResAP(2008)1[55] of the Council of Europe in the summary in section 4.4.

Table 4.2 Highest concentrations during ICP/MS screening analysis, µg/g

Element	Highest concentrations µg/g	Ink no.
Fe	25000	49*
Cu	20000	15
Ca	16000	53*
Al	11000	46
Na	5800	2
Zr	2800	46
B	2600	2
Ba	1800	61
Mg	1700	63
Si	1100	27
Ti	960	4
P	710	4
K	680	12
Zn	53	33
Mn	42	20
Hf	38	59

[55] Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up

Element	Highest concentrations µg/g	Ink no.
Cr	31	20
Pd	21	46
Sr	20	61
Ni	18	20
Ag	11	25
Pb	10	4
La	4.8	3
Rb	4.2	1
Y	4.1	1
Sn	4.1	20
Co	3.6	44
Ce	2.8	39
Mo	2.4	15
Li	2.2	1
V	1.7	20
Se	1.7	1
Th	1.5	5
Sb	1.2	43
Ga	1.1	29
As	0.94	1
Nb	0.80	4
Sc	0.54	59
Cs	0.43	1
W	0.32	50
Nd	0.31	1
Pt	0.30	46
Bi	0.28	10
Cd	0.27	64
U	0.14	4
Eu	0.12	61
Hg	0.11	29
Gd	0.10	1
Sm	0.087	1
Dy	0.082	1
Pr	0.079	1
Yb	0.053	1
Au	0.045	46
Ta	0.044	4
Er	0.043	1
Tl	0.039	51
In	0.014	25
Lu	0.010	6

* Tattoo ink that is registered in connection with skin reactions

Be, Ru, Te, Tb, Ho, Tm, Os or Ir were not demonstrated in any of the tattoo inks.

A content of Ni was demonstrated in all inks. None of the tattoo inks have a label stating that they contain Ni.

The white colours contain titanium dioxide, which corresponds to demonstration of Ti in the white tattoo inks. Ti was also demonstrated in other tattoo inks as titanium dioxide is used to lighten colours. The result of Ti is underestimated, see explanation in the method description of ICP/MS, section 4.2.2.

The high content of Cu is anticipated to originate from phthalocyanines, which is in keeping with the findings in the blue and green tattoo inks that according to the labelling and the safety data sheets contain phthalocyanines. Four selected tattoo inks were analysed to determine the content of Cu more accurately, see results of phtahalocyanines. By means of the applied method, the amount of Cu contained in phthalocyanines and possibly added copper in free form was determined. Therefore, it was not possible to determine soluable copper in the tattoo inks.

Al was demonstrated in high as well as low concentrations in many tattoo inks, but it is presumably not used as pigment. In low concentrations it is most likely due to residue from production, whereas high concentrations might indicate the addition of aluminium (e.g. in the form of aluminium silicate or magnesium aluminium silicate) in order to influence the ability to float (thixotropical properties).

Apart from the above specifications on Ti and Cu there was no connection between tattoo inks and content of certain elements.

4.3.2 Results for carbon black

The quantitative content of carbon black was investigated in five black tattoo inks. The results of the TGA analysis appears in Table 4.3.

Table 4.3 Results of TGA analysis

Ink no.	Colour	% weight loss N_2 (50-200 °C)	% weight loss N_2 (200-500 °C)	% weight loss O_2 (carbon black)	Remaining % weight loss
10	Grey	78.7	-	0.55	20.8
12	Black	47.0	4.0	33.4	15.6
23	Black	46.1	3.6	31.6	18.7
30	Black	68.9	6.2	10.8	14.1
43	Black	39.2	9.1	33.2	18.5

The content of carbon black corresponds to the result of ”carbon black” and is therefore 0.55 %, 33.4 %, 31.6 %, 10.8 % and 33.2 %, respectively. That corresponds to a content of 5.500 µg/g, 334.000 µg/g, 316.000 µg/g, 108.000 µg/g and 332.000 µg/g, respectively.

Ink no. 12 and 23 are black tattoo inks that according to the information on the labels are expected to contain carbon black. No information exists on the content of carbon black in ink no. 43, but from the TGA analysis it appears

that the tattoo ink has a content of carbon black corresponding to ink no. 12 and ink no. 23.

Ink no. 10 is a grey colour which is in keeping with the ink containing a smaller amount of carbon black than the other black colours.

Ink no. 30 is a black ink that is sold to tattooists, but it is not intended for tattooing.

4.3.3 Results for phthalocyanines

Six tattoo inks were investigated. The test results appear in Table 4.4.

Table 4.4 Test results for phthalocyanines

Ink no.	Colour	Phthalocyanine blue pigment (yellow-green colour reaction)	Phthalocyanine green pigment (reddish colour reaction)	Flocculation
31	Green		Positive	Positive
35*	Violet		Positive	Positive
44	Green		Positive	Positive
45	Blue	Positive		Positive
50	Violet		Positive	Positive
60	Green		Positive	Positive

'*' Tattoo ink that is registered in connection with skin reactions

All of the six investigated tattoo inks contain phthalocyanines.

Phthalocyanines contain a metal – e.g. Pigment Blue 15 contains copper. The results from the ICP/MS screening are consistent with the expectations as the content of copper in the colours varies giving bright colours a lower content than the darker colours, see Enclosure C. The highest content of copper was found in the dark blue inks.

On the basis of the information on the labels and from the data sheets, 4 colours were found to contain Phthalocyanine Blue 15:3 (Pigment Blue 15, CAS no. 147-14-8), and simultaneously a high content of copper was found during the ICP/MS screening, see Table 4.5.

The content of copper in these inks was subsequently determined quantitavely by ICP-AES to obtain better quantification. The result of the analysis was used to estimate the content of Phthalocyanine Blue 15:3 in the colours.

The results in Table 4.5 show that the blue tattoo inks have a high content of copper and phthalocyanine followed by the green colour. As expected, the light-blue ink has a lower Cu content than the dark blue ink and therefore a lower phthalocyanine content.

The calculation in Table 4.5 was carried out by using the molar weights for copper (63.5 g/mol) and Phthalocyanine Blue 15:3 (576.1 g/mol) as the result of copper is timed by 576.1 and divided by 63.5.

Table 4.5 Calculation of content of Phthalocyanine Blue 15:3 in selected inks

Ink no.	Colour	Content of Cu µg/g	Calculated content of Phthalocyanine Blue 15:3 µg/g	Percentage by weight % w/w
7	Dark green	12,300	112,000	11.2
8	Blue	19,200	174,000	17.4
15	Blue	20,800	189,000	18.9
25	Pale blue	5,130	46,500	4.65

4.3.4 Results for PAH

The quantitative content of selected polycyclic aromatic hydrocarbons (PAH) was determined in 19 tattoo inks (black, blue, red, orange and violet colours).

A content of PAH exceeding 0.5 µg/g was demonstrated in 14 of the 19 investigated tattoo inks. ResAP(2008)1 of the Council of Europe recommends <0.5 µg/g (<0.5 ppm). The highest content of PAH was demonstrated in the black inks and at the same time the black inks have the highest content of carbon black. That might indicate a connection between the content of carbon black and the demonstration of PAH. It cannot be ruled out that PAH might originate from another source.

The highest content of PAH was demonstrated in two black tattoo inks (ink no. 3 and 11). In ink no. 3 a content of 81 µg/g naphthalene and 27 µg/g pyrene was demonstrated while a content of 28 µg/g pyrene and a content of 5.3 µg/g of benz(a)pyrene were demonstrated in ink no. 11 (the stated results are the average of the analysis in duplicate).

The selected PAHs did not demonstrate a content exceeding the detection limits (0.15-0.5 µg/g) in five tattoo inks, see Table 4.6.

Table 4.6 Tattoo ink with no content of PAH

Ink no.	Colour
30	Black
42	Black
48	Red
49 *	Red
50	Violet

'*' Tattoo ink that is registered in connection with skin reactions

The results of the analyses for PAH appear in Table 4.7-Table 4.9. Ave. means the average of the analysis in duplicate and SD is the calculated standard deviation of the analysis in duplicate. The specification of <DL means that the result is below the detection limit (0.15-0.5 µg/g).

Table 4.7 Result of GC/MS analysis for PAH, CAS no. and detection limits, black and grey inks, µg/g

Ink no.			2, black				3, black				10, grey				11, black			
Name	CAS no.	DL	2a	2b	Ave.	SD	3a	3b	Ave.	SD	10a	10b	Ave.	SD	11a	11b	Ave.	SD
Naphthalene	91-20-3	0.5	<DL	<DL			63	98	81	25	1.2	0.73	1.0	0.3	1.4	1.5	1.4	0.1
Acenaphthylene	208-96-8	0.2	<DL	<DL			1.9	2.0	1.9	0.04	<DL	<DL			<DL	<DL		
Acenaphtene	83-32-9	0.2	<DL	<DL			1.6	1.8	1.7	0.1	<DL	<DL			<DL	<DL		
Fluorene	86-73-7	0.2	<DL	<DL			0.37	0.41	0.39	0.03	<DL	<DL			<DL	<DL		
Phenanthrene/ anthracene	85-01-8 /120-12-7	0.2	<DL	<DL			1.4	1.6	1.5	0.1	<DL	<DL			2.3	2.4	2.3	0.05
Fluoranthene	206-44-0	0.2	<DL	<DL			3.3	3.2	3.3	0.01	<DL	<DL			7.4	7.6	7.5	0.1
Pyrene	129-00-0	0.2	<DL	<DL			28	26	27	2	0.52	0.52	0.52	0.003	29	28	28	1.0
Benz(a)anthracene/ chrysene	56-55-3 /218-01-9	0.2	<DL	<DL			1.3	1.0	1.1	0.3	<DL	<DL			<DL	<DL		
Benz(b)fluoranthene	205-99-2	0.2	0.30	0.34	0.32	0.03	<DL	<DL			<DL	<DL			0.99	1.3	1.1	0.2
Benz(k)flouranthene	207-08-9	0.2	0.32	0.35	0.33	0.02	<DL	<DL			<DL	<DL			0.90	1.2	1.0	0.2
Benz(a)pyrene	50-32-8	0.2	<DL	<DL			<DL	<DL			<DL	<DL			4.7	6.0	5.3	0.9
Indeno(123)pyrene	193-39-5	0.15	0.17	0.18	0.17	0.01	<DL	<DL			<DL	<DL			0.63	0.69	0.66	0.04
Dibenz(ah)anthracene	53-70-3	0.15	0.18	0.21	0.19	0.02	<DL	<DL			<DL	<DL			<DL	<DL		
Benz(ghi)perylene	191-24-2	0.15	0.16	0.19	0.17	0.02	<DL	<DL			<DL	<DL			5.5	5.8	5.6	0.2

Table 4.8 Result of GC/MS analysis for PAH, black inks, µg/g

Ink no.	12, black				23, black				43, black				51, black				58, black			
Name	12a	12b	Ave.	SD	23a	23b	Ave.	SD	43a	43b	Ave.	SD	51a	51b	Ave.	SD	58a	58b	Ave.	SD
Naphthalene	2.1	2.3	2.2	0.1	2.8	3.1	2.9	0.2	3.1	2.9	3.0	0.2	0.8	0.8	0.8	0.1	4.7	5.3	5.0	0.5
Acenaphthylene	1.2	1.4	1.3	0.1	1.4	1.8	1.6	0.3	<DL	<DL			<DL	<DL			<DL	<DL		
Acenaphtene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Fluorene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Phenanthrene/ anthracene	1.6	1.9	1.7	0.2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Fluoranthene	4.1	5.2	4.6	0.8	1.5	2.1	1.8	0.4	<DL	<DL			<DL	<DL			<DL	<DL		
Pyrene	21	26	23	3	12	17	15	4	<DL	<DL			<DL	<DL			0.76	0.71	0.73	0.03
Benz(a)anthracene/ chrysene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benz(b)fluoranthene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benz(k)flouranthene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benz(a)pyrene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Indeno(123)pyrene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Dibenz(ah)anthracene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benz(ghi)perylene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		

Table 4.9 Result of GC/MS analysis for PAH, blue, red and orange inks, µg/g

Ink no.	15, blue				17, red				18*, red				20, orange				45, blue			
Name	15a	15b	Ave.	SD	17a	17b	Ave.	SD	18a*	18b*	Ave.	SD	20a	20b	Ave.	SD	45a	45b	Ave.	SD
Naphthalene	2.0	1.8	1.9	0.2	1.6	1.5	1.6	0.1	1.7	1.6	1.6	0.1	1.8	0.81	1.3	0.7	2.3	3.3	2.8	0.7
Acenaphthylene	<100	<100			<100	<100			<100	<100			<100	<100			<DL	<DL		
Acenaphtene	<100	<100			<100	<100			<100	<100			<100	<100			<DL	<DL		
Fluorene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Phenanthrene/ anthracene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Fluoranthene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Pyrene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benz(a)anthracene/ chrysene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benz(b)fluoranthene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benz(k)flouranthene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benz(a)pyrene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Indeno(123)pyrene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Dibenz(ah)anthracene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benz(ghi)perylene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		

<100 means that the detection limit has been increased due to interference of another substance with the same retention time and ions. The four tattoo inks are from the same colour series

* Tattoo ink that is registered in connection with skin reactions

4.3.5 Results for p-phenylendiamine (PPD)

p-Phenylendiamine (CAS no. 106-50-3) above the detection limit of 4 µg/g was not demonstrated in the 30 investigated tattoo inks, see Table 4.1 for colour number.

4.3.6 Results for PAA liberated from azo colorants

A quantitative analysis was carried out for content of selected primary aromatic amines (PAA) liberated from azo colorants in 19 tattoo inks. The analysis also determined PAA, which is present in free form and not necessarily liberated from the azo colorants, but can be added or be residue or a break-down product.

A content of primary aromatic amines (PAA) was demonstrated in all of the investigated tattoo inks, e.g. aniline and o-toluidine were demonstrated in 13 of the tattoo inks and o-anisidine was demonstrated in 15 tattoo inks.

Five products demonstrated a very high content of PAA – ink no. 26 (green), 27 (yellow) and 49 (red) are in question as o-anisidine was demonstrated and 53 (red) and 57 (brown) in which aniline and 4-Methyl-m-phenylendiamine were demonstrated. Tattoo ink no. 49, 53 and 57 are registered in connection with skin reactions.

Ink no. 53 (red) and 57 (brown) indicate a content of one or more azo colorants that can liberate PAA. The reason is that when analysing for PAA, which is not liberated from the azo colorants, a much smaller content of PAA was demonstrated, see section 4.3.7. Ink no. 53 and 57 are from the same manufacturer and no information exists from the supplier about which pigments the colours contain.

Ink 26, 27 and 49 were not analysed for PAA, which is not liberated from the azo colorants, see section 4.3.7. However, it is estimated that the high content of PAA might indicate that the three colours also can contain an azo colour pigment that can be decomposed to PAA. Ink no. 26 and 27 are from the same manufacturer.

Within the analysis programme of this project it has not been possible to identify the azo colorants and therefore it has not been possible to verify the theory that the azo colorant is the source of the demonstrated high content of PAA in the five products.

On the basis of the results, it is not possible to conclude that certain colours contain specific PAA as the content in the colours differ a lot with regard to concentration and with regard to which PAA has been demonstrated.

The results appear in Table 4.10-Table 4.14. Ave. means the average of the analysis in duplicate and SD is the calculated standard deviation of the analysis in duplicate. The specification of <DL means that the result is below the detection limit.

Table 4.10 Result of GC/MS analysis for amount of free PAA and PAA liberated from azo colorants, CAS no. and detection limits, red inks, µg/g

Ink no.			1, red				5, red				18*, red				24*, red			
Name	CAS no.	DL	1A	1B	Ave.	SD	5A	5B	Ave.	SD	18A*	18B*	Ave. *	SD	24A*	24B*	Ave. *	SD
Aniline	62-53-3	0.5	0.49	0.58	0.54	0.06	0.76	0.76	0.76	0	2.0	2.0	2.0	0	26	24	25	1
4-Aminobiphenyl	92-67-1	1	1.1	<DL	1.1		<DL	<DL			<DL	<DL			<DL	<DL		
Benzidine	92-87-5	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4-Chloro-o-toluidine	95-69-2	2	1.1	1.2	1.2	0.1	<DL	<DL			<DL	<DL			<DL	<DL		
2-Naphthylamine	91-59-8	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
5-Nitro-o-toluidine	99-55-8		<DL	<DL			<DL	<DL			<DL	<DL			15	13	14	1
p-Chloroaniline	106-47-8	1	1.1	1.1	1.1	0	<DL	<DL			<DL	<DL			<DL	<DL		
4-Methoxy-m-phenylenediamine	615-05-4	10	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-methylenedianiline	101-77-9	10	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dichlorobenzidine	91-94-1	1	<DL	<DL			<DL	<DL			<DL	<DL			7.3	5.1	6.2	1.6
3,3'-Dimethoxybenzidine	119-90-4	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylbenzidine	119-93-7	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylenedianiline	838-88-0	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
6-Methoxy-m-toluidine (p-Cresidine)	120-71-8	1	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4,'-Methylenebis(2-chloroaniline)	101-14-4	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Oxydianiline	101-80-4	10	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Thiodianiline	139-65-1	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Toluidine	95-53-4	1	10	10	10	0.3	1.2	0.91	1.1	0.2	<DL	<DL			6.8	6.2		
4-Methyl-m-phenylenediamine	95-80-7	1	1.2	1.2	1.2	0	<DL	<DL			<DL	<DL			2.4	1.9		
2,4-5-Trimethylaniline	137-17-7	1	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Anisidine	90-04-0	0.5	0.55	0.59	0.57	0.03	0.91	0.95	0.93	0.03	93	96	95	2	3.3	2.7	3.0	0.4
2,4-Xylidine/2,6-xylidine	95-68-1/87-62-7	1	0.75	0.74	0.75	0.01	0.68	0.68	0.68	0	<DL	<DL			<DL	<DL		

* Tattoo ink that is registered in connection with skin reactions

Table 4.11 Result of GC/MST analysis for amount of free PAA and PAA liberated from azo colorants, red inks, µg/g

Ink no.	48*, red				49*, red				53*, red			
Name	48A*	48B*	Ave.*	SD	49A*	49B*	Ave.*	SD	53A*	53B*	Ave.*	SD
Aniline	13	9.0	11	3	<DL	<DL			320	280	300	28
4-Aminobiphenyl	<DL	<DL			<DL	<DL			<DL	<DL		
Benzidine	<DL	<DL			<DL	<DL			<DL	<DL		
4-Chloro-o-toluidine	<DL	<DL			<DL	<DL			<DL	<DL		
2-Naphthylamine	<DL	<DL			<DL	<DL			<DL	<DL		
5-Nitro-o-toluidine	<DL	<DL			<DL	<DL			150	150	150	6
p-Chloroaniline	2.6	2.2	2.4	0.3	<DL	<DL			99	100	100	1
4-Methoxy-m-phenylenediamine	<DL	<DL			28	51	40	16	<DL	<DL		
4,4'-methylenedianiline	<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dichlorobenzidine	<DL	<DL			<DL	<DL			5.9	5.6	5.8	0.2
3,3'-Dimethoxybenzidine	<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylbenzidine	<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylenedianiline	<DL	<DL			<DL	<DL			<DL	<DL		
6-Methoxy-m-toluidine (p-Cresidine)	<DL	<DL			<DL	<DL			<DL	<DL		
4,4,'-Methylenebis(2-chloroaniline)	<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Oxydianiline	<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Thiodianiline	<DL	<DL			<DL	<DL			<DL	<DL		
o-Toluidine	4.0	4.6	4.3	0.4	<DL	<DL			21	19	20	1
4-Methyl-m-phenylenediamine	<DL	<DL			<DL	<DL			>400	>400	>400	
2,4-5-Trimethylaniline	<DL	<DL			<DL	<DL			<DL	<DL		
o-Anisidine	42	68	55	18	>424	>425	>424		5.7	1.4	3.6	3.0
2,4-Xylidine/2,6-xylidine	<DL	<DL			<DL	<DL			<DL	<DL		

* Tattoo ink that is registered in connection with skin reactions

Table 4.12 Result of GC/MS analysis for amount of free PAA and PAA liberated from azo colorants, blue and green inks, µg/g

Ink no.	7, green				26, green				44, green				45, blue				60, green			
Name	7A	7B	Ave.	SD	26A	26B	Ave.	SD	44A	44B	Ave.	SD	45A	45B	Ave.	SD	60A	60B	Ave.	SD
Aniline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			2.0	1.4	1.7	0.4
4-Aminobiphenyl	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4-Chloro-o-toluidine	<DL	<DL			<DL	<DL			<DL	<DL			13	17	15	3	<DL	<DL		
2-Naphthylamine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
5-Nitro-o-toluidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
p-Chloroaniline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4-Methoxy-m-phenylenediamine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-methylenedianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dichlorobenzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethoxybenzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylbenzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylenedianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
6-Methoxy-m-toluidine (p-Cresidine)	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4,'-Methylenebis(2-chloroaniline)	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Oxydianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Thiodianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Toluidine	2.6	2.6	2.6	0	<DL	<DL			132	133	133	1	0.92	<DL			42	42	42	0
4-Methyl-m-phenylenediamine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
2,4-5-Trimethylaniline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Anisidine	<DL	<DL			1800	1750	1775	35	9.6	1.4	5.5	5.8	0.96	0.54	0.75	0.30	<DL	<DL		
2,4-Xylidine/2,6-xylidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		

Table 4.13 Result of GC/MS analysis for amount of freePAA and PAA liberated from azo colorants, yellow and orange inks, µg/g

Ink no.	20, orange				27, yellow				36*, yellow				65, orange			
Name	20A	20B	Ave.	SD	27A	27B	Ave.	SD	36A*	36B*	Ave.*	SD	65A	65B	Ave.	SD
Aniline	55	57	56	1	<DL	<DL			3.1	2.9	3.0	0.1	110	110	110	0
4-Aminobiphenyl	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4-Chloro-o-toluidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
2-Naphthylamine	<DL	<DL			<DL	<DL			<DL	<DL			2.8	2.4	2.6	0.3
5-Nitro-o-toluidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
p-Chloroaniline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4-Methoxy-m-phenylenediamine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-methylenedianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dichlorobenzidine	<DL	<DL			<DL	<DL			2.5	2.5	2.5	0	<DL	<DL		
3,3'-Dimethoxybenzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylbenzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylenedianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
6-Methoxy-m-toluidine (p-Cresidine)	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4,'-Methylenebis(2-chloroaniline)	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Oxydianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Thiodianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Toluidine	<DL	<DL			0.70	0.66	0.68	0.03	<DL	<DL			1.4	1.1	1.3	0.2
4-Methyl-m-phenylenediamine	<DL	<DL			<DL	<DL			<DL	<DL			13	18	16	4
2,4-5-Trimethylaniline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Anisidine	<DL	<DL			1050	1250	1150	141	5.6	5.5	5.6	0.1	<DL	<DL		
2,4-Xylidine/2,6-xylidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
p-Phenylenediamine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		

* Tattoo ink that is registered in connection with skin reactions

Table 4.14 Result of GC/MS analysis for amount of free PAA and PAA liberated from azo colorants, violet and brown inks, µg/g

Ink no.	35*, violet				37*, violet				57*, brown			
Name	35A*	35B*	Ave.*	SD	37A*	37B*	Ave.*	SD	57A*	57B*	Ave.*	SD
Aniline	4.4	4.0	4.2	0.3	9.8	10.2	10	0.3	240	220	230	14
4-Aminobiphenyl	<DL	<DL			<DL	<DL			<DL	<DL		
Benzidine	<DL	<DL			<DL	<DL			<DL	<DL		
4-Chloro-o-toluidine	<DL	<DL			<DL	<DL			<DL	<DL		
2-Naphthylamine	<DL	<DL			<DL	<DL			<DL	<DL		
5-Nitro-o-toluidine	<DL	<DL			<DL	<DL			>400	>400		
p-Chloroaniline	<DL	<DL			<DL	<DL			72	72	72	0
4-Methoxy-m-phenylenediamine	<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-methylenedianiline	<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dichlorobenzidine	<DL	<DL			<DL	<DL			4.0	4.0	4.0	0
3,3'-Dimethoxybenzidine	<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylbenzidine	<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylenedianiline	<DL	<DL			<DL	<DL			<DL	<DL		
6-Methoxy-m-toluidine (p-Cresidine)	<DL	<DL			<DL	<DL			<DL	<DL		
4,4,'-Methylenebis(2-chloroaniline)	<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Oxydianiline	<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Thiodianiline	<DL	<DL			<DL	<DL			<DL	<DL		
o-Toluidine	1.5	1.4	1.5	0.1	<DL	<DL			13	13	13	0
4-Methyl-m-phenylenediamine	<DL	<DL			<DL	<DL			>200	>200		
2,4-5-Trimethylaniline	<DL	<DL			<DL	<DL			<DL	<DL		
o-Anisidine	0.54	0.49	0.52	0.04	4.1	4.2	4.2	0.1	1.4	6.6	4.0	3.7
2,4-Xylidine/2,6-xylidine	<DL	<DL			<DL	<DL			<DL	<DL		
p-Phenylenediamine	<DL	<DL			<DL	<DL			<DL	<DL		

'*' Tattoo ink that is registered in connection with skin reactions

4.3.7 Results for PAA from other sources

The quantitative content of selected primary aromatic amines (PAA) that did not emerge from the decomposition of azo colorants was analysed in 24 tattoo inks. In this report, these PAA are called “the free PAA”. The sources can e.g. be residue from the production of pigments or from the decomposition of the pigments. The analysis was carried out together with the analysis for PPD, see results for PPD in section 4.3.5. In addition, the content of free PAA was also demonstrated by analysis for PAA liberated from azo colorants as the results in section 4.3.6 comprise the amount of free and liberated PAA.

Content of PAA above the detecction limit was not demonstrated in 10 of the 24 analysed tattoo inks. Ink no. 5, 7, 8, 10, 12, 13, 15, 20, 30 and 43 (red, green, blue, grey and black) are in question.

Of the 24 tattoo inks, nine colours were analysed as they were registered in connection with skin reactions, see section 1.7. In eight of the nine colours a content of PAA was demonstrated, which does not originate from the decomposition of azo colorants (ink no. 18, 24, 35, 36, 37, 48, 53 and 57).

The highest content of PAA that does not originate from the decomposition of azo colorants was found in tattoo ink 53 (red) and 57 (brown), which also are registered in connection with skin reactions.

In connection with the analysis for PDD, ink no. 53 demonstrated a higher content of 5-Nitro-o-toluidine than was demonstrated in the analysis for PAA liberated from azo colorants including PAA from other sources, see section 4.3.6. The discrepancy is presumably due to problems related to the extraction of homogeneous subsamples, see section 4.2.1.

An additional 6 products were analysed for PAA in connection with analysis for PAA that can be liberated from the azo colorants, see 4.3.6, where PAA was demonstrated in all investigated inks. In total, a PAA content was demonstrated in 20 out of 30 investigated inks.

On the basis of the results, it is not possible to conclude that certain inks contain specific PAA as the content in the inks differs a lot with regard to concentration and with regard to which PAA has been demonstrated.

The results of demonstrated PAA that have not appeared by decomposition of azo colorants can be seen in Table 4.15-Table 4.17. Ave. means the average of the analysis in duplicate and SD is the calculated standard deviation of the analysis in duplicate. The specification of <DL means that the result is below the detection limit.

Table 4.15 Result of GC/MS analysis for PAA that has not appeared from the decomposition of azo colorants. CAS no. and detection limits, black, blue and brown inks, µg/g

Ink no.			23, black				25, blue				45, blue				57*, brown			
Name	CAS #	DL	23A	23B	Aver.	SD	25A	25B	Ave.	SD	45C	45D	Ave.	SD	57C*	57D*	Ave.*	SD
Aniline	62-53-3	1	<DL	<DL			<DL	<DL			<DL	<DL			80	77	79	2
4-Aminobiphenyl	92-67-1	1	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benzidine	92-87-5	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4-Chloro-o-toluidine	95-69-2	2	<DL	<DL			<DL	<DL			5.8	6.0	5.9	0.1	<DL	<DL		
2-Naphthylamine	91-59-8	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Aminoazotoluene	97-56-3	10	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
5-Nitro-o-toluidine	99-55-8	5	<DL	<DL			<DL	<DL			<DL	<DL			58	57	58	0.7
p-Chloroaniline	106-47-8	1	<DL	<DL			<DL	<DL			<DL	<DL			2.2	1.9	2.1	0.2
4-Methoxy-m-phenylenediamine	615-05-4	10	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-methylenedianiline	101-77-9	10	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dichlorobenzidine	91-94-1	1	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethoxybenzidine	119-90-4	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylbenzidine	119-93-7	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylenedianiline	838-88-0	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
6-Methoxy-m-toluidine (p-Cresidine)	120-71-8	1	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4,'-Methylenebis(2-chloroaniline)	101-14-4	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Oxydianiline	101-80-4	10	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Thiodianiline	139-65-1	2	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Toluidine	95-53-4	1	<DL	<DL			<DL	<DL			<DL	<DL			1.0	1.0	1.0	0
4-Methyl-m-phenylenediamine	95-80-7	1	<DL	<DL			<DL	<DL			<DL	<DL			1.7	1.8	1.8	0.1
2,4-5-Trimethylaniline	137-17-7	1	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Anisidine	90-04-0	1	4.6	5.2	4.9	0.4	4.6	5.2	4.9	0.4	<DL	<DL			<DL	<DL		
2,4-Xylidine/2,6-xylidine	95-68-1/87-62-7	1	<DL	<DL			<DL	<DL			<DL	<DL			0.4	0.4	0.4	0.0

* Tattoo ink that is registered in connection with skin reactions

Table 4.16 Result of GC/MS analysis for PAA that has not appeared from the decompostion of azo colorants. Yellow, violet and red, µg/g

Ink no.	36*, yellow				35*, violet				37*, violet				50, violet				18*, red			
Name	36C*	36D*	Ave.*	SD	35C*	35D*	Ave.*	SD	37C*	37D*	Ave.*	SD	50C	50D	Ave.	SD	18C*	18D*	Ave.*	SD
Aniline	2.3	2.2	2.3	0.1	2.0	2.0	2.0	0	1.6	1.6	1.6	0	<DL	<DL			<DL	<DL		
4-Aminobiphenyl	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4-Chloro-o-toluidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
2-Naphthylamine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Aminoazotoluene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
5-Nitro-o-toluidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
p-Chloroaniline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4-Methoxy-m-phenylenediamine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-methylenedianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dichlorobenzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethoxybenzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylbenzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylenedianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
6-Methoxy-m-toluidine p-Cresidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4,'-Methylenebis(2-chloroaniline)	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Oxydianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Thiodianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Toluidine	<DL	<DL			0.90	0.80	0.85	0.07	<DL	<DL			2.0	2.0	2.0	0	<DL	<DL		
4-Methyl-m-phenylenediamine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
2,4-5-Trimethylaniline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Anisidine	4.5	4.7	4.6	0.1	<DL	<DL			0.40	0.30	0.35	0.07	<DL	<DL			4.6	5.2	4.9	0.4
2,4-Xylidine/2,6-xylidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		

* Tattoo ink that is registered in connection with skin reactions

Table 4.17 Result of GC/MS analysis for PAA that have not appeared from the decomposition of azo colorants. Red, µg/g

Colour no.	24*, red				34, red				48*, red				49*, red				53*, red			
Name	24C*	24D*	Ave.*	SD	34A	34B	Ave.	SD	48C*	48D*	Ave.*	SD	49C*	49D*	Ave.*	SD	53C*	53D*	Ave.*	SD
Aniline	4.1	3.3	3.7	1	<DL	<DL			<DL	<DL			<DL	<DL			27	29	28	1
4-Aminobiphenyl	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
Benzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4-Chloro-o-toluidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
2-Naphthylamine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Aminoazotoluene	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
5-Nitro-o-toluidine	6.5	5.9	6.2	0.4	6.0	6.5	6.3	0	<DL	<DL			<DL	<DL			190	190	190	3
p-Chloroaniline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			6.1	6.5	6.3	0.3
4-Methoxy-m-phenylenediamine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-methylenedianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dichlorobenzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			3.7	3.7	3.7	0
3,3'-Dimethoxybenzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylbenzidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
3,3'-Dimethylenedianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
6-Methoxy-m-toluidine p-Cresidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4,'-Methylenebis(2-chloroaniline)	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Oxydianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
4,4'-Thiodianiline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Toluidine	2.8	2.9	2.9	0.1	<DL	<DL			1.1	1.2	1.2	0.1	<DL	<DL			1.4	1.3	1.4	0.1
4-Methyl-m-phenylenediamine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			2.6	2.6	2.6	0
2,4-5-Trimethylaniline	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		
o-Anisidine	0.60	0.50	0.55	0.07	34	34	34	0	8.7	9.3	9.0	0.4	15	15	15	0	<DL	<DL		
2,4-Xylidine/2,6-xylidine	<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL			<DL	<DL		

* Tattoo ink that is registered in connection with skin reactions

4.3.8 Other PAA demonstrated through GC/MS analysis

The GC/MS analyses in section 4.3.6 and 4.3.7 demonstrated a content of other PAA that are determined semi-quantitavely against aniline. The substances were identified by means of their mass spectra by searching in the NIST MS library.

The results show that in eight out of nine tattoo inks, registered in connection with skin reactions, other PAA have been demonstrated (ink no. 18, 24, 35, 37, 48, 49, 53 and 57).

There can be a difference in the content determined by method GC/MS B, see section 4.2.6, and GC/MS C, see 4.2.7, as method C measures the PAA that have not appeared by decomposition of the azo colorants (”free PAA”), while method B is the amount of PAA liberated from azo colorants and PAA from other sources (”free PAA”).

Table 4.18 Semi-quantitative determination of other PAA through method GC/MS B, red colours, µg/g

Name	CAS no.	Ink no.						
		red	red	red	red	red	red	red
		1A	5A	18A*	24A*	48A*	49A*	53A*
2-Ethoxybenzenamine	94-70-2		250	230	10	25	60	25
3-Methoxybenzenamine	536-90-3						140	12
Chloro-toluidine	95-74-9/615-65-6							14
4-Methyl-1,2-benzendiamine	496-72-0/95-70-5/2687-25-4							20
Dichlorobenzamine	95-82-9/95-76-1/608-27-5							130
4-Amino-2-hydroxytoluene	2835-95-2							7
Trichlorobenzamine	634-91-3/634-93-5/636-30-6/634-67-3	20						
Trichlorobenzamine	634-91-3/634-93-5/636-30-6/634-67-3	1100						
2-Nitro-p-toluidine	89-62-3							170
5-Chloro-2,4-dimethoxybenzenamine	97-50-7	240						
4-Chloro-2,5-dimethoxybenzenamine	6358-64-1		80	6				10
1-Amino-2-naphthalenol	2834-92-6	10	14	40	110			

* Tattoo ink that is registered in connection with skin reactions

Table 4.19 Semi-quantitative determination of other PAA through method GC/MS B, other colours, µg/g

Name	Colour no.									
	green	orange	green	yellow	yellow	violet	green	blue	brown	orange
	7A	20A	26A	27A	36A*	37A*	44A	45A	57A*	65A
2-Ethoxybenzenamine	6					45			7	16
3-Methoxybenzenamine			20	14						
Chloro-toluidine								10	7	
Dichlorobenzamine						15			60	6
m-Isopropoxyaniline		100			10	65				
5-Chloro-o-anisidine						340				
4-Chloro-2,5-dimethoxybenzenamine	70				170					
2-Nitro-p-anisidine				14						

Name	Colour no.									
	green	orange	green	yellow	yellow	violet	green	blue	brown	orange
	7A	20A	26A	27A	36A*	37A*	44A	45A	57A*	65A
1-Amino-2-naphthalenol		6								
Pentachloroaniline			10				80			

* Tattoo ink that is registered in connection with skin reactions

Table 4.20 Semi-quantitative determination of other PAA through method GC/MS C, µg/g

Name	CAS no.	Colour no.									
		red	green	blue	red	red	red	yellow	violet	blue	red
		5C	13A	15A	18C*	24C*	34A	36C*	37C*	45C	49C*
2-Ethoxybenzenamine	94-70-2	20			20	35	8		6		30
m-Isopropoxyaniline	41406-00-2			20						500	
5-Chloro-o-anisidine	95-03-4								150		
5-Chloro-2,4-dimethoxybenzenamine	97-50-7							10			
4-Chloro-2,5-dimethoxybenzenamine	6358-64-1							180	5		
2-Nitro-p-anisidine	96-96-8						4				
Pentachloroaniline	527-20-8		15								

* Tattoo ink that is registered in connection with skin reactions

4.4 Summary of results of chemical analyses

A wide range of different metals and other elements, e.g. Ba, Pb, Hg, Cd, Cu, Zn, Cr, Ni, Ag, Au, Sn, Al, Si and As were demonstrated in the tattoo inks, see section 4.3.1 and Table 4.2. No connection was demonstrated between tattoo inks (colour) and content of certain elements exceeding the expected Cu in the tattoo inks that contain phthalocyanines (e.g. green and blue colours) and Ti in the tattoo inks that contain titanium dioxide (e.g. white colour).

A comparison of results was carried out on 61 tattoo inks from analysis for metals and other elements with the recommendations in ResAP(2008)1[56] of the Council of Europe (also refer to Table 1.5 in section 1.10.1):

- As was demonstrated in 51 tattoo inks, however, all of them in concentrations <2 µg/g (<2 ppm), as recommended in ResAP(2008)1 of the European Council.
- Ba was demonstrated in all 61 tattoo inks, and in 53 tattoo inks the concentration was <50 µg/g (<50 ppm), as recommended in ResAP(2008)1 of the Council of Europe. In eight tattoo inks the concentration was >50 µg/g (>50 ppm) (ink no. 13, 26, 27, 28, 33, 34, 61 and 63, two green, two yellow, one orange and three red, respectively). The highest concentration is app. 1800 µg/g (ink no. 61, yellow). The concentration of Ba might be underestimated if the substance appears as barium sulphate.
- Cd was demonstrated in 45 tatoo inks, however, all in concentrations <0.2 µg/g (<0.2 ppm) as recommended in ResAP(2008)1 of the

[56] Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up

Council of Europe. One single colour has a concentration of 0.27 (ink no. 64, peach).

- Co was demonstrated in 43 tattoo inks, however, all in concentrations <25 µg/g (<25 ppm), as recommended in ResAP(2008)1 of the Council of Europe.
- Cr was demonstrated in 57 tattoo inks and the highest concentration was 31 µg/g (ink no. 20, orange). It is not possible to distinguish between Cr III and Cr VI with the applied analysis method (ICP/MS), and therefore it is not possible to compare the results with those of ResAP(2008)1 of the Council of Europe which state a limit value for Cr VI of 0.2 µg/g.
- Cu was demonstrated in all 61 tattoo inks. By means of the applied analysis method (ICP/MS) it is not possible to distinguish between extractable copper and copper from phthalocyanines. A number of tattoo inks that are not expected to contain phthalocyanines as they are not green/blue contain Cu in concentrations <25 µg/g (<25 ppm) as recommended in ResAP(2008)1 of the Council of Europe. Two tattoo inks no. 20 and 57 (orange and brown, respectively) contain 100 and 140 µg/g, respectively, which can be extractable copper or copper from phthalocyanines. None of the two tattoo inks state a content of phthalocyanines or extractable copper.
- Hg was demonstrated in two tattoo inks with a concentration of 0.11 µg/g (0.11 ppm) (ink no. 29, peach) and 0.038 µg/g (0.038 ppm, ink no. 45, blue), which is below the recommended <0.2 ppm in ResAP(2008)1 of the Council of Europe.
- Ni was demonstrated in all 61 tattoo inks. The highest concentration of Ni is 18 µg/g (ink no. 20, orange). ResAP(2008)1 of the Council of Europe recommends that it should be stated on the label of the product if there is a content of Ni, and the detection limit should be as low as technically possible. No tattoo inks have a label stating that they contain Ni.
- Pb was demonstreated in all 61 tattoo inks, but the vast majority contain concentrations of <2 µg/g (<2 ppm) as recommended in ResAP(2008)1 of the Council of Europe. Pb concentrations of 3,2, 5,7, 9,3 and 10 µg/g, respectively, were found in four tattoo inks from the same colour series (ink no. 7, 8, 6 and 4, green, blue, light green and white, respectively).
- Se was demonstrated in 53 tattoo inks, however, all in concentrations of <2 µg/g (<2 ppm) as recommended in ResAP(2008)1 of the Council of Europe.
- Sb was demonstrated in 12 tattoo inks, however, all in concentrations of <2 µg/g (<2 ppm) as recommended in ResAP(2008)1 of the Council of Europe.
- Sn was demonstrated in 21 tattoo inks, however, in concentrations of <50 µg/g (<50 ppm) as recommended in ResAP(2008)1 of the Council of Europe.
- Zn was demonstrated in all 61 tattoo inks, however, all in concentrations of <50 µg/g (<50 ppm) as recommended in ResAP(2008)1 of the Council of Europe, except for one single tattoo ink (ink no. 33, red) with a concentration of 53 µg/g (53 ppm).

The content of carbon black was determined in five inks with 5.500 µg/g, 334.000 µg/g, 316.000 µg/g, 108.000 µg/g and 332.000 µg/g, respectively, (ink no. 10, 12, 23, 30 and 43, respectively), see section 4.3.2. The three colours with the highest content of carbon black are the black tattoo inks from the most frequently used colour series (ink no. 12, 23 and 43). The colour with the lowest content (5.500 µg/g) is grey (ink no. 10).

Six tattoo inks were investigated for phthalocyanines (blue, green and violet). It is not stated on their labels or in their safety data sheets if they contain phthalocyanines, see section 4.3.3. The analysis showed that all six investigated tattoo inks contain phthalocyanines.

The phthalocyanines often contain copper and in the light of the Cu discovered through ICP/MS screening, the highest content of phthalocyanines was demonstrated in the blue colours, followed by the green. The highest content was demonstrated in a blue colour (ink no. 15), where the content of phthalocyanine Blue 15:3 was estimated to 189.000 µg/g, see section 4.3.3.

Quantitative analyses for selected polycyclic aromatic hydrocarbons (PAH) demonstrated a content of PAH exceeding 0.5 µg/g in 14 of the 19 investigated tattoo inks (black, red, blue, orange and violet), see section 4.3.4. ResAP(2008)1 of the Council of Europe recommends <0.5 µg/g (<0.5 ppm). The black inks have the highest content and that corresponds to a high content of carbon black. The two highest concentrations that were demonstrated were 81 µg/g of naphtalene and 27 µg/g of pyrene in ink no. 3 (the results are the averages of the analyses in duplicate).

p-Phenylendiamine (PPD) was not demonstrated in the 30 investigated tattoo inks (black, red, blue, green, orange and violet), see section 4.3.5.

A content of primary aromatic amines (PAA) was demonstrated in all 19 investigated tattoo inks (in the colours red, yellow, orange, blue, green, violet and brown), see section 4.3.6., which were analysed quantitavely for content of selected PAA liberated from azo colorants. Aniline and o-toluidine were e.g. demonstrated in 13 of the tattoo inks and o-anisidine was demonstrated in 15 tattoo inks. The ResAP(2008)1 of the Council of Europe recommends that there should be no content of the demonstrated PAA. The analysis includes PAA, which can be present in free form or as residue, and therefore the result is no evidence that all 19 tattoo inks contain azo colorants that can liberate PAA.

In five of the 19 investigated products a very high content of PAA was demonstrated – concerning ink no. 26 (green), 27 (yellow) and 49 (red), in which o-anisidine was demonstrated, and 53 (red) and 57 (brown), in which aniline and 4-Methyl-m-phenylendiamine were demonstrated. Tattoo ink no. 49, 53 and 57 are registred in connection with skin reactions, see section1.7. It is estimated that the demonstrated PAA could be liberated from the azo colorants. It has not been possible within the analysis programme of this project to verify the theory on content of azo colorants that can liberate PAA.

24 tattoo inks were analysed for content of free PAA, i.a. PAA that are not liberated from azo colorants, but originate from another source, e.g. added directly, residue during production of pigments or decomposition of pigments. A content of free PAA above the detection limit was demonstrated in 14 of the analysed tattoo inks. During the analysis for PAA that can be

liberated from the azo colorants another 6 colours were investigated for a content of PAA and all colours contained PAA. Therefore, PAA was demonstrated in 20 out of the 30 investigated inks.
All of the nine inks registered in connection with skin reactions when using tattoo inks, see section 1.7 and chapter 6, demonstrated a content of free PAA (ink no. 18, 24, 35, 36, 37, 48, 49, 53 and 57), see section 4.3.6, 4.3.7 and 4.3.8.

In addition to the PAA mentioned in ResAP(2008)1 of the Council of Europe, a number of tattoo inks demonstrated other primary aromatic amines, see section 4.3.8.

It is not possible on the basis of the results for analysis of PAA to conclude that certain inks contain specific PAA as the content in the inks differ a lot with regard to concentration and with regard to which PAA were demonstrated.

5 Health Effect Assessment: Selected chemical substances in tattoo inks

The health risks associated with chemical substances in tattoo inks are often discussed. Since the tattoo inks are introduced directly into the skin, the absorption from the tattood skin area as well as the critical effect(s) of a specific chemical substance in the tattoo inks might differ compared to the situation where the chemical substance is
applied directly on the skin either neat or incorporated in, e.g. colorants.

Tattoo inks contain generally one or more colorants (pigments) as well as coformulants such as, e.g. binders (usually barium sulphate), additives (substances loosely bound to the pigment in order to modify the properties of the pigment), and solvents (usually ethanol and isopropanol). In addition, the finished tattoo inks may also contain chemical impurities. (Kemikalieinspektionen 2010[57]).

The purpose of the health effect assessment conducted in this project was to assess the possible health risks that might be associated with exposure to the selected chemical substances after tattooing with the analysed tattoo inks.

5.1 Health effect assessment: Principles

The health effect assessments have been conducted according to the principles outlined in the Danish EPA Guideline for the preparation of reports in the series of "Survey of Chemical Substances in Consumer Products" of 18 June 2009[58].

According to this Guideline, the health effect assessment should be conducted according to the same principles as laid down for health effect assessments of chemical substances in the REACH Regulation. These principles are described in detail in the REACH Guidance Documents, available on the European Chemicals Agency's (ECHA) website[59].

A health effect assessment (risk assessment) consists of a hazard assessment, an exposure assessment and a risk characterisation. The principles for the hazard assessment and the risk characterisation are briefly described in the following sections (5.1.1 and 5.1.2). The hazard assessments of the selected chemical substances in the analysed tattoo inks are described in Section 5.3, and the risk characterisations are described in Section 5.4.

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[57] Farliga ämnen i tatueringfärger. Utredning av tellsynsansvar samt behov av ytterligare reglering – rapport från ett regeringsupdrag som utförts i samråd med Läkemedelsverket, Socialstyrelsen och Konsumentverket. Kemikalieinspektionen Rapport Nr 3/10, 2010.

[58] Vejledning til udarbejdelse af "Kortlægning af kemiske stoffer i forbrugerprodukter". MILJØstyrelsen, Kemikalier, Forbrugergruppen, 18. juni 2009.

[59] http://reach.jrc.it/docs/guidance_document/information_requirements_en.htm?time=1222948859

5.1.1 Hazard assessment: Principles

A hazard assessment (effect assessment) is generally based on data elucidating the toxicological effects in humans and experimental animals of a given chemical substance.

Exposure to a chemical substance can result in a broad spectrum of toxicological effects varying from mild effects such as e.g. irritation to more severe effects as e.g. allergy and eventually to fatal poisonings. The type and severity of the effects observed is generally correlated with the dose or exposure concentration. (Nielsen et al. 2005[60], Danish EPA 2006[61]).

Most effects are considered as having a threshold, i.e., a dose or exposure concentration below which the effect is not observed (threshold effects). The highest dose not resulting in an adverse effect is often referred to as the NOAEL (No Observed Adverse Effect Level), and the lowest dose resulting in an effect is often referred to as the LOAEL (Lowest Observed Adverse Effect Level). (Nielsen et al. 2005, Danish EPA 2006).

For certain effects, a threshold cannot be identified, e.g. many genotoxic effects as well as carcinogenic effects caused by damage of the genetic material (non-threshold effects). For these effects, a dose-dependent response at all doses above zero is assumed and thus, some risk is considered to exist at any exposure level. Consequently, a NOAEL or LOAEL cannot be established for such effects. Instead a Benchmark Dose (e.g. $BMDL_{10}$ or T_{25}) is often established. (Nielsen et al. 2005, Danish EPA 2006).

When all the relevant data have been evaluated, the "critical effect" is identified, i.e. the effect considered as being the essential one for the subsequent risk assessment. For critical effects with a threshold, a NOAEL or LOAEL is then established; for the critical effects without a threshold, a $BMDL_{10}$ or a T_{25} is then established.

Based on the established NOAEL/LOAEL/$BMDL_{10}$/T_{25}, often referred to as the 'Point of Departure' (PoD), a "Derived No Effect Level" (DNEL, for threshold effects) or "Derived Minimal Effect Level" (DMEL, for non-threshold effects) is derived by applying appropriate uncertainty factors, often called assessment factors (AF). (ECHA, 2008[62]).

5.1.2 Risk characterisation: Principles

Based on the hazard (effect) assessment, i.e. the identification of the critical effect and establishment of DNEL/DMEL, and an exposure assessment, a risk characterisation is carried out.

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[60] Principper for sundhedsmæssig vurdering af kemiske stoffer med henblik på fastsættelse af kvalitetskriterier for luft, jord og vand. Elsa Nielsen, Grete Østergaard, John Christian Larsen og Ole Ladefoged. Afdeling for Toksikologi og Risikovurdering, Danmarks Fødevareforskning. Miljøprojekt Nr. 974 2005. ***In Danish with an English summary***.

[61] Metoder til fastsættelse af kvalitetskriterier for kemiske stoffer i jord, luft og drikkevand med henblik på at beskytte sundheden. Vejledning fra Miljøstyrelsen Nr. 5 2006. ***In Danish***.

[62] Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health. European Chemicals Agency, 2008.

The DNEL/DMEL represents the maximum exposure to which an individual should be exposed. In the risk characterisation, the exposure estimates are compared with the DNEL/DMEL and the so-called risk characterisation ratio (RCR) is calculated, where RCR = exposure/DN(M)EL. If the exposure estimate is lower than the DNEL/DMEL, i.e. RCR <1, the exposure is not considered to pose a health risk in the given situation. (ECHA, 2008[63]).

5.2 Selection of chemical substances for the health effect assessment

The chemical analyses (Chapter 4) have revealed a great variation in the chemical composition of the analysed tattoo inks as well as in the concentration of the chemical substances detected in the analysed tattoo inks. Thus, it was not possible in this project to conduct a health effect assessment for all the chemical substances found in the 61 analysed tattoo inks. Therefore, only a number of the chemical substances has been selected for the health effect assessment. The rationale for the selection of the substances is described below.

In the survey phase of this project (Chapter 1) is was investigated which tattoo inks the professional Danish tattooists used in 2010. Subsequently, 65 of these tattoo inks were purchased (listed in Table 1.3) and the concentration of selected chemical substances / substance groups were analysed in 61 of the 65 tattoo inks in the analytical phase of the project (Chapter 4).

The selection of substances for the health effect assessment was primarily based on the concentrations of the substances found in the analysed tattoo inks. Substances for which the concentration in the analysed tattoo inks was higher than the recommendations given in the Council of Europe's "Resolution ResAP(2008)1 on Requirements & Criteria for Safety of Tattoos & Permanent Make Up"[64] were selected for the health effect assessment. In addition, other substances that were considered to pose a health risk at the concentrations found in the analysed tattoo inks, based on expert judgement, were also selected.

One or more of the following chemical substances / substance groups have been analysed in 61 of the purchased tattoo inks (Table 4.1):

- Selected elements (61 inks)
- Carbon black (4 black inks and one gray)
- Phthalocyanines (6 inks)
- Polycyclic aromatic hydrocarbons (PAH) (19 inks)
- Primary aromatic amines (PAA) released from azo dyes (19 inks)
- PAA not released from azo dyes, called 'free' PAA (24 inks).

The 61 tattoo inks were analysed for 66 elements, including the 13 elements in the Council of Europe's ResAP(2008)1 Table 3 'Maximum allowed

[63] Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health. European Chemicals Agency, 2008.

[64] The Council of Europe Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up. Adopted by the Committee of Ministers on 20 February 2008 at the 1018th meeting of the Ministers' Deputies.

concentrations of impurities in products for tattoos' (reproduced in Table 1.5 of this report).

Selected tattoo inks were analysed for 16 different PAH. In the Council of Europe's ResAP(2008)1 Table 3 'Maximum allowed concentrations of impurities in products for tattoos', the maximum concentration for PAH is 0.5 ppm and for benzo(a)pyrene 5 ppb. The recommended maximum concentration for PAH is a total concentration and it has not been further specified which PAH are included in the total concentration.

Selected tattoo inks were analysed for 23 different PAA (free PAA as well as the sum of free PAA and PAA released from azo dyes). The 23 PAA represent 15 of the 20 aromatic amines included in the Council of Europe's ResAP(2008)1 Table 1 'List of aromatic amines, particularly with regard to their carcinogenic, mutagenic, reprotoxic and sensitising properties, which should neither be present in tattoos and PMU products nor released from azo-colorants' as well as the seven aromatic amines in the Council of Europe's ResAP(2008)1 Table 1 mentioned as 'Other substances classified as carcinogens in Categories 1, 2, and 3 by the European Commission and mentioned in the Council Directive 1967/548/EEC', including p-phenylenediamine.
In addition, the selected tattoo inks were also analysed for aniline, which is not included in the Council of Europe's ResAP(2008)1 Table 1.

The following substances / substance groups were found in higher concentrations than the maximum allowed concentrations in the Council of Europe's ResAP(2008)1 Table 3: Chromium (56/61 inks), nickel (all 61 inks), barium (8/61 inks), copper (17/61 inks), lead (4/61 inks), cadmium (2/61 inks), PAH (14/19 analysed inks), and benzo(a)pyrene (1 /19 analysed inks).
For chromium, it should be noted that the applied analytical method could not distinguish between Cr(III) and Cr(VI). The maximum allowed concentration in the Council of Europe's ResAP(2008)1 Table 3 is for Cr(VI).
For copper, it should be noted that the applied analytical method could not distinguish between soluble copper and copper released from phthalocyanines. The maximum allowed concentration in the Council of Europe's ResAP(2008)1 Table 3 is for soluble copper.
These six elements and PAH were selected for the health effect assessment.

The analyses of free PAA (24 inks) as well as of the sum of free PAA and PAA released from azo dyes (19 inks) revealed that up to 2/3 of the tattoo inks contained one or more of the aromatic amines listed in the Council of Europe's ResAP(2008)1 Table 1 of aromatic amines that should neither be present in tattoos nor released from azo-colorants.
The following PAA were found: o-anisidine (14 inks: sum 11 / free 5), 5-nitro-o-toluidine (4 inks: sum 3 / free 4), p-chloroaniline (4 ink: sum 4 / free 2) , 3.3 '-dichlorobenzidine (4 inks: sum 4 / free 1), 4-methyl-m-phenylenediamine (5 inks: sum 5 / free 2), 4-methoxy-m-phenylenediamine (a single ink: sum 1 / free 0), 4-chloro-o-toluidine (a single ink: sum 1 / free 1), 2-naphthylamine (a single ink: sum 1 / free 0) and o-toluidine (12 inks: sum 11 / free 5). It should be noted that p-phenylenediamine (PPD) was not found in any of the analysed tattoo inks.
Aniline (not included in the Council of Europe's ResAP(2008)1 Table 1) was also found in the analysed inks (11 inks: sum 11 / free 6).

These 10 PAA were selected for the health effect assessment.

Aluminum (> 10 µg/g) was found in most of the tattoo inks (52/61) and titanium (> 10 µg/g) in about half of the inks (34/61). Both elements were selected for the health assessment.

For seven of the black tattoo inks it was noted on the packaging and/or the data sheet that carbon black was used as a pigment (Annex B). Carbon black was found in very high concentrations (> 100,000 µg/g) in the four analysed black inks as well as in the gray ink (5,500 µg/g). Carbon black was selected for the health effect assessment, as it occurs in the majority of black inks and in high concentrations in the analysed inks, and since black ink is the most commonly used one in tattooing (Section 1.6.2).

For 13 tattoo inks (6 blue, 6 green, 1 purple) it wwas noted on the packaging and/or the data sheet that they contained phthalocyanines (Annex B). Phthalocyanines were analysed in six inks, where neither the packaging nor the data sheet informed that the ink contained phthalocyanines; phthalocyanines were found in all six inks.
Phthalocyanines were selected for the health effect assessment as they occur in many inks.

5.3 Hazard assessment of the selected chemical substances

For the selected chemical substances, the hazard assessment included an identification of the critical effect(s) in relation to tattooing and establishment of a DNEL or DMEL for the critical effect(s), if possible. Some types of effects such as, e.g. acute toxicity and local irritation of eyes and airways were not considered as being relevant in relation to tattooing and therefore, these types of effects were not addressed further in this report.

The identification of the critical effect(s) were based on the EU classification of substances according to Annex I of the Council Directive 1967/548/EEC[65] (short form: 67-Directive in section 5.3) and the IARC classification for carcinogenic effects when available, as well as on the critical effects identified in selected expert opinions from national and international bodies. The NOAELs / LOAELs presented below are generally those established in the selected expert opinions from national and international bodies.

5.3.1 Elements

5.3.1.1 Aluminium

Aluminium is not classified for health effects according to Annex I of the 67-Directive, and has not been evaluated by IARC.

In Denmark, a health-based quality criterion for aluminium and inorganic compounds in drinking water has been set (Beltoft and Nielsen 2001[66]). The

[65] Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L 196, 16.8.1967, p. 1.

[66] Beltoft V and Nielsen E (2001): Evaluation of health hazards by exposure to aluminium and inorganic compounds and estimation of a quality criterion in drinking water. The Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration. Prepared for the Danish EPA.

critical effect was considered to be the neurological effects observed in humans. The available human data were considered inadequate for the establishment of a NOAEL or LOAEL for the critical effect.

JECFA has more recently evaluated aluminium as a food additive (JECFA 2007[67]). The critical effects were considered to be the effects on the reproductive system and the effects on the developing nervous system. JECFA has stated that the available studies have many limitations and are not adequate for defining the dose-response relationships. Based on studies with rats, mice and dogs given aluminium in the diet, JECFA considered the LOEL to be in the range of 50-75 mg Al/kg bw per day.
Based on the lower end of the range of the LOELs (50 mg Al/kg bw per day) and application of an overall uncertainty factor of 300 (10 for interspecies differences, 10 for interindividual differences, 3 for deficiencies in the database), and because of the potential for bioaccumulation, JECFA established a PTWI (Provisonal Tolerable Weekly Intake) of 1 mg Al/kg bw.

The PTWI can be converted to a daily dose of approximately 0.2 mg Al/kg bw, which, in principle, corresponds to a DNEL.

In the Danish document (Beltoft and Nielsen 2001[68]), it is mentioned that some individuals are unusual sensitive to some types of aluminium containing antiperspirants and develop skin rashes, which may be related to aluminium.

A well-documented case of aluminium-induced granulomas in a tattoo has been described in Chapter 6 of this report. Aluminium can induce allergic reactions in the skin as well as eczema and inflammation; however, it is still unclear whether these reactions actually are allergic or might be toxicological based on the special physico-chemical reactions that can take place on the surface of particles.

Conclusion:
Based on the above, the critical effect of aluminium in relation to tattooing is considered to be the granulomas in tattoos developed as a result of a local foreign body reaction (see Chapter 6). It cannot be excluded that an allergic reaction might also be involved. A DNEL cannot be established for the critical effect.
The critical systemic effects of aluminium are considered to be the effects on the reproductive system and the developing nervous system. The PTWI set by JECFA is 1 mg Al/kg bw (approximately 0.2 mg Al/kg bw per day, which, in principle, corresponds to a DNEL).

5.3.1.2 Barium

Barium is not classified for health effects according to Annex I of the 67-Directive.

Barium compounds (except barium sulphate, some organic compounds, and other compounds independently included in Annex I) are classified 'Xn, R20/22 - harmful by inhalation and if swallowed'.

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[67] JECFA (2007). Aluminium from all sources, including food additives (addendum). In: WHO Food Additive Series 58, pp. 119-207.
[68] Beltoft V and Nielsen E (2001): Evaluation of health hazards by exposure to aluminium and inorganic compounds and estimation of a quality criterion in drinking water. The Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration. Prepared for the Danish EPA.

Barium chloride is classified 'T; R25 - toxic if swallowed' and 'Xn, R20 - harmful by inhalation'.

Barium has not been evaluated by IARC.

In Denmark, a health-based quality criterion for barium and inorganic water-soluble barium compounds in drinking water has been set (Nielsen and Ladefoged 2006[69]). The critical effect was considered to be the cardiovascular effects observed in humans. Based on a NOAEL of 0.21 mg Ba/kg bw per day and a total uncertainty factor of 10 (for interindividual differences), a TDI (Tolerable Daily Intake) of 0.021 mg Ba/kg bw was calculated.

The TDI corresponds, in principle, to a DNEL.

Conclusion:
Based on the above, the critical effect of barium in relation to tattooing is considered to be the cardiovascular effects. The DNEL is set at 0.02 mg Ba/kg bw per day.

5.3.1.3 Cadmium

Cadmium is not classified for health effects according to Annex I of the 67-Directive.

Cadmium compounds (except compounds independently included in Annex I) are classified 'Xn; R20/21/22 - harmful by inhalation, in contact with skin and if swallowed'.
Cadmium chloride and cadmium sulphate are classified 'Carc. Cat. 2; R45 - may cause cancer', 'Muta. Cat. 2; R46 - may cause heritable genetic damage', 'Repr. Cat. 2; R60-61 - may impair fertility and may cause harm to the unborn child', 'T+; R26 - very toxic by inhalation', 'T; R25 - toxic if swallowed' and 'T; R48/23/25 - toxic: danger of serious damage to health by prolonged exposure through inhalation and if swallowed'.

IARC (IARC 1993[70]) has classified cadmium and cadmium compounds in Group 1 'carcinogenic to humans' (human evidence: sufficient; evidence in experimental animals: sufficient). The Working Group noted that, in making the overall evaluation, they took into consideration the evidence that ionic cadmium causes genotoxic effects in a variety of types of eukaryotic cells, including human cells. This note should probably be seen in light of the fact that cadmium and cadmium compounds primarily have shown carcinogenic effects in the lungs after inhalation, but tumours have also been observed in other organs (prostate and kidney). The IARC evaluation was not changed at the more recent IARC Expert Meeting in March 2009 (Straif et al. 2009[71]).

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[69] Nielsen E and Ladefoged O (2006): Evaluation of health hazards by exposure to Inorganic water-soluble barium compounds. Department of Toxicology and Risk Assessment, Danish Institute for Food and Veterinary Research. Prepared for the Danish EPA.

[70] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 58, Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry. IARC, Lyon, France, 1993, p. 119.

[71] Straif K, Benbrahim-Tallaa L, Baan R, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Guha N, Freeman C, Galichet L, Cogliano V (2009). A review of human carcinogens - Part C: metals, arsenic, dusts, and fibres, on behalf of the WHO International Agency for Research on Cancer Monograph Working Group. International Agency for Research on Cancer, Lyon, France. Lancet 10, 453-454.

Cadmium and cadmium oxide have been included in the EU's risk assessment program for existing substances (EU-RAR 2007[72]). The risk assessment report also addressed the harmful health effects of ionic cadmium.
The critical effects after repeated exposure over a prolonged time were considered to be the effects on bones and kidneys observed in humans; the LOAEL was set at 2 µg Cd/g creatinine. In the risk characterisation, a reference MOS (Margin of Safety) of 3 was considered because a NOAEL could not be established. The reference MOS corresponds, in principle, to an overall uncertainty factor.
With regard to reproductive and developmental toxicity, it was noted that the effects have been observed at high doses in studies of experimental animals that received water-soluble cadmium compounds orally. A NOAEL of 1 mg Cd/kg bw per day was established for effects on fertility and sex organs in experimental animals (rats). In the risk characterisation, a reference MOS of 100 (10 for interspecies variation, 10 for interindividual variation) was considered.
With regard to the carcinogenic effects and genotoxicity, no threshold was considered to exist and hence, a NOAEL or LOAEL was not established. It should be noted that cadmium and cadmium compounds primarily causes cancer in the lungs after inhalation. The carcinogenic effect of cadmium is therefore, not considered as a critical effect in relation to tattooing.

Based on the LOAEL of 2 µg Cd/g creatinine (approximately 40 µg Cd/day, corresponding to approximately 0.6 µg Cd/kg bw per day for an adult person weighing 70 kg) and application of an overall uncertainty factor of 3, a DNEL of approximately 0.2 µg Cd/kg bw per day can be established. It should be noted that since cadmium accumulates in the body, the DNEL should be set as an average value over a week or a month.

In 2009, EFSA established a TWI (Tolerable Weekly Intake) of 2.5 µg Cd/kg bw (approximately 0.4 µg Cd/kg bw per day) based on effects on the kidneys observed in humans (EFSA 2011[73]) . JECFA (JECFA 2010[74]) has established a PTMI (Provisional Tolerable Monthly Intake) of 25 µg Cd/kg bw (approximately 0.8 µg Cd/kg bw per day) based on the same data as EFSA used in establishing their TWI (EFSA 2011).

It should be noted that the DNEL (approximately 0.2 µg Cd/kg bw per day) is of the same magnitude as the EFSA TWI (converted to a daily dose of approximately 0.4 µg Cd/kg bw per day) as well as the JECFA PTMI (converted to a daily dose of approximately 0.8 µg Cd/kg bw per day).

Conclusion:
Based on the above, the critical effects of cadmium in relation to tattooing is considered to be the effects on bones and kidneys. The DNEL is set at 0.2 µg Cd/kg bw per day. It should be noted that since cadmium accumulates in the body, the DNEL should be set as an average value over a week or a month.

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[72] European Union Risk Assessment Report. Cadmium oxide. CAS No.: 1306-19-0, EINECS No: 215-146-2. European Communities, 2007.
[73] Scientific Opinion: Statement on tolerable weekly intake for cadmium. EFSA Panel on Contaminants in the Food Chain (CONTAM), EFSA Journal 2011;9(2):1975.
[74] Cadmium. In: Joint FAO/WHO Expert Committee on Food Additives. Seventy-third meeting, Geneva, 8-17 June 2010. Summary and Conclusions, p. 17. Issued 24 June 2010.

5.3.1.4 Chromium

Chromium is not classified for health effects according to Annex I of the 67-Directive.
Chromium compounds (except compounds independently included in Annex I) are classified 'Carc. Cat. 2; R49 - may cause cancer by inhalation' and 'R43 - may cause sensitisation by skin contact'.

IARC (IARC 1990[75]) has classified chromium (VI) compounds in Group 1 'carcinogenic to humans' (human evidence: sufficient; evidence in experimental animals: sufficient), and metallic chromium and chromium (III) compounds in Group 3 'not classifiable as to their carcinogenicity to humans' (human evidence: inadequate; evidence in experimental animals: inadequate). The IARC evaluation was not changed at the more recent IARC Expert Meeting in March 2009 (Straif et al. 2009[76]).
It should be noted that chromium (VI) compounds have primarily shown a carcinogenic effect in the airways (lung, nasal cavity, sinuses) after inhalation.

Five specific chromium (VI) compounds (chromium trioxide, sodium chromate, sodium dichromate, ammonium dichromate, potassium dichromate) have been included in the EU's risk assessment program for existing substances (EU-RAR 2005[77]). In the risk assessment report it is noted that chromium (VI) becomes reduced to chromium (III) after entering the body, and that repeated exposure leads to accumulation of chromium in several tissues and organs.
In the risk characterisation, skin sensitisation resulting from contact with chromium (VI) is mentioned as being relatively common in humans working with chromium compounds.
The critical effect after repeated exposure over a prolonged period of time was considered to be the effects in the kidneys observed in workers. The available data were not considered sufficient for establishing a NOAEL or LOAEL.
With regard to the carcinogenic effect, no threshold was considered to exist and hence, a NOAEL or LOAEL was not established. It should be noted that chromium (VI) compounds are classified as carcinogenic in the respiratory tract after inhalation. The carcinogenic effect of chromium (VI) compounds is therefore, not considered as a critical effect in relation to tattooing.

Conclusion:
Based on the above, the critical effect of chromium (VI) in relation to tattooing is considered to be sensitisation. A DNEL cannot be established for the critical effect.

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[75] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 49, Chromium, nickel and welding. IARC, Lyon, France, 1990. pp. 257-446.
[76] Straif K, Benbrahim-Tallaa L, Baan R, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Guha N, Freeman C, Galichet L, Cogliano V (2009). A review of human carcinogens - Part C: metals, arsenic, dusts, and fibres, on behalf of the WHO International Agency for Research on Cancer Monograph Working Group. International Agency for Research on Cancer, Lyon, France. Lancet 10, 453-454.
[77] European Union Risk Assessment Report. Chromium trioxide, sodium chromate, sodium dichromate, ammonium dichromate and potassium dichromate. CAS-No.: 1333-82-0, 7775-11-3, 10588-01-9, 7789-09-5 and 7778-50-9, EINECS-No.: 215-607-8, 231-889-5, 234-190-3,232-143-1 and 231-906-6. European Communities, 2005.

5.3.1.5 Copper

Copper is not classified for health effects according to Annex I of the 67-Directive.
Copper chloride is classified 'Xn, R22 - harmful if swallowed'.
Copper sulphate is classified 'Xn, R22 - harmful if swallowed' and 'Xi, R36/38 - irritating to eyes and skin'.

Copper or copper compounds have not been evaluated by IARC.

In Denmark, a health-based quality criterion for copper in drinking water has been set (Nielsen 1997[78]). The critical effect following intake of excess copper in drinking water was considered to be the irritative effects in the gastrointestinal tract observed in humans. A NOAEL or LOAEL for the critical effect could not be established based on the available data. The only systemic effects reported were effects in the liver in young children. It should be noted that it is still discussed whether the effects observed in the liver can be attributed to copper in drinking water.

Copper sulphate, but not copper chloride is classified as a skin irritant. Copper and four specific copper salts (copper (II) sulphate, copper (I) oxide, copper (II) oxide and dicopper chloride trihydroxide) have been included in the EU's risk assessment program for existing substances (the copper industry has, on a voluntarily basis, submitted a risk assessment report V-RAR 2007[79]). According to the risk assessment report, no human data on skin irritation are available. Data from experimental animal studies conducted according to current test guidelines indicated that copper (II) sulphate and copper (I) oxide are mild skin irritants (based on results from only a single study). Based on these data it was concluded (in the risk assessment report) that a classification for skin irritation according to EU classification criteria is not warranted for these two copper salts.

The justification for a classification of copper sulphate, but not copper chloride as skin irritants is not known. Based on the data on skin irritation as reflected in the risk assessment report (V-RAR 2007), it is considered that skin irritation is not likely to be a critical effect of copper in relation to tattooing.

Conclusion:
Based on the above, no critical health effects of copper in relation to tattooing have been identified.

5.3.1.6 Lead

Lead is not classified for health effects according to Annex I of the 67-Directive.

Lead compounds (except compounds independently included in Annex I) are classified 'Repr. Cat. 1; R61 - may cause harm to the unborn child', 'Repr.

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[78] Nielsen E (1997). Evaluation of health hazards by exposure to copper and estimation of a limit value in drinking water. The Instituttet of Toxicology, National Food Agency, Denmark. Prepared for the Danish EPA.

[79] European Union Risk Assessment Report. Copper, copper II sulphate pentahydrate, copper(I)oxide, copper(II)oxide, dicopper chloride trihydroxide. CAS No.: 7440-50-8, 7758-99-8, 1317-39-1, 1317–38–0, 1332-65-6, EINECS No: 231–159–6, 231–847–6, 215-270-7, 215–269–1, 215-572-9. Voluntary Risk Assessment, European Copper Institute, June 2007.

Cat. 3; R62 - possible risk of impaired fertility', 'Xn, R20/22 - harmful by inhalation and if swallowed' and 'R33 – danger of cumulative effects'.

IARC (IARC 2006[80]) has classified inorganic lead compounds in Group 2A 'probably carcinogenic to humans' (human evidence: limited; evidence in experimental animals: sufficient) and organic compounds in group 3 'not classifiable as to their carcinogenicity to humans' (human evidence: inadequate; evidence in experimental animals: inadequate). For the organic lead compounds, the Working group has noted that these are metabolised, at least in part, to ionic lead both in humans and animals and may consequently exert the toxicities associated with inorganic lead.

In Denmark, a health-based quality criterion for lead and inorganic lead compounds in soil has been set (Nielsen 2004[81]).
The critical effects were considered to be the effects on the nervous, haematopoietic and reproductive systems, and the carcinogenic effect. It should be noted that the mode of action for the carcinogenic effect is not completely understood and that tumours have only been seen at relatively high doses. The carcinogenic effect of lead is therefore, not considered as a critical effect in relation to tattooing.
The most critical effect of lead at low concentrations was considered to be the effects on the developing nervous system. It is still discussed whether there is a threshold for the effects on the developing nervous system. Therefore, a NOAEL or LOAEL for the most critical effect could not be established.

In their most recent opinion, the EFSA's CONTAM Panel concluded that there is no evidence for a threshold for the critical effects of lead, including developmental neurotoxicity and nephrotoxicity in adults (EFSA 2010[82]). Based on the available data, a $BMDL_{01}$ (95th percentile lower confidence limit of the benchmark dose (BMD) of 1% extra risk) for the critical effects on the developing nervous system (children as well as the unborn child) was calculated at 12 µg B-Pb/liter. Using an "Integrated Exposure Uptake Biokinetic (IEUBK) model" for lead in children, the $BMDL_{01}$ of 12 µg B-Pb/liter was converted to a dietary intake value of 0.50 µg Pb/kg bw per day.

In an opinion on lead and lead compounds in jewellery, adopted by the ECHA Committee for Risk Assessment (RAC) in the spring of 2011, it was concluded that no threshold for the adverse effect has been identified in humans (ECHA 2011[83]). In their risk assessment, RAC used 1/10 of the EFSA $BMDL_{01}$ of 0.50 µg Pb/kg bw per day, i.e. 0.05 µg Pb/kg bw per day as a sort of a tolerable DMEL.

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[80] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 87, Inorganic and Organic Lead Compounds. IARC, Lyon, France, 2006.
[81] Nielsen E (2004): Evaluation of health hazards by exposure to lead and inorganic lead compounds and estimation of a quality criterion in soil. Department of Toxicology and Risk Assessment, Danish Institute for Food and Veterinary Research. Prepared for the Danish EPA.
[82] EFSA (2010). Scientific Opinion on Lead in Food. EFSA Panel on Contaminants in the Food Chain (CONTAM), EFSA Journal 2010; 8(4):1570, European Food Safety Authority (EFSA), Parma, Italy.
[83] ECHA (2011). Opinion on an Annex XV dossier proposing restrictions on lead and lead compounds in jewellery. ECHA/RAC/RES-O-0000001304-85-03/F adopted 10 March 2011. European Chemicals Bureau, Committee for Risk Assessment (RAC), Helsinki, Finland.

Conclusion:
Based on the above, the critical effect of lead in relation to tattooing is considered to be the effects on the developing nervous system (children as well as the unborn child). A DNEL for the critical effect cannot be established.
ECHA/RAC has set 0.05 µg Pb/kg bw per day as a sort of a tolerable DMEL.

5.3.1.7 Nickel

According to Annex I of the 67-Directive, nickel is classified 'Carc. Cat. 3; R40 - limited evidence of a carcinogenic effect', 'T; R48/23 - toxic: danger of serious damage to health by prolonged exposure through inhalation' and 'R43 - may cause sensitisation by skin contact'.
Nickel sulphate is classified 'Carc. Cat. 1; R49 - may cause cancer by inhalation', 'Muta. Cat. 3; R68 - possible risk of irreversible effects', 'Repr. Cat. 2; R61 - may cause harm to the unborn child', 'T; R48/23 - toxic: danger of serious damage to health by prolonged exposure through inhalation', 'Xn, R20/22 - harmful by inhalation and if swallowed', 'Xi, R38 – irritating to skin' and 'R42/43 - may cause sensitisation by inhalation and skin contact'.
Nickel chloride is classified 'Carc. Cat. 1; R49 - may cause cancer by inhalation', 'Muta. Cat. 3; R68 - possible risk of irreversible effects', 'Repr. Cat. 2; R61 - may cause harm to the unborn child', 'T; R23/25 - toxic by inhalation and if swallowed', 'T; R48/23 - toxic: danger of serious damage to health by prolonged exposure through inhalation', 'Xi, R38 – irritating to skin' and 'R42/43 - may cause sensitisation by inhalation and skin contact'.
Nickel nitrate is classified 'Carc. Cat. 1; R49 - may cause cancer by inhalation', 'Muta. Cat. 3; R68 - possible risk of irreversible effects', 'Repr. Cat. 2; R61 - may cause harm to the unborn child', 'T; R48/23 - toxic: danger of serious damage to health by prolonged exposure through inhalation', 'Xn, R20/22 - harmful by inhalation and if swallowed', 'Xi, R38 – irritating to skin', 'Xi; R41 - risk of serious damage to eyes' and 'R42/43 - may cause sensitisation by inhalation and skin contact'.

IARC (IARC 1990[84]) has classified nickel(II) compounds in Group 1 'carcinogenic to humans' (human evidence: sufficient; evidence in experimental animals: limited), and metallic nickel in group 2B 'possibly carcinogenic to humans' (human evidence: inadequate; evidence in experimental animals: sufficient). The IARC evaluation was not changed at the more recent IARC Expert Meeting in March 2009 (Straif et al. 2009[85]). It should be noted that nickel(II) compounds have primarily shown a carcinogenic effect in the airways (lung, nasal cavity, sinuses) after inhalation.

Metallic nickel and four specific nickel compounds (nickel sulphate, nickel chloride, nickel nitrate, nickel carbonate) have been included in the EU's risk assessment program for existing substances. Denmark was the rapporteur and thus responsible for the preparation of the risk assessment reports. Based on these reports, a background document for the setting of a health-based quality

[84] IARC (1990). IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 49, Chromium, nickel and welding. IARC, Lyon, France, 1990. pp. 257-446.
[85] Straif K, Benbrahim-Tallaa L, Baan R, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Guha N, Freeman C, Galichet L, Cogliano V (2009). A review of human carcinogens - Part C: metals, arsenic, dusts, and fibres, on behalf of the WHO International Agency for Research on Cancer Monograph Working Group. International Agency for Research on Cancer, Lyon, France. Lancet 10, 453-454.

criterion for inorganic nickel salts in drinking water has been prepared (Nielsen and Larsen 2010[86]).
With regard to sensitisation, nickel is noted as being well known as a skin sensitiser in humans, and is one of the most frequent skin sensitisers in humans.
The critical effect after repeated exposure over a prolonged period of time was considered to be the developmental toxicity as severe effects were seen in the offspring of rats in a two-generation study. Based on the NOAEL of 1.1 mg Ni/kg bw per day and application of a total uncertainty factor of 200 (10 for interspecies variation, 10 for interindividual variation, 2 because of the severity of the effects observed (deaths) at the LOAEL, which is only twice as high as the NOAEL), a TDI (Tolerable Daily Intake) of 5.5 µg Ni/kg bw per day was established.
With regard to the carcinogenic effect, it should be noted that nickel and nickel compounds are classified as carcinogenic in the respiratory tract after inhalation. The carcinogenic effect of nickel and nickel compounds is therefore, not considered as a critical effect in relation to tattooing.

The TDI is in principle similar to a DNEL.

Conclusion:
Based on the above, the critical effect of nickel in relation to tattooing is considered to be sensitisation. A DNEL for the critical effect cannot be established.
Nickel sulphate, nickel chloride and nickel nitrate are classified as skin irritants and thus, it cannot be excluded that skin irritation might be a critical effect in relation to tattooing as well.
The critical systemic effects of nickel are considered to be the developmental effects. The DNEL is set at 5.5 µg Ni/kg bw per day for the systemic effects.

5.3.1.8 Titanium
Titanium and titanium dioxide are not classified for health effects according to Annex I of the 67-Directive.

IARC (IARC 1989[87]) has classified titanium dioxide in group 3 'not classifiable as to its carcinogenicity in humans' (human evidence: inadequate; evidence in experimental animals: limited). It should be noted that a carcinogenic effect was only observed in the lungs after inhalation. The possible carcinogenic potential of titanium dioxide is therefore, not considered as a critical effect in relation to tattooing.

Titanium dioxide can be manufactured to form two crystal structures, anatase and rutile.
JECFA has evaluated titanium dioxide as a food additive (JECFA 1969[88]). Establishment of an ADI (Acceptable Daily Intake) was considered unnecessary by JECFA because titanium dioxide is a very insoluble compound and because studies in several species, including humans, showed

[86] Nielsen E and Larsen PB (2010): Evaluation of health hazards by exposure to nickel, inor-ganic and soluble salts and proposal of a health-based quality criterion for drinking water. Department of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark / Danish EPA. Prepared for the Danish EPA.
[87] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 47, Titanium dioxide. IARC, Lyon, France, 1989. p. 307.
[88] 150. Titanium dioxide. FAO Nutrition Meetings Report Series 46a.

neither significant absorption nor tissue storage following ingestion. In their evaluation, JECFA did not distinguish between the anatase and rutile form of titanium dioxide.
In the EU (Directive 94/36/EC[89]), titanium dioxide (E 171) is adopted on the list of permitted food colours. According to the specification, only the anatase form was approved as a food additive.
EFSA has more recently assessed the safety in use of the rutile form as an alternative to the permitted anatase form (EFSA 2004[90]). EFSA considered that the two forms were similar chemically, but differed in their crystalline structure and light reflectance. It was agreed that the bioavailability of the two forms was essentially the same and therefore, the toxicological database would be applicable to either form. On this basis EFSA noted that the platelet form of rutile titanium dioxide could be used to replace the anatase titanium dioxide in any of its current applications. This has subsequently been endorsed in the EU legislation, i.e. the rutile form is now also permitted as a food additive (pigment) (Directive 2006/33/EC[91]).

Titanium dioxide is generally considered as being an inert substance and thus, not to possess any harmful health effects. Titanium dioxide occurs often in the form of nanoparticles in tattoo inks. It was recently reported that titanium dioxide nanoparticles caused pulmonary effects (changes in expression of genes related to inflammation and immune reactions) after inhalation (Halappanavar et al. 2011[92]). Based on the available knowledge, it is, however, not possible to assess the possible critical health effects of titanium dioxide as nanoparticles.

Conclusion:
Based on the above, the possible critical health effects of titanium or titanium dioxide (nanoparticles) in relation to tattooing cannot be identified.

5.3.2 Carbon black

Carbon black is not classified for health effects according to Annex I of the 67-Directive.

IARC (IARC 2006[93]) has classified carbon black in Group 2B 'possibly carcinogenic to humans' (human evidence: inadequate; evidence in experimental animals: sufficient). The classification is probably based on a carcinogenic effect in studies of laboratory animals (rats) exposed to carbon

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[89] European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. Official Journal of the European Communities L 237/13, 10.9.1994.
[90] Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and materials in Contact with Food on a request from the Commission related to the safety in use of rutile titanium dioxide as an alternative to the presently permitted anatase form. The EFSA Journal (2004) 163:1-12.
[91] Kommissionens direktiv 2006/33/EF af 20. marts 2006 om ændring af direktiv 95/45/EF for så vidt angår sunset yellow FCF (E 110) og titandioxid (E 171) (EØS-relevant tekst). EU-Tidende nr. L 082 af 21/03/2006 s. 0010-0013.
[92] Halappanavar S. Jackson P., Williams A., Jensen K.A., Hougaard K.S., Vogel U., Yauk C.L., Wallin H. Pulmonary response to surface-coated nanotitanium dioxide particles includes induction of acute phase response genes, inflammatory cascades, and changes in microRNAs: A toxicogenomic study. Environ Mol Mutagen 2011; DOI 10.1002/em.20639.
[93] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 93, Carbon Black. IARC, Lyon, France, 2006.

black by inhalation. The carcinogenic effect of carbon black is therefore, not considered as a critical effect in relation to tattooing.

JECFA has evaluated carbon black as a food additive (JECFA[94]). An ADI (Acceptable Daily Intake) was not established for the following reasons:
1) Carbon black from hydrocarbon sources has been shown to contain different amounts of known carcinogens. In the selection of chemical substances and tattoo inks for analyses in this project it is noted (Section 1.10.4) that a recently published study has demonstrated PAH in a number of black tattoo inks containing carbon black.
2) Knowledge is lacking on the ability of humans to extract such carcinogens upon ingestion.
3) Only limited feeding studies in experimental animals with defined carbon black are available.

No other expert opinions of carbon black of relevance in relation to carbon black in tattoo inks have been located.

Carbon black has generally been considered as being an inert compound and thus, not to possess any harmful health effects. Carbon black occurs often in the form of nanoparticles in tattoo inks. It was recently reported that carbon black nanoparticles can cause DNA damage in cells from the lungs of experimental animals (Jacobsen et al. 2008[95], Jacobsen et al. 2007[96]). It cannot be excluded that carbon black as nanoparticles also may cause DNA damage in cells from other organs and tissues. Based on the available knowledge it is, however, not possible to assess the possible critical health effects of carbon black as nanoparticles.

Conclusion:
Based on the above, the possible critical health effects of carbon black (nanoparticles) in relation to tattooing cannot be identified. It should be noted that carbon black originating from hydrocarbon sources may contain different amounts of known carcinogens (e.g. PAH). This might be a critical effect of carbon black in relation to tattooing. A DNEL/DMEL for the possible critical effect of carbon black cannot be established.

5.3.3 Phthalocyanines

Phthalocyanines form complexes with most of the elements in the periodic table. In general, all the complexes are of very low solubility in most solvents, including water.

Phthalocyanines are not classified for health effects according to Annex I of the 67-Directive, and have not been evaluated by IARC.

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[94] 636. Carbon Black. WHO Food Additive Series 22.
[95] Jacobsen N.R., Pojana G., White P., Møller P., Cohn C.A., Korsholm K.S., Vogel U., Marcomini A., Loft S., Wallin H. Genotoxicity, cytotoxicity and reactive oxygen species induced by single- walled carbon nanotubes and C60 fullerenes in the FE1-MutaTMMouse lung epithelial cells. Environ Mol Mutagen 2008;49:476-87.
[96] Jacobsen N.R., Saber A.T., White P., Møller P., Pojana G., Vogel U., Loft S., Gingerich J., Soper L., Douglas G.R., Wallin H. Increased mutant frequency by carbon black, but not quartz, in the lacZ and cII transgenes of muta mouse lung epithelial cells. Environ Mol Mutagen 2007;48:451-61.

Copper phthalocyanine (CAS No. 147-14-8) has been evaluated in the OECD SIDS program (OECD[97]). The most relevant data in relation to tattooing are summarised here:
The pigment is insoluble in water and stable in most solutions, i.e. is not degraded.
In rats, a reduced number of red blood cells was observed after oral administration of the pigment by gavage (1000 mg/kg bw) daily for 28 days. The NOAEL was established at 200 mg/kg bw per day.
In rats and mice, no effects were seen after administration of the pigment in the feed (0.3 to 5%) for 13 weeks.
No tumours were observed in mice given the pigment for 8 months.
No genotoxic effects were observed in a variety of tests.
In rats, no effects on fertility and no effects in offspring were observed after oral administration of the pigment by gavage (0, 40, 200, 1000 mg/kg bw) daily for 42 days (males) and from 14 days before mating to 3 days after giving birth (females). The NOAEL was established at 1000 mg/kg bw per day for offspring as well as for the parents.

The critical effect after repeated exposure over a prolonged time period is considered to be the decreased number of red blood cells. Based on the NOAEL of 200 mg/kg bw per day and application of a total uncertainty factor of 100 (10 for interspecies variation, 10 for interindividual variation), a DNEL of 2 mg/kg bw per day can be established. It should be noted that, in the OECD SIDS report, the magnitude of the decrease in the number of red blood cells in exposed animals compared to controls is not presented. Therefore, it cannot be evaluated whether the decrease is statistically and biologically significantly different compared to the control group and thus, the DNEL might be overestimated.

Conclusion:
Based on the above, the critical effect of phthalocyanines in relation to tattooing is considered to be the decreased number of red blood cells. The DNEL is set at 2 mg/kg bw per day; however, this DNEL might be overestimated.

5.3.4 Polycyclic Aromatic Hydrocarbons (PAH)

In the analytical phase of this project (Chapter 4), the following 16 PAH were analysed in selected tattoo inks:
Acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, chrysene, dibenz(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene and pyrene.

PAH as a group is not classified for health effects according to Annex I of the 67-Directive.
Benz(a)pyrene (BaP) is classified ‘Carc. Cat. 2; R45 - may cause cancer’, ‘ Muta. Cat. 2; R46 - may cause heritable genetic damage’ and ‘Repr. Cat. 2; R60-61 - may impair fertility and may cause harm to the unborn child’.

IARC (IARC 2005[98]) has evaluated 60 different PAH.

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[97] Copper phthalocyanine. CAS No.: 147-14-8. OECD SIDS, UNEP Publications.
[98] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 92, Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures. IARC, Lyon, France, 2010.

BaP is the only PAH classified in Group 1 ‘arcinogenic to humans’.
Three PAH (including dibenz(a,h)anthracene) are classified in Group 2A ‘probably carcinogenic to humans'.
Eleven PAH (including benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, and indeno(1,2,3-cd)pyrene) are classified in Group 2B ‘possibly carcinogenic to humans’.
The remaining 45 PAH (including acenaphthene, anthracene, benzo(ghi)perylene, fluoranthene, fluorene, phenanthrene and pyrene) are classified in group 3 ‘not classifiable as to their carcinogenicity to humans.
IARC places substances in Group 3 when it, based on the available data, cannot be concluded whether the substance is not (group 4) / possibly (group 2B) / probably (group 2A) / is (Group 1) carcinogenic to humans.
Of the PAH analysed in this project, the following are not included in the IARC assessment: Acenaphthylen, naphthalene.
The IARC Working Group noted that PAH congeners and mixtures vary widely in the level of carcinogenic response induced by the given dose, i.e. that there is great difference in the doses leading to carcinogenic effects of the different PAH congeners and mixtures.

In Denmark, a health-based quality criterion for PAH in soil has been set (Larsen 2004[99]).
The critical effect was considered to be the carcinogenic effect, an effect which is very likely attributable to damage of the genes. The target organs and tissues for the tumourigenic effect depend on the route of exposure. Thus, tumours are primarily observed in the gastrointestinal tract after ingestion, primarily in the airways and lungs after inhalation and primarily in the skin after dermal contact, i.e. the tumours are primarily observed locally at the site of first contact. However, for some individual PAH, tumours have also been observed systemically (in organs and tissues in the body), primarily in the liver. Based on the available data, on the carcinogenic effects of PAH as a group or of BaP, it is not possible to set a lower limit (threshold) for the carcinogenic effect.
For substances / substance groups where it is considered that there is no threshold for the carcinogenic effects, the average daily lifetime dose resulting in one additional incidence of cancer among one million individuals (10^{-6} lifetime risk) is generally considered as a tolerable dose, often termed the ‘virtually safe dose’. From the most recent studies in mice and rats of BaP, the ‘virtually safe dose’ was estimated at 0.6-5 ng/kg bw per day for a lifetime risk of 10^{-6} when based on all tumours combined, i.e. the tolerable dose. This dose corresponds in principle to a DMEL.

BaP has generally been regarded as being the most potent PAH and is the most widely used marker in relation to health effect assessments of PAH; however, there are also other equally potent or even more potent PAH (EHC 1998[100]). In the following list, the potency of the various PAH relative to the potency of BaP is presented, where a factor of 0.1 means that the potency is 10 times less the potency of BaP, a factor of 1.0 that the potency is the same as for BaP, and a factor of 100 that the potency is 100 times greater than the potency of BaP (note: the list is reproduced in EHC (1998) from an older reference, but not evaluated by the WHO/IPCS Task Group):

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[99] Evaluation of health hazards by exposure to PAH and estimation of a quality criterion in soil. Department of Toxicology and Risk Assessment, Danish Institute for Food and Veterinary Research. Prepared for the Danish EPA.
[100] Polycyclic aromatic hydrocarbons, selected non-heterocyclic. Environmental Health Criteria 202. IPCS, WHO, 1998.

- Benz(a)pyrene (BaP) 1.0
- Benz(a)anthracene 0.1
- Benz(b)fluoranthene 0.1
- Benz(j)fluoranthene 0.1
- Benz(k)fluoranthene 0.1
- Chrysene 0.1
- Cyclopenta(cd)pyrene 0.1
- Dibenz(a,h)anthracene 1.0
- Dibenz(a,e)fluoranthene 1.0
- Dibenz(a,e)pyrene 1.0
- Dibenz(a,h)pyrene 1.0
- Dibenz(a,i)pyrene 0.1
- Dibenz(a,l)pyrene 100
- Indeno(1,2,3-cd)pyrene 0.1

Seven of the 16 PAH analysed in this project are included the above list: BaP, benzo(a)anthracene, benzo(b), fluoranthene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracene and indeno(1,2,3-cd)pyrene.
Based on the relative potencies presented in the above list, a DMEL could, in principle, be calculated for mixtures of these seven PAH. However, as another nine PAH also were analysed in selected tattoo inks, and as not all the 16 analysed PAH were found in all the analysed tattoo inks, any calculations of DMELs for mixtures of different PAH in the analysed tattoo inks have not been performed.

Conclusion:
Based on the above, the critical effect of BaP, as well as for the other PAH, in relation to tattooing is considered to be the carcinogenic effect. The DMEL for BaP is set at 0.6-5 ng/kg bw per day. DMELs for the other PAH found in the analysed tattoo inks, as well as DMELs for mixtures of different PAH in the analysed inks, cannot be established.

5.3.5 Primary aromatic amines (PAA)

Azo compounds are widely used as pigments in tattoo inks. Azo compounds are characterised by containing one or more azo groups, i.e. double bonds between two nitrogen atoms. The azo group is not stable and will under certain conditions be degraded to the original building blocks of the azo compound, i.e. the aromatic amines. These amines may also occur as impurities in azo dyes (residues from the manufacturing).

5.3.5.1 Aniline
According to Annex I of the 67-Directive, aniline is classified ‘Carc. Cat. 3; R40 - limited evidence of a carcinogenic effect’, ‘Muta. Cat. 3; R68 - possible risk of irreversible effects’, ‘T; R23/24/25 - toxic by inhalation, in contact with skin and if swallowed’, ‘T; R48/23/24/25 - toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed’, ‘Xi; R41 - risk of serious damage to eyes’ and ‘R43 - may cause sensitisation by skin contact’.

IARC (IARC 1987[101]) has classified aniline in Group 3 'not classifiable as to its carcinogenicity to humans' (human evidence: inadequate; evidence in experimental animals: limited).

Aniline has been included in the EU's risk assessment program for existing substances (EU-RAR 2004[102]).
It was concluded that aniline causes contact allergy in humans, often associated with para-group cross reactivity.
The critical effect after repeated exposure over a prolonged period of time was considered to be the damage to red blood cells, including haemolytic anaemia and methaemoglobinaemia as observed in experimental animals; a LOAEL of 7 mg/kg bw per day was established based on a 2-year study in rats. In the risk characterisation for workers, a reference MOS (Margin of Safety) of 150 was applied (10 for interspecies variation, 5 for interindividual variation (factor 5 is the 'default' value for workers), 3 as a LOAEL was used instead of a NOAEL). The total factor of 150 corresponds, in principle ,to an overall uncertainty factor. In the risk characterisation for consumers (general population), a reference MOS for oral exposure has not been evaluated.
With regard to carcinogenicity, it was concluded that aniline is probably carcinogenic to humans (tumours primarily in the spleen) and that there is no threshold for this effect. A T_{25} (for rats) of 46 mg/kg bw per day was estimated, and a HT_{25} (T_{25} for humans) of 4.6 mg/kg bw per day for oral exposure by applying a factor of 10 for extrapolation of the T_{25} for rats to HT_{25} for humans.

Based on the LOAEL of 7 mg/kg bw per day and application of a total uncertainty factor of 300 (10 for interspecies variation, 10 for interindividual variation, 3 because a LOAEL was used instead of a NOAEL), a DNEL of approximately 0.02 mg/kg bw per day can be established.

Based on the HT_{25} of 4.6 mg/kg bw per day and application of a HtLF (High to low dose risk extrapolation factor) of 250,000 (the 'default' for the 10^{-6} lifetime risk when T_{25} is used as a PoD), a DMEL of approximately 2×10^{-5} mg/kg bw per day (approximately 20 ng/kg bw per day) can be established.

Conclusion:
Based on the above, the critical effects of aniline in relation to tattooing are considered to be sensitisation and carcinogenic effects. A DNEL for sensitisation cannot be established. The DMEL is set at approximately 2×10^{-5} mg/kg bw per day for the carcinogenic effects.

5.3.5.2 o-Anisidine

According to Annex I of the 67-Directive, o-anisidine is classified 'Carc. Cat. 2; R45 - may cause cancer', 'Muta. Cat. 3; R68 - possible risk of irreversible effects' and 'T; R23/24/25 - toxic by inhalation, in contact with skin and if swallowed'.

IARC (IARC 1999[103]) has classified o-anisidine in Group 2B 'possibly carcinogenic to humans' (human evidence: inadequate; evidence in experimental animals: sufficient).

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[101] IARC Monographs on the evaluation of carcinogenic risks to humans, Supplement 7, Aniline. IARC, Lyon, France, 1987, p. 99.
[102] European Union Risk Assessment Report. Aniline. CAS No.: 62-53-3, EINECS No.: 200-539-3. European Communities, 2004.

o-Anisidine has been included in the EU's risk assessment program for existing substances (EU-RAR 2002[104]).
With regard to sensitisation, there are indications of sensitising properties in a study with guinea pigs, while no human data are available. On this basis it was concluded that o-anisidine has not been tested adequately for sensitising properties.
The critical effect after repeated exposure over a prolonged period of time was considered to be the damage to red blood cells, including haemolytic anaemia and methaemoglobinaemia as observed in experimental animals; a NO(A)EL of 16 mg/kg bw per day was derived based on a 28-day study in rats. It is noted that this NO(A)EL is used in risk characterisation, partly because longer-term studies (up to 2 years) showed that the toxicity of o-anisidine did not increase significantly with the duration of exposure, partly because a NOAEL could not be derived from the longer studies (higher doses used than in the 28-day study). In the risk characterisation for consumers, a reference MOS (Margin of Safety) for oral exposure has not been evaluated. But the NO(A)EL of 16 mg/kg bw per day was converted to a so-called 'humane NAEL' of 0.07 mg/kg bw per day by applying a factor of 4 for interspecies variation, a factor of 6 because a 28-day study was used instead of a longer term study, and a factor of 10 because humans are much more sensitive to the formation of methaemoglobin than rats. This overall factor of 240 corresponds, in principle, to an overall uncertainty factor.
The critical effect of o-anisidine for the assessment of human health was concluded to be the carcinogenic effect (mainly tumours in the bladder), and that there is no threshold for this effect. A T_{25} (for rats) of 39.7 mg/kg bw per day was estimated, and a HT_{25} (T_{25} for humans) of 9.9 mg/kg bw per day by applying a factor of 4 for extrapolation of the T_{25} for rats to HT_{25} for humans.

Based on the NOAEL of 16 mg/kg bw per day and application of a total uncertainty factor of 600 (10 for interspecies variation, 10 for interindividual variation, 6 because the basis is a 28-day study), a DNEL of approximately 0.03 mg/kg bw per day can be established. It should be noted that this DNEL is not equal to the 'human NAEL' derived in the EU risk assessment report. The differences is due to that an assessment factor to acoount for the interindividual variation was not considered in the derivation of the 'human NAEL' in the EU risk assessment report.

Based on the HT_{25} of 9.9 mg/kg bw per day and application of a HtLF (High to low dose risk extrapolation factor) of 250,000 (the 'default' for the 10^{-6} lifetime risk when T_{25} is used as a PoD), a DMEL of approximately 4×10^{-5} mg/kg bw per day (approximately 40 ng/kg bw per day) can be established.

Conclusion:
Based on the above, the critical effect of o-anisidine in relation to tattooing is considered to be the carcinogenic effect. The DMEL is set at approximately 4×10^{-5} mg/kg bw per day. It should be noted that o-anisidine has not been tested adequately for an evaluation of the sensitising properties.

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[103] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 73, ***ortho***-Anisidine. IARC, Lyon, France, 1999, p. 49.
[104] European Union Risk Assessment Report. ***o***-Anisidine. CAS No.: 90-04-0, EINECS No.: 201-963-1. European Communities, 2002.

5.3.5.3 p-Chloroaniline

According to Annex I of the 67-Directive, p-chloroaniline is classified 'Carc. Cat. 2; R45 - may cause cancer', 'T; R23/24/25 - toxic by inhalation, in contact with skin and if swallowed' and 'R43 - may cause sensitisation by skin contact'.

IARC (IARC 1993[105]) has classified p-chloroaniline in Group 2B 'possibly carcinogenic to humans' (human evidence: inadequate; evidence in experimental animals: sufficient).

p-Chloroaniline has been evaluated in the CICAD programme (an international programme sponsored by UNEP/ILO/WHO jointly) (CICAD 2003[106]).
Based on the available data, p-chloroaniline was considered to be a skin sensitiser.
The critical effect after repeated exposure over a prolonged period of time was considered to be the damage to red blood cells, including haemolytic anaemia and methaemoglobinaemia as observed in experimental animals; a LOAEL of 2 mg/kg bw per day was established based on a 2-year study in rats. Based on the LOAEL of 2 mg/kg bw per day and application of a total uncertainty factor of 1000 (10 for interspecies variation, 10 for interindividual variation, 10 because a LOAEL was used instead of a NOAEL), a tolerable intake of 0.002 mg/kg bw was derived.
p-Chloroaniline is carcinogenic in male rats, with the induction of unusual and rare tumours of the spleen, which is typical for aniline and related substances. Whether the mechanism for the carcinogenic effect is mediated through genotoxic or non-genotoxic events is unresolved. No PoD (e.g. T_{25}) for the carcinogenic effect has been considered.

Conclusion:
Based on the above, the critical effects of p-chloroaniline in relation to tattooing are considered to be sensitisation and carcinogenic effects. A DNEL/DMEL for the critical effects cannot be established.

5.3.5.4 4-Chloro-o-toluidine

According to Annex I of the 67-Directive, 4-chloro-o-toluidine is classified 'Carc. Cat. 2; R45 - may cause cancer', 'Muta. Cat. 3; R68 - possible risk of irreversible effects' and 'T; R23/24/25 - toxic by inhalation, in contact with skin and if swallowed'.

IARC (IARC 2000[107], IARC 2008[108]) has classified 4-chloro-o-toluidine in group 2A 'probably carcinogenic to humans' (human evidence: limited; evidence in experimental animals: sufficient).

The U.S. National Cancer Institute (NCI 1979[109]) has investigated 4-chloro-o-toluidine for carcinogenic effects after dietary administration. Tumours

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[105] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 57, ***para***-Chloroaniline. IARC, Lyon, France, 1993, p. 305.
[106] 4-Chloroaniline. Concise International Chemical Assessment Document 48. WHO, 2003.
[107] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 77, 4-Chloro-ortho-toluidine. IARC, Lyon, France, 2000, p. 323.
[108] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 99, Some Aromatic Amines, Organic Dyes, and Related Exposures. IARC, Lyon, France, 2010.

(haemangiosarcomas, a rare tumour type developed from blood vessels into the surrounding tissue) were observed in mice but not in rats.

No other expert opinions of 4-chloro-o-toluidine of relevance in relation to 4-chloro-o-toluidine in tattoo inks have been located.

Conclusion:
Based on the above, the critical effect of 4-chloro-o-toluidine in relation to tattooing is considered to be the carcinogenic effect. A DMEL for the critical effect cannot be established.

5.3.5.5 3,3'-Dichlorobenzidine

According to Annex I of the 67-Directive, 3,3'-dichlorobenzidine is classified 'Carc. Cat. 2; R45 - may cause cancer', 'Xn, R21 - harmful in contact with skin' and 'R43 - may cause sensitisation by skin contact'.

IARC (IARC 1987[110]) has classified 3,3'-dichlorobenzidine in Group 2B 'possibly carcinogenic to humans' (human evidence: inadequate; evidence in experimental animals: sufficient).

IARC (IARC 2008[111]) has classified benzidine in Group 1 'carcinogenic to humans' (human evidence: sufficient; evidence in experimental animals: sufficient). In this more recent IARC monograph, it is mentioned that the evidence for a carcinogenic effect of 3,3'-dichlorobenzidine in experimental animals is sufficient; however, an evaluation of the human evidence as well as an overall evaluation of the carcinogenic effect of 3,3'-dichlorobenzidine has not been provided.

3,3'-Dichlorobenzidine has been evaluated in the CICAD programme (an international programme sponsored by UNEP/ILO/WHO jointly) (CICAD 2003[112]).
Dermatitis has been reported among workers (one limited study, no further details); no data on the sensitisation in experimental animals were identified. The available data were considered as being inadequate to assess the effects after repeated exposure over a prolonged time period.
The critical effect of 3,3'-dichlorobenzidine was considered to be the carcinogenic effect for which there is no threshold. The $TD_{0.05}$ (the dose associated with a 5% increase in tumour incidence in rats) was estimated to be in the range of 0.74 to 1.4 mg/kg bw per day depending on the tumour type used as the basis for the $TD_{0.05}$ (mammary tumours: 0.74; leukaemia: 1.4). Based on the $TD_{0.05}$ of 0.74 mg/kg bw per day and application of a total factor of 5000-50000, a guidance value of 1.48×10^{-4} - 1.48×10^{-5} mg/kg bw was derived. It was noted that the limitations of the critical study upon which this guidance value is based should be borne in mind in its interpretation (only a single dose level and the exposure was shorter than 2 years (up to 488 days)).

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[109] Bioassay of 4-chloro-o-toluidine hydrochloride for possible carcinogenicity, CAS No. 3165-93-3. National Cancer Institute, Carcinogenesis Technical Report Series No. 165, 1979.
[110] IARC Monographs on the evaluation of carcinogenic risks to humans, Supplement 7, 3,3'-Dichlorobenzidine. IARC, Lyon, France, 1987, p. 193.
[111] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 99, Some Aromatic Amines, Organic Dyes, and Related Exposures. IARC, Lyon, France, 2010.
[112] 3,3'-Dichlorobenzidine. Concise International Chemical Assessment Document 2. WHO, 1998.

The guidance value corresponds, in principle, to a DMEL. It should be noted, however, that the underlying study does not live up to today's quality standards and thus, a DMEL cannot be established based on this study.

Conclusion:
Based on the above, the critical effects of 3,3'-dichlorobenzidine in relation to tattooing is considered to be sensitisation and carcinogenic effects. A DNEL/DMEL for the critical effects cannot be established.

5.3.5.6 4-Methyl-m-phenylenediamine

According to Annex I of the 67-Directive, 4-methyl-m-phenylenediamine (2,4-diaminotoluene / 2,4-toluenediamine) is classified 'Carc. Cat. 2; R45 - may cause cancer', 'Muta. Cat. 3; R68 - possible risk of irreversible effects', 'Repr. Cat. 3; R62 - possible risk of impaired fertility', 'T; R25 - toxic if swallowed', 'Xn, R21 - harmful in contact with skin', 'Xn, R48/22 - harmful: danger of serious damage to health by prolonged exposure if swallowed' and 'R43 - may cause sensitisation by skin contact'.

IARC (IARC 1978[113]) has classified 4-methyl-m-phenylenediamine in Group 2B 'possibly carcinogenic to humans' (human evidence: no data; evidence in experimental animals: sufficient).

Diaminotoluenes have been evaluated by WHO/IPCS (EHC 1987[114]). The most relevant data in relation to tattooing are summarised here:
Dermal contact may possibly cause skin sensitisation.
After repeated exposure over a prolonged time period, methaemoglobinaemia and the effects in the kidneys have been observed.
2,4-Diaminotoluene is carcinogenic in experimental animals (rats and mice, tumours in the liver) and all three isomers have been shown to be genotoxic.

No other expert opinions of 4-methyl-m-phenylenediamine of relevance in relation to 4-methyl-m-phenylenediamine in tattoo inks have been located.

Conclusion:
Based on the above, the critical effects of 4-methyl-m-phenylenediamine in relation to tattooing is considered to be sensitisation and carcinogenic effects. A DNEL/DMEL for the critical effects cannot be established.

5.3.5.7 4-Methoxy-m-phenylenediamine

According to Annex I of the 67-Directive, 4-methoxy-m-phenylenediamine (2.4-diaminoanisol) is classified 'Carc. Cat. 2; R45 - may cause cancer', 'Muta. Cat. 3; R68 - possible risk of irreversible effects' and 'Xn, R22 - harmful if swallowed'.

IARC (IARC 2001[115]) has classified 4-methoxy-m-phenylenediamine in Group 2B 'possibly carcinogenic to humans' (human evidence: inadequate; evidence in experimental animals: sufficient).

No other expert opinions of 4-methoxy-m-phenylenediamine of relevance in relation to 4-methoxy-m-phenylenediamine in tattoo inks have been located.

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[113] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 16, 2,4-Diaminotoluene. IARC, Lyon, France, 1978. pp. 83.
[114] Diaminotoluenes. Environmental Health Criteria 74. IPCS, WHO, 1987.
[115] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 79, 2,4-Diaminoanisole. IARC, Lyon, France, 2001, p. 621.

Conclusion:
Based on the above, the critical effect of 4-methoxy-m-phenylenediamine in relation to tattooing is considered to be the carcinogenic effect. A DMEL for the critical effect cannot be established.

5.3.5.8 2-Naphthylamine

According to Annex I of the 67-Directive, 2-naphthylamine is classified 'Carc. Cat. 2; R45 - may cause cancer' and 'Xn, R22 - harmful if swallowed'.

IARC (IARC 1987[116], IARC 2008[117]) has classified 2-naphthylamine in Group 1 'carcinogenic to humans' (human evidence: sufficient; evidence in experimental animals: sufficient).

No other expert opinions of 2-naphthylamine of relevance in relation to 2-naphthylamine in tattoo inks have been located.

Conclusion:
Based on the above, the critical effect of 2-naphthylamine in relation to tattooing is considered to be the carcinogenic effect. A DMEL for the critical effect cannot be established.

5.3.5.9 5-Nitro-o-toluidine

According to Annex I of the 67-Directive, 5-nitro-o-toluidine is classified 'Carc. Cat. 3; R40 - limited evidence of a carcinogenic effect' and 'T; R23/24/25 - toxic by inhalation, in contact with skin and if swallowed'.

IARC (IARC 1990[118]) has classified 5-nitro-o-toluidine in group 3 'not classifiable as to its carcinogenicity to humans' (human evidence: no data; evidence in experimental animals: limited.)

No other expert opinions of 5-nitro-o-toluidine of relevance in relation to 5-nitro-o-toluidine in tattoo inks have been located.

Conclusion:
Based on the above, the critical effect of 5-nitro-o-toluidine in relation to tattooing is considered to be the carcinogenic effect. A DMEL for the critical effect cannot be established.

5.3.5.10 o-Toluidine

According to Annex I of the 67-Directive, o-toluidine is classified 'Carc. Cat. 2; R45 - may cause cancer', 'T; R23/25 - toxic by inhalation and if swallowed' and 'Xi; R36 - irritating to eyes'.

IARC (IARC 2008[119]) has classified o-toluidine in group 1 'carcinogenic to humans' (human evidence: sufficient; evidence in experimental animals: sufficient).

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[116] IARC Monographs on the evaluation of carcinogenic risks to humans, Supplement 7, 2-Naphthylamine. IARC, Lyon, France, 1987, p. 261.
[117] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 99, Some Aromatic Amines, Organic Dyes, and Related Exposures. IARC, Lyon, France, 2010.
[118] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 48, 5-Nitro-***ortho***-toluidine. IARC, Lyon, France, 1990. p. 169.
[119] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 99, Some Aromatic Amines, Organic Dyes, and Related Exposures. IARC, Lyon, France, 2010.

o-Toluidine has been evaluated in the CICAD programme (an international programme sponsored by UNEP/ILO/WHO jointly) (CICAD 1998[120]).
The available data were not considered valid for an evaluation of the sensitisation potential.
The critical effect of o-toluidine was considered to be the carcinogenic effects. The mechanism for the carcinogenic effect is not clear, but involvement of a genotoxic mechanism cannot be eliminated. No PoD (e.g. T_{25}) for the carcinogenic effect has been considered.

o-Toluidine has been evaluated in the OECD SIDS program (OECD 2004[121]).
The available data were not considered valid for an evaluation of the sensitisation potential.
The critical effect after repeated exposure over a prolonged period of time was considered to be the marked damage to red blood cells, including methaemoglobinaemia as observed in laboratory animals; a LOAEL of approximately 25 mg/kg bw per day was derived based on a 14-day study in rats.
o-Toluidine is carcinogenic in experimental animals (rats and mice, tumours in several organs) and the carcinogenic effect is probably due to genotoxic events.

Based on the LOAEL of 25 mg/kg bw per day and application of a total uncertainty factor of 1800 (10 for interspecies variation, 10 for interindividual variation, 3 because a LOAEL was used instead of a NOAEL, 6 because the basis is a 14-day study instead of a long-term study), a DNEL of approximately 0.01 mg/kg bw per day can be established.

Conclusion:
Based on the above, the critical effect of o-toluidine in relation to tattooing is considered to be the carcinogenic effect. A DMEL for the critical effect cannot be established.

5.3.6 Summary: Hazard Assessment

Twenty-one substances / substance groups were selected for the health effect assessment: Eight elements, ten aromatic amines, carbon black, PAH and phthalocyanines.

For the selected chemical substances, the identification of the critical effect(s) were based on the EU classification of substances according to Annex I of the 67-Directive and the IARC classification for carcinogenic effects when available, as well as on the critical effects identified in selected expert opinions from national and international bodies. The NOAELs / LOAELs presented are generally those established in the selected expert opinions from national and international bodies.

Carcinogenic effect was considered as the critical effect in relation to tattooing for PAH and the ten selected PAA (aniline, o-anisidine, p-chloroaniline, 4-chloro-o-toluidine, 3,3'-dichlorobenzidine, 4-methyl-m-phenylenediamine, 4-methoxy-m-phenylenediamine, 2-naphthylamine, 5-nitro-o-toluidine and o-toluidine).

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[120] o-Toluidine. Concise International Chemical Assessment Document 7. WHO, 1998.
[121] o-Toluidine, CAS No.: 95-53-4. OECD SIDS 2004, UNEP Publications.

For these two groups of substances (PAH and PAA) it is considered that there is no lower limit (threshold) for the carcinogenic effects and therefore, a DNEL cannot be established. For one of the PAH (benzo(a)pyrene) and for two of the PAA (aniline and o-anisidine), a DMEL has been established. For the remaining substances in these two groups, a DMEL could not be established based on the available data.

Also other of the selected substances were considered as being carcinogenic. Cadmium, chromium (VI) and nickel are classified as carcinogens according to Annex I of the 67-Directive, and by IARC in Group 1 'carcinogenic to humans'. It should be noted that for these three substances, the carcinogenic effects are (primarily) observed in the respiratory tract and/or lungs after inhalation. Therefore, the carcinogenic effects of these three substances are not considered as a critical effect in relation to tattooing.
Lead is classified by IARC in Group 2A 'probably carcinogenic to humans', but not classified for carcinogenicity in the EU according to Annex I of the 67-Directive. It should be noted that the mode of action for the carcinogenic effect of lead is not completely understood. Furthermore, tumours have only been seen at relatively high doses. Therefore, the carcinogenic effect of lead is not considered as a critical effect in relation to tattooing.

Sensitisation was considered as a critical effect in relation to tattooing for a number of the selected substances: Aluminium, chromium, nickel, aniline, p-chloroaniline, 3,3'-dichlorobenzidine and 4-methyl-m-phenylenediamine. In the EU, these substances are, with the exception of aluminium, classified 'R43 - may cause sensitisation by skin contact' according to Annex I of the 67-Directive.
It is disputed whether contact allergy is a threshold effect for which a certain dose has to be passed for triggering an effect or not. The existence of a threshold for induction of allergy (sensitisation) has been shown for some contact allergens. Similarly, studies have shown that a threshold also exists for triggering allergy (elicitation) in sensitised individuals. Since contact allergens varies widely in their relative potency, they probably also exhibit a large variation in relation to a threshold for both induction and elicitation of allergy. The health effect assessment of chemical contact allergens can only be performed if the potency and the threshold value have been carefully examined for the specific chemical allergen. (Nielsen et al. 2005[122]).
For the selected substances, the available data are not sufficient for an evaluation of neither the potency nor the threshold value and therefore, a DNEL for sensitisation cannot be established for these substances.
It should be noted that, in general, there is a lack of knowledge regarding sensitisation in relation to tattooing (described in Section 3.6 and 6.2), and that allergy to permanently deposited substances/pigments in the skin probably is clinically different from ordinary contact eczema.

For a number of the selected substances, other effects than carcinogenicity and sensitisation were considered as the critical effect in relation to tattooing: Barium (effects on the cardiovascular system), lead (effects on the developing nervous system), cadmium (effects on bones and kidney) and phthalocyanines (decreased number of red blood cells). For barium, cadmium

[122] Principper for sundhedsmæssig vurdering af kemiske stoffer med henblik på fastsættelse af kvalitetskriterier for luft, jord og vand. Elsa Nielsen, Grete Østergaard, John Christian Larsen og Ole Ladefoged. Afdeling for Toksikologi og Risikovurdering, Danmarks Fødevareforskning. Miljøprojekt Nr. 974 2005. In Danish with an English summary.

and phthalocyanines, a DNEL was established for the critical effect. For lead, a DNEL could not be established for the critical effect as a threshold for the critical effect (effects on the developing nervous system) has not been identified.

For some substances, a critical effect in relation to tattooing could not be identified: copper, titanium (titanium dioxide) and carbon black.
Some copper salts may cause skin irritation. However, based on the available data it ws considered that skin irritation is not likely to be a critical effect of copper in relation to tattooing.
Titanium dioxide occurs often in the form of nanoparticles in tattoo inks. It was recently reported that titanium dioxide nanoparticles caused pulmonary effects (changes in expression of genes related to inflammation and immune reactions) after inhalation. Based on the available knowledge, it was not possible to assess the possible critical health effects of titanium dioxide as nanoparticles.
Carbon black originating from hydrocarbon sources may contain different amounts of known carcinogens (e.g. PAH). This might be a critical effect of carbon black in relation to tattooing. Carbon black occurs often in the form of nanoparticles in tattoo inks. It was recently reported that carbon black nanoparticles can cause DNA damage in cells from the lungs of experimental animals. Based on the available knowledge it is not possible to assess the possible critical health effects of carbon black as nanoparticles.
On this basis, a DNEL/DMEL for the possible critical effects of copper, titanium (titanium dioxide) or carbon black could not be established.

5.4 Risk characterisation of the selected chemical substances

The health effect assessment (risk assessment) consists of a hazard assessment, an exposure assessment and a risk characterisation. In relation to tattooing, where chemical substances in the tattoo inks are deposited in the skin, the health effect assessment is an assessment of whether a given chemical substance deposited in the skin is associated with a health risk. The health effect assessment includes local effects in the skin as well as systemic effects, i.e. effects that occur in tissues and organs in the body after absorption of the substance from the tattooed skin area. The outcome of the hazard assessment is the identification of the critical effect and the establishment of a DNEL/DMEL for the critical effect.

In the risk characterisation, the exposure estimate is compared with the DNEL/DMEL and the so-called risk characterisation ratio (RCR) is calculated. RCR is thus, the ratio between the exposure estimate and the DNEL/DMEL (RCR = exposure/DN(M)EL). If the exposure estimate is lower than the DNEL/DMEL, i.e. the RCR <1, the exposure is not considered to pose a health risk in the given situation. The DNEL/DMEL established for the critical (systemic) effects for the selected chemical substances in the analysed tattoo inks is generally expressed in the unit ‘mg/kg bw per day’. Therefore, the exposure estimates also have to be expressed in the same unit for an RCR to be calculated.

In order to express the exposure estimate in the form of a systemic dose expressed in the unit ‘mg/kg bw per day’, it is necessary to know how much of the substance deposited in the tattooed skin area following tattooing is subsequently absorbed, i.e. to know the percentage of the deposited substance

that is transported from the tattooed skin area to the tissues and organs in the body via the blood circulation and/or the lymphatic system.

Knowledge about the absorption of a given substance from a tattooed skin area is thus, an essential part of the health effect assessment of this substance in relation to tattooing. This project has revealed a number of limitations as well as lack of knowledge for a valid evaluation of the uptake, as well as transport of substances from a tattooed skin area to the tissues and organs in the body, to be performed. Consequently, it is not possible with currently available knowledge to perform a valid quantitative exposure assessment, i.e. to estimate the systemic dose of the selected chemical substances in the analysed tattoo inks. In addition, the project has also revealed limitations and lack of knowledge in relation to the hazard assessment (identification of the critical effect in relation to tattooing and establishment of a DNEL/DMEL for the critical effect) for a number of the selected chemical substances.

The limitations and lack of knowledge in relation to the exposure assessment for the selected chemical substances in the analysed tattoo inks (further addressed in section 5.4.1) as well as in relation to the hazard assessment for a number of the selected substances (further addressed in section 5.4.2) imply that a valid risk characterisation according to the REACH guidance documents (calculation of RCR) cannot be performed.

5.4.1 Limitations / lack of knowledge: Exposure assessment

Tattoo inks contain generally one or more colorants (pigments) as well as coformulants. In addition, the finished tattoo inks may also contain chemical impurities.

The basis for the exposure assessment of the selected chemical substances in the analysed tattoo inks is the concentration of the substances found by the analyses of the tattoo inks.

The PAA, PAH and elements found in the analysed tattoo inks are not used as colorants/pigments or coformulants as such, but may occur in the tattoo inks following degradation of the pigments/coformulants (for example PAA from azo dyes), or as chemical impurities in the tattoo inks and/or pigments (for example PAH in carbon black, PAA as residues from the manufacturing). Some of the substances found in tattoo inks are probably released due to the analytical method used for the analyses of the tattoo inks (for example titanium from titanium dioxide, barium from barium sulphate). This means that the analytical program chosen in this project does not reveal the pigments, coformulants and chemical impurities that are actually present in the analysed tattoo inks and to which a tattooed person is exposed to. A limitation in relation to the exposure assessment is thus, the lack of knowledge about the pigments, coformulants and chemical impurities that a tattooed person actually is exposed to following tattooing with these tattoo inks.

The pigments in tattoo inks often occur as particles with a particle size down to 20 nanometers, i.e. nanoparticles (section 1.5). The pigments used for tattooing are generally of low solubility or insoluble, as it is the intention that the pigments should remain in the skin, otherwise the tattoo would faint and disappear within a relatively short time. In addition, coformulants as well as chemical impurities in the tattoo inks may also occur as particles. The very low solubility of the pigments (particles in general) means that the pigments deposited in the skin will behave significantly different from substances that

are soluble, at least partly, in biological fluids such as e.g. blood or lymph. The pigments used for tattooing are often coated in order to inhibit dissolution and degradation and consequently, this will affect the release of degradation products and chemical impurities from the pigments. Both solubility and coating are thus, of great importance for the potential absorption of pigments deposited in the skin and the subsequent transport and distribution to tissues and organs in the body.

Knowledge about the uptake and distribution of pigments deposited in the skin are described in detail elsewhere in this report (section 3.3 and 3.4). Below is a brief summary with focus on the uncertainties, limitations and lack of knowledge for a valid quantitative exposure assessment to be performed for the pigments.

In a study of an azo dye (Pigment Red 22), the amount of pigment deposited in the skin (the human and pig) was found to range from 0.60 to 9.42 mg/cm^2 (mean 2.53 mg/cm^2), depending on the tattooing technique. Based on the mean value (2.53 mg/cm^2) and an average area of a tattoo (454 cm^2), the estimated average amount of pigment deposited in the skin was calculated to 1,149 mg. For the group with tattoos covering a large skin area (1,090 cm^2) and the highest value of pigment deposited in the skin (9.42 mg/cm^2), the estimated amount of pigment deposited in the skin was calculated to 10,268 mg. Obviously, there is great variation in the amount of pigment deposited in the skin depending on the tattoo technique, as well as a big differences in the tattooed skin area from person to person. Both conditions thus imply that there is even a very great variation among tattooed individuals with regard to the amount of pigment(s) deposited in the skin. A limitation in relation to a valid quantitative exposure assessment of the pigments is thus, the uncertainty due to the large individual variation in the amount of pigment(s) deposited in the tattooed skin area. The same limitation will also apply to the coformulants and chemical impurities that occur in tattoo inks as particles.

Within the first weeks after tattooing, the pigments will move locally in the skin. Part of the deposited pigments is degraded locally in the skin to other chemical substances, for instance under influence of (sun)light. Although the intention is that the pigments should remain in the skin, part of the deposited pigments will be transferred to the lymphatic vessels and blood circulation, particularly nanoparticles, and thus, the pigments are absorbed and distributed to the tissues and organs in the body. For example, the pigments can be deposited in the regional lymph nodes from which there is a direct contact with the blood-forming system (bone marrow) and immune system (lymph nodes).

The kinetics have also been studied in mice by using the azo dye ‘Pigment Red 22’. The amount of the pigment in the skin was reduced to 32% of the initial dose at 42 days after the tattooing was performed. When the tattooed skin area was exposed to sunlight the reduction was greater. There is, however, no information about the percentage of the initial dose that was absorbed or the percentage of the initial dose that was degraded locally in the skin. A limitation in relation to a quantitative exposure assessment of Pigment Red 22, in this study as well as in general, is thus, the lack of knowledge about the ratio between the percentage of the pigment absorbed and the percentage of the pigment degraded locally in the skin. The same limitation will also apply to other pigments, as well as to the coformulants and chemical impurities that occur in tattoo inks as particles.

Furthermore, it should be noted that the structure of mouse skin is very different from the structure of human skin. This means that the uptake and degradation of Pigment Red 22 in human skin can be quite different from that in mouse skin. Another limitation in relation to a human exposure assessment of Pigment Red 22 is thus, the lack of knowledge regarding the importance of the structural differences between human skin and mouse skin in terms of absorption and degradation of Pigment Red 22 deposited in the skin. The same limitation will also apply to other pigments, as well as to the coformulants and chemical impurities that occur in tattoo inks as particles.

Most of the pigments in the purchased tattoo inks belong to one of the following chemical groups: Carbon black, phthalocyanines, azo dyes, acridines and inorganic pigments (e.g. titanium dioxide) (Annex B). These pigments are both chemically and structurally very different. A great uncertainty is thus encumbered with respect to provide specific and valid estimates/assumptions for deposition, absorption, distribution, metabolism and excretion of the different pigments in the analysed tattoo inks in tattooed humans based on a single study in mice of a single pigment (Pigment Red 22), as these parameters very likely will vary for the different pigments due to the chemical and structural differences of the pigments. The same uncertainty will also apply to coformulants and chemical impurities that occur in tattoo inks as particles. It should be noted that acridines were not included in the analysis program in this project and therefore, nor in the health effect assessment.

Nanoparticles injected in the skin can be distributed to the organs in the body (liver, kidneys, spleen), whereas larger particles are not transferred to the blood circulation (Section 3.4). The distribution of nanoparticles in the body is different from dissolved substances, as well as for larger particles. Therefore, the content of nanoparticles in the tattoo inks is particularly contributing to the uncertainty in terms of which organs in the body (in addition to the skin and the lymph nodes that drain the tattooed area) that are exposed to the pigments/(nano)particles. Another limitation in relation to a valid quantitative exposure assessment for pigments, as well as for the coformulant and chemical impurities that occur in tattoo inks as particles, is thus, the lack of knowledge about how nanoparticles are absorbed and distributed to the tissues and organs in the body.

From a precautionary approach it could be assumed that the pigments, as well as other chemical substances occurring as particulates in tattoo inks, behave like substances that are completely dissolved in biological fluids and thus, absorbed completely (100%) from the tattooed skin area and subsequently transported and distributed to the tissues and organs in the body. With regard to the pigments, this is, however, a completely unrealistic ‘worst case’ assumption as pigments with very low solubility particularly are selected for tattooing in order to ensure that they remain in the skin after tattooing. The same condition will also apply to the coformulants and chemical impurities that occur in tattoo inks as particles.

Based on the kinetic study with Pigment Red 22 in mice it could be assumed that approximately one-third of a given pigment would be deposited in the skin and that the remaining two-third would not be degraded locally in the skin and consisting exclusively of nanoparticles. In this case, the percentage absorbed could amount up to two-third of the initial amount of pigment injected in the skin, i.e. could be transported and distributed to the tissues and

organs in the body and there exert an effect. This ‘worst case’ assumption is, however, as described above, encumbered with a high degree of uncertainty related to the extrapolation of the results for a single pigment in a single study in mice to a human exposure assessment for the various pigments, as well as for the coformulants and chemical impurities that occur as particles in the analysed tattoo inks.

Due to the uncertainties and variables that are related to the exposure assessment as described above, the current knowledge is considered as being insufficient for a valid quantitative exposure assessment of the selected chemical substances in the analysed tattoo inks, as well as for pigments, coformulants and chemical impurities that occur in tattoo inks (as particles) in general. Quantitative exposure assessments based on ‘worst case’ estimates and assumptions are considered likely to be more misleading than indicative of the real human situation. Acknowledging this, quantitative exposure assessments of the selected chemical substances in the analysed tattoo inks have not been performed in this project.

5.4.2 Limitations / lack of knowledge: Hazard assessment

The outcome of the hazard assessment is the identification of the critical effect and the establishment of a DNEL/DMEL for the critical effect.
In the hazard assessments of the selected chemical substances, the critical effect(s) in relation to tattooing wa identified and a DNEL/DMEL for the critical effect(s) was established if possible (Section 5.3).

This project has revealed that a limitation in relation to the hazard assessment for most of the selected chemical substances/groups is that it has not been possible to establish a DNEL/DMEL for the critical effect(s), usually carcinogenicity and/or sensitisation.
With regard to carcinogenicity, the data for the majority of the selected substances for which the carcinogenic effect was considered as the critical effect in relation to tattooing (PAH and the 10 selected PAA) were not sufficient for establishing a DMEL. Thus, it has only been possible to establish a DMEL for one of the 16 analysed PAH and for two of the 10 analysed PAA.
With regard to sensitisation, the data for the selected substances for which sensitisation has been considered as the critical effect in relation to tattooing (aluminium, chromium, nickel, aniline, p-chloroaniline, 3,3’-dichlorobenzidine and 4-methyl-m-phenylenediamine) were not sufficient for an evaluation of the potency or threshold value and thus, a DNEL/DMEL could not be established.

For one substance (lead), a DNEL could not be established as a threshold for the critical effect of lead (effects on the developing nervous system) has not been identified.
For three substances (copper, titanium (titanium dioxide) and carbon black), a critical effect in relation to tattooing could not be identified based on the current knowledge and thus, a DNEL/DMEL could not be established for these substances.

In general, knowledge is lacking regarding the development of cancer in relation to tattooing. As described elsewhere (Section 3.4, 5.4.1), the pigments in tattoo inks can be transported from the tattooed skin area to the regional lymph nodes, in which tumours can develop. Whether the development of tumours in the lymph nodes is related to the intrinsic properties of the

pigment or is a result of deposition of foreign particles in the lymph nodes cannot be evaluated based on the current knowledge. A possible association between the development of skin cancer and tattooing has been addressed (section 3.6); an association has neither been proved nor indicated.

In general, knowledge is also lacking regarding sensitisation in relation to tattooing. As described elsewhere (section 3.6 and 6.2), there is a high degree of uncertainty on how allergic reactions in relation to tattooing appear clinically and how an allergic reaction can be documented. This is due to the fact that the usual tests for contact allergy are not suitable for testing particulates such as e.g. pigments in tattoo inks as they have neither been developed nor validated for this purpose. In addition, it is assumed that allergy to a permanently deposited substance/pigment in the skin probably is clinically different from ordinary contact eczema. Based on the current knowledge it is thus, not possible to evaluate whether sensitisation due to skin contact with the selected substances actually is a critical effect in relation to tattooing where the substances are deposited in the skin. However, from a precautionary perspective, sensitisation should be considered as a possible critical effect of the above-mentioned substances in relation to tattooing for the time being.

5.4.3 Limitations / lack of knowledge: The selected chemical substances

In this project, 21 substances / groups of substances were selected for the health effect assessment: Carbon black, phthalocyanines, 10 PAA, PAH and eight elements. A number of uncertainties, limitations and lack of knowledge have been identified in relation to the exposure assessments for the selected chemical substances (Section 5.4.1) as well as in relation to the hazard assessment for a number of the selected substances (section 5.4.2). The uncertainties, limitations and lack of knowledge imply that a valid risk characterisation according to the REACH guidance documents (calculation of RCR) cannot be performed.

The main uncertainties, limitations and lack of knowledge for each of the selected chemical substances / substance groups are summarised in this section.

5.4.3.1 Carbon black

Carbon black is used as a pigment in tattoo ink and has been analysed as such in the selected tattoo inks (four black inks and one gray). In principle, it should be possible to estimate a systemic exposure based on the results of the analyses. However, as described in section 5.4.1, the current knowledge is, in general, considered as being insufficient for a valid quantitative estimate of the systemic exposure to particles deposited in the skin. In addition, carbon black often occurs in the form of nanoparticles in tattoo inks. Due to the uncertainty on how nanoparticles are absorbed and distributed to the tissues and organs in the body from a tattooed skin area it is not possible to provide a valid quantitative exposure estimate for carbon black (nanoparticles) based on the current knowledge.

It was recently reported that carbon black nanoparticles can cause DNA damage in cells from the lungs of experimental animals. Based on the available knowledge it is not possible to assess the possible critical health effects of carbon black as nanoparticles. It should also be noted that carbon black originating from hydrocarbon sources may contain different amounts of known carcinogens (e.g. PAH). This might be a critical effect of carbon black

in relation to tattooing; however, the current knowledge is not sufficient for an evaluation of this aspects. Consequently, a DNEL/DMEL for the possible critical effect of carbon black cannot be established.

Based on the current knowledge it cannot be evaluated whether carbon black would pose a health risk following tattooing with carbon black-containing tattoo inks.

5.4.3.2 Phthalocyanines

Phthalocyanines are used as pigment in tattoo inks. Phthalocyanines form complexes with most of the elements in the periodic table. In general, all the complexes are of very low solubility in most solvents, including water.

Phthalocyanines have been analysed qualitatively in a blue ink, in three green inks and in two purple inks. All six inks contained phthalocyanines. This qualitative analysis can, however, not be used as a basis for a quantitative exposure assessment.

In addition, the content of phthalocyanines in a green and in three blue inks has been calculated. The calculation was based on the concentration of copper found in the chemical analyses in these inks. The results of this calculation could, in principle, be used as a basis for an estimate of the systemic exposure for the copper-containing phthalocyanines. However, as described in section 5.4.1, the current knowledge is, in general, considered as being insufficient for a valid quantitative estimate of the systemic exposure to particles deposited in the skin.

The critical effect of phthalocyanines in relation to tattooing was considered to be the decreased number of red blood cells. The DNEL was set at 2 mg/kg bw per day. It should be noted that the DNEL might be overestimated (see Section 5.3.3).

Based on the current knowledge it cannot be evaluated whether phthalocyanines would pose a health risk following tattooing with phthalocyanine-containing tattoo inks.

5.4.3.3 Primary aromatic amines (PAA)

Many azo compounds are used as pigments in tattoo inks. Azo compounds are characterised by containing one or more azo groups, i.e. double bonds between two nitrogen atoms. The azo group is not stable and will under certain conditions be degraded to the original building blocks of the azo compound, i.e. aromatic amines.

In this project, azo dyes have not been analysed as such and therefore, the analyses cannot provide a basis for an exposure assessment of azo dyes as such.

An indication of the presence of azo dyes in tattoo inks was in this project represented by the analyses of PAA released from azo dyes. However, PAA can also occur as impurities in the tattoo inks (for example as residues from the manufacturing) or they might for some purpose have been added to the tattoo inks deliberately, in this project represented by the analyses of free PAA. Based on the analyses in this project, it is only possible for some of the analysed tattoo inks to evaluate whether a detected PAA in the tattoo inks occurs as such as an impurity of the tattoo inks or in the azo dyes, or whether a detected PAA occurs in the analysed tattoo inks as a result of degradation of

an azo dye in the analytical process. There is a lack knowledge whether the degradation of azo dyes and the resulting release of the building blocks, PAA, could occur in the skin after tattooing. Furthermore, there is a lack of knowledge about how the selected PAA are absorbed and distributed to the tissues and organs in the body from a tattooed skin area. Based on the current knowledge it is therefore, not possible to provide a valid quantitative exposure estimate for the selected PAA.

A number of PAA are known or suspected human carcinogens. In the hazard assessment of the ten selected PAA, the carcinogenic effect was considered as the critical effect in relation to tattooing for all ten PAA; however, only for two of the substances a DMEL could be established. For the remaining PAA it was not possible to established a DMEL based on the available data. IARC (IARC 2008[123]) has evaluated a number of aromatic amines for their carcinogenicity, including three of the ten selected PAA (4-chloro-o-toluidine, 2-naphthylamine and o-toluidine). In the introductory section to the IARC monograph it was concluded that most, if not all, aromatic amines are carcinogenic and that the mode of action for the carcinogenic effect apparently is the same for the aromatic amines. It is therefore likely that there might be a cumulative effect provided that more than one PAA is present in a tattoo ink.

Sensitisation by skin contact was also considered as a critical effect in relation to tattooing for four of the ten selected PAA. The data are, however, not sufficient for an evaluation of neither the potency nor the threshold value and therefore, a DNEL for sensitisation cannot be established for these substances. In general, knowledge is also lacking regarding sensitisation in relation to tattooing and, based on the current knowledge, it is therefore not possible to evaluate whether sensitisation due to skin contact with the selected substances actually is a critical effect in relation to tattooing where the substances are deposited in the skin.

Based on the current knowledge it cannot be evaluated whether the selected PAA would pose a health risk (cancer, sensitisation) following tattooing with PAA-containing tattoo inks.

5.4.3.4 Polycyclic aromatic hydrocarbons (PAHs)

PAHs are not used as such as pigments in tattoo inks. However, in a recently published study PAH were detected in a number of black tattoo inks containing carbon black (section 1.10.4), in which they presumably occur as impurities in carbon black. In this project, 16 different PAH were analysed in 19 different tattoo inks. The highest PAH content was found in black inks that also had a high content of carbon black. This could indicate a correlation between the content of carbon black and PAH. However, it cannot be excluded whether PAH in the tattoo inks originate from other sources. BaP was detected in only a single tattoo ink.

Based on the analyses, it is not possible to evaluate whether the detected PAH occur as such as impurities in the tattoo inks or are released from carbon black during the analytical process. There is also a lack knowledge whether the release of PAH from carbon black could occur in the skin after tattooing. Furthermore, there is a lack of knowledge on how the PAH are absorbed and

[123] IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 99, Some Aromatic Amines, Organic Dyes, and Related Exposures. IARC, Lyon, France, 2010.

distributed to the tissues and organs in the body from a tattooed skin area. Based on the current knowledge it is therefore, not possible to provide a valid quantitative exposure estimate for the PAH.

The critical effect of BaP, as well as for several other PAH, in relation to tattooing is considered to be the carcinogenic effect. A DMEL has been established for one of the PAH (BaP). For the other PAH found in the analysed inks, a DMEL could not be established based on the available data. The mode of action for the carcinogenic effect is probably common for all the carcinogenic PAH, although the potency may vary considerably (see Section 5.3.4). It is therefore likely that there might be a cumulative effect provided that more than one PAH is present in a tattoo ink. However, based on the current knowledge it is not possible to set a DMEL for mixtures of different PAH in the analysed tattoo inks.

Based on the current knowledge it cannot be evaluated whether PAH would pose a health risk (cancer) following tattooing with PAH-containing tattoo inks.

5.4.3.5 Elements

The selected elements (aluminium, barium, cadmium, chromium, copper, lead, nickel, titanium) are not used as such in tattoo inks or as coformululants. Based on the analyses in this project, it is not possible to evaluate whether the detected elements occur as such as impurities in the pigments and/or coformulants or in the tattoo inks. Or whether the detected elements occur in the analysed tattoo inks as a result of degradation of the pigments, coformulants and/or chemical impurities in the tattoo inks during the analytical process. This means that the analyses actually cannot form the basis for exposure assessments of the selected elements, as well as of the pigments, coformulants and/or chemical impurities from which the detected elements might be released. Moreover, there is a lack knowledge whether the release of the various elements from pigments, coformulants and/or chemical impurities in the tattoo inks or the colorants could occur in the skin after tattooing. Based on the current knowledge it is therefore, not possible to provide valid quantitative exposure estimates for the selected elements, as well as for the pigments, coformulants and/or chemical impurities from which the detected elements might be released.

The critical effect of aluminium in relation to tattooing was considered to be the granulomas developed in tattoos as a result of a local foreign body reaction. It cannot be excluded that an allergic reaction might also be involved. A DNEL could not be established for the critical effect.
The critical systemic effects of aluminium are considered to be the effects on the reproductive system and the developing nervous system. The PTWI set by JECFA, converted to a daily dose, corresponds, in principle to a DNEL.

Barium sulphate is insoluble and thus, relatively inert in contrast to ionic barium and soluble barium salts such as e.g. the chloride, nitrate and hydroxide (from which the barium ion is released in the body). Ionic barium is toxic to several organs and tissues, including the nervous system, kidney and heart. The critical effect of barium in relation to tattooing was considered to be the cardiovascular effects; a DNEL was established for the critical effects.
Some barium salts are alkaline and therefore, might exert local effects in the skin. Barium can react with titanium dioxide thereby forming the insoluble salt barium titanate. This might be of particular importance in tattoo inks in

which both soluble barium salts and titanium dioxide occur. The extent of degradation of the insoluble barium sulphate and barium titanate locally in the skin thereby releasing the toxic barium ion is not known.

The critical effects of cadmium in relation to tattooing was considered to be the effects on bones and kidneys. A DNEL was established for the critical effects. It should be noted that since cadmium accumulates in the body, the DNEL should be set as an average value over a week or a month.

The critical effect of chromium (VI) in relation to tattooing was considered to be sensitisation. A DNEL could nnot be established for the critical effect. In general, knowledge is lacking regarding sensitisation in relation to tattooing and thus, it is not possible to evaluate whether sensitisation due to skin contact with chromium (VI) actually is a critical effect in relation to tattooing. It should be noted that the analytical method for chromium in this project could not distinguish between Cr(III) and Cr(VI) and thus, there is a lack of knowledge whether chromium occurs as chromium (III) and/or chromium (VI) in the analysed tattoo inks. In addition, there is a lack of knowledge regarding possible health effects of chromium (III).

Based on the current knowledge, no critical health effects of copper in relation to tattooing was identified. It is considered likely that the copper found in the analysed tattoo inks may originate from degradation of copper-containing phthalocyanines. Whether such a degradation also could take place in the skin is not known.

The critical effect of lead in relation to tattooing was considered to be the effects on the developing nervous system (children as well as the unborn child). A DNEL for the critical effect could not be established.

The critical effect of nickel in relation to tattooing was considered to be sensitisation. A DNEL could not be established for the critical effect. In general, knowledge is lacking regarding sensitisation in relation to tattooing and thus, it is not possible to evaluate whether sensitisation due to skin contact with nickel actually is a critical effect in relation to tattooing. Nickel sulphate, nickel chloride and nickel nitrate are classified as skin irritants. It cannot be excluded that skin irritation might also be a critical effect of nickel in relation to tattooing.

It was recently reported that titanium dioxide nanoparticles caused pulmonary effects (changes in expression of genes related to inflammation and immune reactions) after inhalation. Titanium dioxide occurs often as nanoparticles in tattoo inks. Based on the current knowledge, it is not possible to assess the possible critical health effects of titanium dioxide as nanoparticles. It should be noted that titanium dioxide has not been analysed as such in this project. It is, however, considered likely that the elemental titanium found in the analysed tattoo inks may originate from degradation of titanium dioxide.

Overall, based on the current knowledge, it could not be evaluated whether the eight selected elements would pose a health risk following tattooing with tattoo inks containing the these elements as such, or containing pigments, coformulants, and/or chemical impurities from which these elements might be released in the skin following tattooing.

5.4.4 Risk characterisation: Conclusion

Twenty-one substances / substance groups were selected for the health effect assessment: Eight elements, ten aromatic amines, carbon black, PAH and phthalocyanines.

This project has revealed a number of limitations as well as lack of knowledge in order to perform quantitative exposure assessments. Due to the uncertainties and variables that are related to the exposure assessments as described in detail in Section 5.4.1, the current knowledge is considered as being insufficient for a valid quantitative exposure assessment of the selected chemical substances in the analysed tattoo inks, as well as for pigments, coformulants and chemical impurities that occur in tattoo inks in general. Quantitative exposure assessments based on 'worst case' estimates and assumptions are considered likely to be more misleading than indicative of the real human situation. Acknowledging this, quantitative exposure assessments of the selected chemical substances / substance groups in the analysed tattoo inks have not been performed in this project.

This project has also revealed a number of limitations and lack of knowledge in relation to the hazard assessment (identification of critical effect in relation to tattooing and establishment of DNEL/DMEL for the critical effects) for a number of the selected chemical substances (section 5.4.2). One limitation is that for most of the selected substances / substance groups it has not been possible to establish a DNEL/DMEL for the critical effect(s), usually a carcinogenicity and/or sensitisation. In addition, knowledge is, in general, lacking regarding the development of cancer as well as of sensitisation in relation to tattooing.

The uncertainties, limitations and lack of knowledge in relation to the exposure assessment for the selected chemical substances / substance groups in the analysed tattoo inks, as well as in relation to the hazard assessment for a number of the selected substances, imply that a valid risk characterisation according to the REACH guidance documents (calculation of RCR) could not be performed.

Overall, based on the current knowledge, it could not be evaluated whether the selected chemical substances / substance groups would pose a health risk following tattooing with tattoo inks containing the selected chemical substances / substance groups as such, or containing pigments, coformulants, and/or chemical impurities from which the selected chemical substances / substance groups might be released in the skin following tattooing.

It should be noted that several case studies have described adverse reactions among tattooed individuals who have been tattooed with several of the tattoo inks analysed in this project, see the following chapter.

6 Health assessment: Patient reactions

During the survey, a number of tattoo inks were registered as they are associated with patient reactions arising after tattooing with the relevant colour. The results of the investigations are described in this chapter.

6.1 Background

A high degree of evidence regarding the composition of tattoo colours and possible side-effects can be obtained by directly studying people who have experienced visible side-effects from tattoos and where the applied ink also can be procured and analysed. Changes in the skin and reaction patterns can be unambiguous and follow one or several patterns related to a certain pigment, a certain chemical type of pigment or a certain colour, or they can be multiple and therefore difficult to identify and characterise. The consolidation of knowledge of specific patterns requires additional observations with the same conclusion.

As the skin's barrier to penetration of chemical substances from the outside is very dense, it makes a great difference if the chemical substance gets into contact with the skin from the outside, e.g. through contact with cosmetic products, or by injecting the substance into the skin as is the case when injecting tattoo ink with a tattoo needle and when injecting medicine with a needle and syringe.

At the Danish hospital called Bispebjerg Hospital, Department of Dermatology D, a large number of people with reactions to tattoos have been examined as part of a department project. In addition, a number of paraclinical examinations have been carried out in the form of i.a. allergy tests and biopsies. Usually, it is not possible for the examined people to procure exact information about which colour they have been tattooed with, but in this case some could. The colours that were identified during the survey were purchased and the compounds were examined in this project.

The selected cases have been supplemented with cases from other sources where information about reactions and applied colours exists and on that basis it will be estimated if general conclusions can be drawn about possible relations between tattoo ink and clinical risks.

Some general observations will initially be presented and general patterns and mechanisms will be outlined.

6.2 General conditions

Tattooing itself is accompanied by pain and under tattooing everybody immediately experiences a reddening and certain swelling of the skin due to the numerous needle pricks in the skin. During such an inflammatory condition histamine is liberated in the skin – this substance is liberated in

connection with all skin traumas of a certain degree and in addition to pain and suffering also results in a temporary reaction in the vessels of the skin followed by reddening and a sensation of heat. In the days after and several weeks after tattooing, a rejection reaction of damaged skin appears and in addition to that there is a certain rejection of surplus pigment existing in the superficial layers of the skin and in scabs. At the beginning, the skin can be humid and suppurating. During healing, there is a phase with dryness and creation of scale and chaps might arise. A moisturising cream is often used according to the recommendation of the tattooist. In uncomplicated cases, the skin heals after 3-4 weeks. Healing can be complicated by infections from the bacteria of the skin or from micro organisms coming from the tattoo ink.

A couple of months pass before cronical complications of permanent character appear. In those cases, the skin often thickens, possibly becomes extremely thick, with scale creation and troublesome itching and pain. Such complications require dermatological treatment.

Cronical adverse events such as swelling and itching when the tattoo is exposed to sunlight are other well-konwn complications or adverse events. Often, the tattooed person does not consult a doctor about the problem, but merely uses a sun creme on the tattoo or according to routine covers the tattoo with clothing. The reactions calm down in a few hours and leave no after-effects. They consistently appear during exposure to sun.

The chronical reactions of permanent character can in principle be induced either by the particles or by the chemical substances in the colours or possibly by a chemical substance on the surface of the particles.

Particles are foreign bodies. In general, foreign bodies liberate a so-called foreign body reaction in the skin as the organism trys either to encapsulate and inactivate the particle or attempts to find its way to the skin surface and expel the foreign body. Under the microscope, the creation of encapsulated particle and cell nests can be seen, so-called granuloma. This type of tattoo reaction is rather frequent. Clinically, tattoo granuloma appear as tissue in the size of a pea formed as a dome in a certain colour in the tattoo.

A frequent, permanent type of reaction is the lichenoid reaction, where epidermis thickens with squamous, cornified formations of up to a thickness of 5-10 mm in all parts of the tattoo where a certain colour exists, most often red[124]. Under the microscope, it appears that epidermis cells have proliferated and there is a great occurrence of white blood cells, so-called inflammation. The mediators of inflammation give rise to itching and adverse events. This type of reaction is not explained as a foreign body reaction and can be due to several mechanisms:

- Chemical irritation from the pigment and/or chemical substances in the tattoo ink of an unspecified character causing a chronical inflammatory condition. The irritation might originate from too much colour being tattooed into the skin. The irritation can vary from light to severe and can possibly manifest itself as a lichenoid reaction.

- Allergic reaction aimed at a chemical substance (e.g. pigment or residue) in the colour, chemical substance that is split off the pigment

[124] Examinations carried out at Bispebjerg Hospital, Department of Dermatology D

or exposed on the surface of the pigment, or as residue contained in the pigment particles. The allergy-causing agent can be the chemical substance itself or the substance in connection with a tissue protein or an amino acid, a hapten. The immune system regards the allergenic agent as a foreign body and trys to eliminate it by creating lymphocytes that are specifically aimed at the allergy-causing agent and that concentrate in the tattoo resulting in a chronical reaction possibly in the form of a lichenoid reaction, clinically speaking. Allergic reaction in tattoos can also result from other substances than the pigment, including residue from the production of e.g. nickel and chromium. Tattoo inks are not suited for allergy tests in the form of contact allergy tests/patch tests as the colorant in the ink is partly unknown, particulate and not in soluble form and as the particles can be chemically coated. In addition, some tattoo colours are mixed colours that contain several different pigments. Therefore, a patch test would not be able to conclude which content in the tatto colour specifically induces the allergic reaction and to transfer it to other tattoo inks. Complex allergic reactions in the body mediated by tattooing in the form of widespread reactions in the vascular system, vasculitis, and in the form of iritis in the eye, also after tattooing, can be induced through an allergic reaction by a pigment protein complex and allergic reactions to tattoo colours are not obligatory limited to a simple chemical substance as mediator. The above indicates that an allergic reation to tattoo inks can comprise several types of allergic mechanisms and not merely type IV allergy (contact allergy).

- Reaction with deep wounds in the skin, i.e. ulceration and possibly necrosis with a wound in subcutis that will not heal. The mechanism in these very severe reactions can be a direct effect on the tissue from a colour that constitutes a severe basic chemical irritant, a severe allergic reaction or exposure to a chemical substance in the colour with a cytotoxic effect. The rare appearance of these reactions and their long and therapy resistant courses indicate that the reactions typically are allergic and that the allergy is serious. Manifestation can result in disablement and the need for surgical treatment, including amputation, might arise. A generalised allergic reaction can develop.

Special mechanisms and reactions to tattooing comprise:

- The influence of pigment and/or chemical substances on the cells and tissue of the skin with the induction of an abnormal biological reaction in the skin through the influence of cells and tissue of the skin as a well-defined proliferative response with increased proliferation of certain cells – by increased proliferation of epidermis cells (a lichenoid reaction - see above), by stimulation of the collagen creating cells of the skin, fibroblasts, abnormally increased cicatrization in the tattoo in the form of a keloid, by initiating the concentration of white blood cells in the skin the creation of pseudolymphoma, a benign type of leukaemia.

- Carcinogenic effect on the cells of the skin resulting in an uninhibited regeneration of cells and aggressive growth with skin cancer, especially basal cell carcinoma and melanoms, or by further development of pseudolymphoma to an actual leucemic condition. However, the frequency of this and the casual relation between tattoo ink and cancer

originating in a tattoo has currently not been concluded as only sparse observations exist. A carcinogenic effect could be manifested in the lymph glands that drain the tattooed skin area, especially if the area of the tattoo is large.

- Congenital malformations, teratogenic effect, presuppose systematic circulation of substance from the tattoo. The effect, which could be of clinical importance to tattoos made during the first three months of a pregnancy and which could be induced by a small substance amount, has not been systematically investigated and has therefore not been clarified and can therefore not be excluded.

6.3 Cases

Table 6.1 shows the findings in eight persons with nine reactions to tattoos of which five were investigated and elucidated at Bispebjerg Hospital, Department of Dermatology D. A clinical evaluation was carried out and when feasible it was supplemented with skin biopsy and standard allergy tests (patch tests) for contact allergy, including tests for nickel allergy and chromium as well as tests on specific tattoo inks used by a specific tattooist in the specific case that caused problems.

On the basis of the information stated on the labels of the tattoo ink used on 5 persons, 12 different pigments formed part of the applied tattoo inks established by their CI number. However, CI 77891 was the only pigment that recurred in two applied colours. That might be an incidental observation and the table shows that neither one nor a few pigments exist that have the main responsibility or the general responsibility for the reactions.

None of the five persons who were examined at Bispebjerg Hospital by means of standard allergy tests showed a reaction to nickel or chromium and an active allergic reation to those metals was therefore unlikely as the reason for the tattooing reactions, irrespective of the analytic content of these metals in the tattoo inks. Either the expsosure to these metal ions during tattooing was below a possible sensitization limit, or the metal ions were not in free form in the tattoo inks, but bound in a chemical combination (pigment/subsidary ingredient/chemical impurities). Finally, an explanation could be that the persons were not susceptible to this specific allergy.

With regard to type of reaction, there were three different cases of lichenoid reaction of different degrees. In connection with lichenoid reactions, the changes developed over weeks to months and consisted of a nodular, squamous firm swelling in the tattoo in all areas where the causative tattoo colour had been used. Under the microscope, a pronounced reactive thickening of epidermis and infiltration with white bloodcells was seen in the underlying dermis, where the pigment that induced the reaction was found. In connection with inflammatory reaction a quick reddening and swelling of the skin appeared followed by healing after a shorter or longer period of time. In connection with ulceration with necrosis (dead tissue) there was considerable reddening and swelling of the skin with severe ulceration and dead tissue in the tattoo, rejection of the tattoo and skin in full thickness in the course of a few weeks resulting in a deep wound down to subcutis. The wound healed slowly after months.

Table 6.1 Observed or reported clinical reactions to tattoo inks

Ink no.	Text on label	Source	Clinical reaction
18 red	CI# 12390 Alcohol, glycerin	BBH/Patient 1	Lichenoid reaction, severe
24 red	CI# 73915 CI# 21110 CI# 77891 CI# 12477 Proprietary, glycerin, isopropanol	Interview	Reaction type uninformed
35 Violet	CI# 73900 Proprietary, glycerin, isopropanol	BBH/Patient 2	Lichenoid reaction, moderate
36 Yellow	CI# 21108 CI# 77891 Alcohol, glycerin	BBH/Patient 3	Inflammatory reaction (previous vaccination granuloma)
37 Violet	CI# 15880 CI# 74160 CI# 77891 CI#74260 Alcohol, glycerin	BBH/Patient 4	Lichenoid reaction, severe
48 red	Uninformed	Internet	Uninformed
49 red	Uninformed	Internet	Uninformed
53 red	Uninformed	BBH/Patient 5	Wound with skin necrosis, 3+ allergy test
57 brown	Uinformed	BBH/Patient 5	Inflammatory reaction, 2+ allergy test

BBH = Bispebjerg Hospital, Department of Dermatology D

The chemical analyses in this report were carried out on in-duplicate purchased fresh samples of tattoo inks and not directly on the samples, which the persons suffering from the reactions obtained from their tattooists. That means that the samples were not strictly authentic. During allergy testing, the authentic samples were used.

Two out of three persons with lichenoid reactions were tattooed with colours with a high content of aluminium, while analysis of PAA did not show special findings.

The person with known vaccination granuloma caused by foreign bodies in the form of aluminium particles from previous vaccination did not have an especially high content of aluminium in the applied tattoo ink and did not react with a granulomatous reaction in the tattoo. The cause of the person's inflammatory reaction is unsolved, but hardly related to aluminium.

The person with a wound and skin necrosis in the tattoo differed partly by having a serious, invasive complication in his tattoo and partly by having a very positive allergy test reaction to the applied red colour besides a very positive allergy test reaction to brown colour in the same series, irrespective of the person not being tattooed with brown (the brown colour is assumed to contain the same pigment as in red colour in mixtures with other pigments, including carbon black as darkener). The series was imported from Taiwan. The label did not state the name of the manufacturer or the CI number and therefore there was no information about the chemical pigment.

The series consisted of a total of 6 colours, of which the 4 non-red colours in allergy tests showed a negative reaction. Allergy tests of the red and brown colour showed a positive reaction and the tattoo colours were analysed. A very high content of PAA was demonstrated in both colours, which can indicate a

content of azo colorant. The conclusion concerning that person was that the person probably had been allergically sensitized against red azo colorant or PAA's (e.g. present in the form of residue or decomposed from azo colorant) and that the person had developed the allergy as a consequence of the tattoo with red colour as there was a latent period of some weeks from tattooing took place and till the reaction developed. The reaction resolved without other complications as the skin in its entire thickness had been rejected and the pigment thus eliminated.

The persons who had lichenoid reactions were all tested with the applied colour. Two showed uncertain toxic reactions of a mild degree, but neither had reactions that could be interpreted as allergic.

The mentioned cases indicate the existence of allergic reactions to azo colorant or PAA's (e.g. present in the form of residue or decomposed from the azo colorant) in red tattoo ink and can give a heavy reaction with wounds and skin nicrosis as the allergy-causing agent is deposited directly in the skin. Allergic sensibilization hardly lies behind the lichenoid reactions, but it is rather a reactive condition in the skin, whereby an attempt is made to eliminate foreign matter in the skin transepidermally resulting in a special reaction in epidermis with nodular thickening and lichenoid squamation. However, it is not impossible that lichenoid reactions can hide reactions against weak allergy-causing agents or allergenic agents with little discharge in the skin, for instance due to coating of the pigment. Just as in the case of pseudolymphomas (i.e. leukaemia-like reactions) in tattoos, it is not possible in the light of the data this report is based on to further illustrate granulomatous tattoo reactions that have much in common with vaccination granuloma. However, on the basis of literature on i.a. vaccination granulona it is probable that granulomatous reactions in tattoos are foreign body reactions to i.a. particulate aluminium contained in the colours.

It is not possible to illustrate the possible injurous effect of tattoo pigment deposited in lymph nodes or of pigment distributed systematically, possibly in the form of nanoparticles, as that has not been clarified in studies.

In the course of the project, an analysis was not carried out on which azo colorant formed the red pigment, which in the case of the person with an allergic reaction with a wound and skin necrosis was the guilty allergy-causing agent. A characterization of that could be of vital importance to solve why red tattoo inks very frequently are reported to have side-effects.

Allergy similar reactions of the granulomatous type have been reported among a number of tattooed persons. The reactions are probably related to aluminium, as aluminium exists in all tattoo inks with only a few exceptions, please see the results of the chemical analyses in Enclosure C. In tattoo inks it is probably mainly a question of particulate aluminium in the form of silicate or as another connection that as in paint is added to adjust the viscosity and to make the product tixotropic. A well-documented case of aluminium-induced granulomas in a tattoo has been published by McFadden et al. (1989)[125].

Since the 1970s, it is a well-known fact that aluminium in DiTePol vaccines that are injected in subcutis can give long-term nodosity with granuloma tissue structure that is interpreted as foreign body granuloma created with a

[125] McFadden N., Lyberg T., Hensten-Pettersen A. Aluminium-induced granulomas in a tattoo. J Am Acad Dermatol, 1989;20:903-8.

starting point in particles in aluminium hydroxide hydrate vaccines (Chong et al. 2006[126]).

Aluminium can induce reactions in the skin with eczema and inflammation, but it is still unclear if the reactions are allergic or of another nature, e.g. toxic with a background in the special physical-chemical reactions that can take place on the surface of the particles. Aluminium granulomas have been connected with granuloma creation in connection with sarcoidosis with lung affection and it is possible that an individual disposition for granuloma creation exists, a disposition that also can be of importance for tattoo granuloma.

6.4 Conclusion and summary

Reactions to tattoos comprise immediate reactions and delayed reactions and the clinical picture is rather manifold and does not have the same clinical appearance. That speaks in favour of not being able to relate the reactions to one individual chemical substance or type of substance, to one individual physical property or to one individual mediator mechanism.

That is confirmed by observating eight cases. The pigments in the colours showed great variation – only one pigment, CI 77891, recurred in two cases, perhaps due to coincidence. The tattoo reactions could not be related to a certain pigment as characterised by the CI number stated by the manufacturer.

Six persons were allergy tested (patch tests) with the tattoo colour that gave one person a serious reaction and with a general allergy test panel with the 42 most frequent contact allergy adverse events including nickel and chromium. The tests showed a negative reaction except for the person who earlier had reacted to the tattoo colour. That indicates that allergic mechanisms are ordinary especially as the colours were placed concentrated on the skin. However, tattoo ink is particulate and possibly coated and therefore not suited for patch tests. Nickel or chromium allergy do not seem to have any importance.

The person with a serious reaction in the form of a wound with necrosis in the skin in the red tattoo, had a serious reaction to (3+ reaktion) to the patch test with the applied tattoo colour. Demonstration of a high content of PAA in the tattoo colour by analysis indicated that the red colorant was of the type azo colorant. However, that does not mean that the specifically found PAA induced the reaction.

Cases confirm that reactions in red colour or red mixed colours are frequent and possibly related to content of azo colorant or the PAAs of the breaking down products. The particular form of azo colorants, their possible coating and other systematic conditions concerning these pigments, currently unknown, can be particularly significant to the occurance of clinical reactions. The cases do not unambiguously point at one specific pigment.

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[126] Chong H et al. Persistent nodules at injection sites (aluminium granuloma) – clinicopathological study of 14 cases with a diverse range of histological reaction patterns. Histopathology 2006;48:182-88.

Comparison of pigments is tattoo ink with the Executive Order on Cosmetics

Explanation to Table 1, "Area of application cosmetics":

1. Ink permitted in all cosmetic products.
2. Ink permitted in all cosmetic products with the exeption of cosmetic products for use around the
   eyes, especially eye make-up and cleansers for that purpose.
3. Ink that solely is permitted in cosmetic products that are not intended for contact with the mucous
   membranes.
4. Ink that solely is permitted in cosmetic products that are intended for brief skin contact.

Table 1 Pigments used in cosmetic tattoo inks and pigments that appear from the Executive Order on Cosmetics.

Name	CAS no.	Chemical name	Field of application cosmetics	CI number
Pigment Violet 23	6358-30-1	8,18-dichloro-5,15-diethyl-5,15-dihydrodiindol[3,2-b:3',2'-m]triphenodioxazine	Group 4	51319
Pigment Red 122	980-26-7	5,12-Dihydro-2,9-dimethylquino[2,3-b]acridin-7,14-dione	Group 4	73915
Pigment Yellow 1	2512-29-0	2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-N-phenylbutyramide	Group 3	11680
Pigment Orange 43	4424-06-0	Bisbenzimidazo[2,1-b:2',1'-i]benzo[lmn][3,8]phenanthroline-8,17-dione	Group 3	71105
Pigment Green 7	1328-53-6	Polychloro copper phthalocyanine	Group 2	74260
Pigment White 6	13463-67-7	Titanium dioxide	Group 1	77891
Pigment Red 101	1309-37-1	Iron(III)Oxide	Group 1	77491
Pigment Blue 15	147-14-8	Tetrabenzo-5,10,15,20-diazaporphyrinephthalocyanine	Group 1	74160
Pigment Blue 15:3	147-14-8	tetrabenzo-5,10,15,20-diazaporphyrinephthalocyanine	Group 1	74160
Pigment Black 7	1333-86-4	Carbon Black	Group 1	77266
Pigment White 6	13463-67-7	Titanium dioxide	Group 1	77891
Pigment Brown 6	52357-70-7	Iron oxide	Group 1	77499
Pigment Red 101	1309-37-1	Iron (III)oxide	Group 1	77491
Jernoxid	1345-25-1	Iron(II)oxide	Group 1	77489
Pigment Yellow 42	51274-00-1	Iron (III)oxide, monohydrate	Group 1	77492
Sudan Rød	1229-55-6	1-[(2-methoxyphenyl)azo]-2-Naphthalenol	Group 1	12150
Food Yellow 13	8004-92-0	2-(1,3-Dioxoindan-2-yl) quinolinedisulfonic acid sodium salt;	Group 1	47005
Mangan Violet	10101-66-3	Manganese ammonium pyrophosphate	Group 1	77742
Food Red 17	25956-17-6	2-Naphthalensulfonic acid, 6-hydroxy-5-((6-methoxy-4-sulfo-m-tolyl)azo)-, disodium salt	Group 1	16035
Food Blue 2	3844-45-9	Disodium bis[4-(N-ethyl-N-3-sulfonatophenylmethyl)aminophenyl]- 2- sulfonatophenylmethylium	Group 1	42090

Name	CAS no.	Chemical name	Field of application cosmetics	CI number
Acid Red 87	17372-87-1	2,4,5,7-Tetrabromofluorescein	Group 1	45380
Pigment Yellow 83	5567-15-7	2,2'-[(3,3'-Dichlor[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-chlor-2,5-dimethoxyphenyl)-3-oxobutyramid]	Group 4	21108
Pigment red 5	6410-41-9	N-(5-Chlor-2,4-dimethoxyphenyl)-4-[[5-[(diethylamino)sulfonyl]-2-methoxyphenyl]azo]-3-hydroxy-2-naphthalencarboxamid	Group 1	12490
Pigment violet 19	1047-16-1	5,12-Dihydroquino[2,3-b]acridin-7,14-dion	Group 4	73900
Pigment red 63:1	6417-83-0	Calcium 3-hydroxy-4-[(1-sulfonat-2-naphthyl)azo]-2-naphthoat	Group 1	15880
Pigment Orange 5	3468-63-1	1-[(2,4-Dinitrophenyl)azo]-2-naphthol	Not permitted	12075

Table 2 Pigments in cosmetic tattoo inks and pigments that do not appear from the Executive Order on Cosmetics

Name	CAS no.	Chemical name	CI number
Pigment Orange 36	12236-62-3	2-((4-chloro-2-nitrophenyl)azo)-N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxobutanamide	11780
Pigment Yellow 74	6358-31-2	2-[(2-methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	11741
Pigment Red 146	2786-76-7	4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2- carboxamide	12475
Pigment Yellow 97	12225-18-2	N-(4-chloro-2,5-dimethoxyphenyl)-2-[[2,5-dimethoxy-4-[(phenylamino)-sulfonyl]-phenyl]-azo]-3-oxo-butanamide	11767
Pigment Red 146	5280-68-2	N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide	12485
Pigment Brown 25	6992-11-6	4-[(2,5-dichlorophenyl)-azo]-N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxy-2-naphthalenecarboxamide	12510
Pigment Red 266	36968-27-1	Naphthol red	12474

Information on the pigments in the purchased inks

Content of pigment in an ink is marked with X in the tables.
The information is a copy from the labels and safety data sheets.
There is not in all cases agreement between the pigments stated on the labels and the pigments stated in the data safety sheets. That is the case for 10 of the purchased tattoo inks. In connection with those 10 tattoo inks, information that is not stated on the label, but merely originates from the safety data sheet, is indicated with *.

Table 1 Green colours

Info on label or safety data sheet	Pigment	Type af pigment	Green, Colour no.									
			6	*7*	*13*	*16*	*26*	*31*	*41*	*44*	*55*	*60*
Titanium Dioxide CI# 77891 CAS# 13463-67-7	Titanium dioxide	Inorganic pigment	X	X			X		X			X
Pigment Green 7 / Phthalocyanine Green 7 CI# 74260 CAS# 1328-53-6	Phthalocyanine	Phthalocyanine	X		X	X	X*		X			
Phthalocyanine Blue 15:3 / Pigment Blue 15 CI# 74160 CAS# 147-14-8	(29H,31H-phthalocyaninato (2-)-N29,N30,N31,N32) Copper	Phthalocyanine		X			X					
Pigment yellow 151 CI# 13980 CAS# 31837-42-0	2-[[1-[[(2,3-Dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]benzoic acid	Azo colorant		X								
Pigment yellow 83 CI# 21108 CAS# 5567-15-7	2,2'-[(3,3'-Dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-chloro-2,5-dimethoxyphenyl)-3-oxobutyramide]	Azo colorant		X			X*					
Pigment Yellow 74 / Arylide Yellow CI# 11741 CAS# 6358-31-2	2-[(2-Methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	Azo colorant				X						
Pigment yellow 65 CI# 11740 CAS# 6528-34-3	2-[(4-Methoxy-2-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxo-butyramide	Azo colorant					X					
Pigment black 7 CI# 77266	Carbon black	Carbon black						X				
Carbon black 7 CI# 77226	Carbon black	Carbon black										X

Info on label or safety data sheet	Pigment	Type af pigment	Green, Colour no.									
			6	*7*	*13*	*16*	*26*	*31*	*41*	*44*	*55*	*60*
Pigment red 5 CI# 12490 CAS# 6410-41-9	N-(5-Chloro-2,4-dimethoxyphenyl)-4-[[5-[(diethylamino)sulfonyl]-2-methoxyphenyl]azo]-3-hydroxy-2-naphthalenecarboxamide	Azo colorant						X				
Pigment orange 13 CI# 21110 CAS# 3520-72-7	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]	Azo colorant							X			
Pigment yellow 14 CI# 21095 CAS# 5468-75-7	2,2'-[(3,3'-Dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-methylphenyl)-3-oxobutyramide]	Azo colorant										X
Pigment orange 16 CI# 21160 CAS# 6505-28-8	2,2'-((3,3'-Dimethoxy(1,1'-biphenyl)-4,4'-diyl)bis(azo))bis(3-oxo-N-phenylbutyramide)	Azo colorant										X*
Pigment Orange 5 CI# 12075 CAS# 3468-63-1	1-[(2,4-Dinitrophenyl)azo]-2-naphthol	Azo colorant										X

*According to data sheet

Table 2 Red colours

Info on label or safety data sheet	Pigment name	Type of pigment	Red, Colour no.											
			1	5	17	18¤	24¤	33	34	39	48¤	49 ¤	53¤	63
Pigment Red 170 CI# 12475 CAS# 2786-76-7	4-[[4-(Aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide	Azo colorant		X			X*		X*	X				X
Pigment red 210 CI# 12477 CAS# 61932-63-6	Permanent Red F 6RK; Pigment Red 5S; Red 5S; Sunbrite Red 210				X		X		X					
Pigment red 17 CI# 12390 CAS# 6655-84-1	3-Hydroxy-4-[(2-methyl-5-nitrophenyl)azo]-N-(2-methylphenyl)-2-naphthalenecarboxamide; 3-Hydroxy-4-[(2-methyl-5-nitrophenyl)azo]-N-(o-tolyl)naphthalene-2-carboxamide	Azo colorant				X								
Titanium Dioxide CI# 77891 CAS# 13463-67-7	Titanium Dioxide	Inorganic pigment				X*	X			X				
Pigment red 2 CI# 12310 CAS# 6041-94-7	4-[(2,5-Dichlorophenyl)azo]-3-hydroxy-N-phenylnaphthalene-2-carboxamide	Azo colorant				X*								
Pigment Red 122 CI# 73915 CAS# 980-26-7	5,12-Dihydro-2,9-dimethylquino[2,3-b]acridine-7,14-dione	Acridine					X							
Pigment orange 13 CI# 21110 CAS# 3520-72-7	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]	Azo colorant					X		X					
Pigment Yellow 97 CI# 11767 CAS# 12225-18-2	N-(4-Chloro-2,5-dimethoxyphenyl)-2-[[2,5-dimethoxy-4-[(phenylamino)sulphonyl] phenyl]azo]-3-oxobutyramide	Azo colorant					X*							

Info on label or safety data sheet	Pigment name	Type of pigment	Red, Colour no.											
			1	5	17	18¤	24¤	33	34	39	48¤	49 ¤	53¤	63
Pigment red 146 CI# 12485 CAS# 5280-68-2	N-(4-Chloro-2,5-dimethoxyphenyl) -3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl] phenyl]azo]naphthalene-2-carboxamide	Azo colorant						X						
Pigment red 5 CI# 12490 CAS# 6410-41-9	N-(5-Chloro-2,4-dimethoxyphenyl)-4-[[5-[(diethylamino)sulfonyl]-2-methoxyphenyl]azo]-3-hydroxy-2-naphthalenecarboxamide	Azo colorant						X						
Pigment black 7 CI# 77266	Carbon black	Carbon black						X						
Pigment yellow 65 CI# 11740 CAS# 6528-34-3	2-[(4-Methoxy-2-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxo-butyramide	Azo colorant							X					

*According to data sheet

¤ Tattoo inks, registered in connection with skin reactions

Table 3 Blue colours

Info on label or safety data sheet	Pigment name	Type af pigment	Blue, Colour no.							
			8	15	25	32	38	45	56	62
Titanium Dioxide CI# 77891 CAS# 13463-67-7	Titanium Dioxide	Inorganic pigment	X	X	X	X	X			X
Phthalocyanine Blue 15:3 / Pigment Blue 15 CI# 74160 CAS# 147-14-8	(29H,31H-phthalocyaninato (2-)-N29,N30,N31,N32) Copper	Phthalocyanine	X	X	X		X			X
Pigment black 7 CI# 77266		Carbon black				X				
Pigment red 146 CI# 12485 CAS# 5280-68-2	N-(4-Chloro-2,5-dimethoxyphenyl) -3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl] phenyl]azo]naphthalene-2-carboxamide	Azo colorant				X				
Pigment Green 7 / Phthalocyanine Green 7 CI# 74260 CAS# 1328-53-6	Accosperse Cyan green g; Brilliant green phthalocyanine	Phthalocyanine				X				X

Table 4 Black colours

Info on label or safety data sheet	Pigment name	Black, Colour no.										
		2	3	10	11	12	23	30	42	43	51	58
Pigment black 7 CI# 77266	Carbon black		X	X					X			
Carbon black 7 CI# 77226	Carbon black				X	X	X					X

Table 5 White colours

Info on label or safety data sheet	Pigment name		White, Colour no.					
		Type af pigment	4	14	22	46	52	59
Titanium Dioxide CI# 77891 CAS# 13463-67-7	Titanium dioxide	Inorganic pigment	X	X	X			

Table 6 Yellow colours

Info on label or safety data sheet	Pigment name	Type af pigment	Yellow, Colour no.							
			9	19	27	36¤	40	47	54	61
Pigment orange 16 CI# 21160 CAS# 6505-28-8	2,2'-((3,3'-Dimethoxy(1,1'-biphenyl)-4,4'-diyl)bis(azo))bis(3-oxo-N-phenylbutyramide)	Azo colorant	X							X*
Pigment yellow 83 CI# 21108 CAS# 5567-15-7	2,2'-[(3,3'-dichlorobiphenyl-4,4'-diyl)diazene-2,1-diyl]bis[N-(4-chloro-2,5-dimethoxyphenyl)-3-oxobutanamide]		X			X				
Pigment yellow 151 CI# 13980 CAS# 31837-42-0	2-[[1-[[(2,3-Dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]benzoic acid	Azo colorant	X							
Pigment Yellow 74 / Arylide Yellow CI# 11741 CAS# 6358-31-2	2-[(2-Methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	Azo colorant		X			X			
Titanium Dioxide CI# 77891 CAS# 13463-67-7	Titanium Dioxide	Inorganic pigment		X*	X	X	X			X
Pigment yellow 65 CI# 11740 CAS# 6528-34-3	2-[(4-Methoxy-2-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxo-butyramide	Azo colorant			X					
Pigment Yellow 97 CI# 11767 CAS# 12225-18-2	N-(4-Chloro-2,5-dimethoxyphenyl)-2-[[2,5-dimethoxy-4-[(phenylamino)sulphonyl]phenyl]azo]-3-oxobutyramide	Azo colorant			X*					
Pigment yellow 14 CI# 21095 CAS# 5468-75-7	2,2'-[(3,3'-Dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-methylphenyl)-3-oxobutyramide]	Azo colorant								X
Pigment Orange 5 CI# 12075 CAS# 3468-63-1	1-[(2,4-Dinitrophenyl)azo]-2-naphthol	Azo colorant								X

*According to data sheet

¤ Tattoo ink that is registered in connection with skin reactions

Table 7 Orange colours and peach colours

Info on label or safety data sheet	Pigment name	Type af pigment	Colour no.					
			Orange	Peach	Orange	Peach	Orange	Peach
			20	21	28	29	65	64
Pigment red 210 CI# 12477 CAS# 61932-63-6	Permanent Red F 6RK; Pigment Red 5S; Red 5S; Sunbrite Red 210		X	X				
Ferric oxide CI# 77491 CAS# 1309-37-1	Iron(III)oxide	Inorganic pigment	X					
Titanium Dioxide CI# 77891 CAS# 13463-67-7	Titanium dioxide	Inorganic pigment		X	X	X		X
Pigment Yellow 74 / Arylide Yellow CI# 11741 CAS# 6358-31-2	2-[(2-Methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide	Azo colorant		X				
Pigment yellow 65 CI# 11740 CAS# 6528-34-3	2-[(4-Methoxy-2-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxo-butyramide	Azo colorant			X			
Pigment orange 13 CI# 21110 CAS# 3520-72-7	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]	Azo colorant			X	X		
Pigment Yellow 97 CI# 11767 CAS# 12225-18-2	N-(4-Chloro-2,5-dimethoxyphenyl)-2-[[2,5-dimethoxy-4-[(phenylamino)sulphonyl] phenyl]azo]-3-oxobutyramide	Azo colorant			X*			
Pigment Red 122 CI# 73915 CAS# 980-26-7	5,12-Dihydro-2,9-dimethylquino[2,3-b]acridine-7,14-dione	Acridin				X		
Pigment orange 16 CI# 21160 CAS# 6505-28-8	2,2'-((3,3'-Dimethoxy(1,1'-biphenyl)-4,4'-diyl)bis(azo))bis(3-oxo-N-phenylbutyramide)	Azo colorant					X	X*
Pigment yellow 14 CI# 21095 CAS# 5468-75-7	2,2'-[(3,3'-Dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-methylphenyl)-3-oxobutyramide]	Azo colorant					X*	
Pigment Red 170 CI# 12475 CAS# 2786-76-7	4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxy-2-Naphthalenecarboxamide	Azo colorant					X	X*
Pigment Orange 5 CI# 12075 CAS# 3468-63-1	1-[(2,4-Dinitrophenyl)azo]-2-naphthol	Azo colorant						X

Info on label or safety data sheet	Pigment name	Type af pigment	Colour no.					
			Orange	Peach	Orange	Peach	Orange	Peach
			20	21	28	29	65	64
Acid Brown 14 CI# 20195 CAS# 5850-16-8	4,4'-[(2,4-dihydroxy-1,3-phenylene)bis(azo)]bis-1-naphthalenesulfonicacid	Azo colorant					X	

*According to data sheet

Table 8 Violet and brown colours

Info on label or safety data sheet	Pigment name	Type af pigment	Colour no.			
			Violet	Violet	Violet	Brown
			35¤	37¤	50	57¤
Pigment violet 19 CI# 73900 CAS# 1047-16-1	5,12-Dihydroquino[2,3-b]acridine-7,14-dione	Acridine	X			
Pigment red 63:1 CI# 15880 CAS# 6417-83-0	Calcium 3-hydroxy-4-[(1-sulphonato-2-naphthyl)azo]-2-naphthoate	Azo colorant		X		
Phthalocyanine Blue 15:3 / Pigment Blue 15 CI# 74160 CAS# 147-14-8	(29H,31H-phthalocyaninato (2-)-N29,N30,N31,N32) copper	Phthalocyanine		X		
Titanium Dioxide CI# 77891 CAS# 13463-67-7	Titanium Dioxide	Inorganic pigment	X*	X		
Pigment Green 7 / Phthalocyanine Green 7 CI# 74260 CAS# 1328-53-6	Accosperse Cyan green g; Brilliant green phthalocyanine	Phthalocyanine		X		
Pigment Red 122 CI# 73915 CAS# 980-26-7	5,12-Dihydro-2,9-dimethylquino[2,3-b]acridine-7,14-dione	Acridine	X*			

*According to data sheet

¤Tattoo ink that is registered in connection with skin reactions

Results of ICP/MS screening

This enclosure states the results of the ICP/MS screening, see Chapter 4.

The results of the ICP/MS screening analyses appear in the following tables as the results are classified according to colour. Tattoo inks that were registered in connection with skin reactions have a *. Indication of <DL are results below the detection limit.

Table 1 Result of ICP/MS screening analysis for black and grey tattoo inks, µg/g

Element	DL	Colour no.										
		Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Grey
		2	3	11	12	42	23	30	43	51	58	10
Li	0.04	1.6	<DL	<DL	<DL	1.4	<DL	1.4	0.083	<DL	<DL	<DL
B	1	2600	5.8	<DL	<DL	2200	<DL	2200	6.7	<DL	<DL	<DL
Na	1	5800	140	410	510	4500	140	5600	890	290	120	330
Mg	0.1	7.5	10	15	17	6.2	7.7	9.8	87	6.6	18	25
Al	0.2	1.7	47	11	46	1.4	16	2.6	34	5.8	310	4.0
Si	0.4	8.0	130	34	34	25	29	13	58	19	96	44
P	0.2	11	13	5.9	290	11	<DL	9.8	<DL	0.79	8.4	150
K	1	31	120	45	680	20	89	25	87	39	150	12
Ca	1	58	30	68	63	91	30	100	190	28	37	360
Sc	0.01	0.012	0.041	0.026	0.026	0.021	0.015	0.016	0.026	0.013	0.021	0.032
Ti	0.02	0.088	3.1	0.77	2.07	0.40	1.4	1.2	2.7	0.65	1.7	1.1
V	0.1	<DL	0.69	0.41	0.57	<DL	0.74	<DL	0.50	<DL	0.12	0.12
Cr	0.04	1.1	1.9	1.8	3.02	0.74	1.8	1.1	8.9	4.4	2.3	1.4
Mn	0.01	0.16	0.30	2.1	2.16	0.092	0.15	0.19	1.2	0.62	0.87	0.73
Fe	1	13	25	380	360	6.3	17	8.9	240	36	160	32
Co	0.01	<DL	0.018	0.024	0.061	<DL	0.017	<DL	0.037	0.035	0.022	0.031
Ni	0.02	1.3	0.50	0.48	0.69	0.96	0.46	1.2	0.72	2.5	0.63	0.33
Cu	0.02	0.25	0.61	1.0	3.47	0.24	0.63	0.30	18	0.40	0.92	0.48
Zn	0.2	0.89	2.7	1.1	0.45	1.2	0.84	2.3	2.9	23	0.99	5.3
Ga	0.01	<DL	<DL	<DL	0.019	<DL	<DL	<DL	0.012	0.051	0.053	<DL
As	0.04	0.086	0.19	0.085	0.24	0.14	0.12	0.095	0.067	0.25	0.050	0.40
Se	0.04	0.081	0.49	0.045	0.37	0.13	0.25	0.068	0.15	0.13	<DL	1.3
Rb	0.01	0.028	0.032	0.013	0.087	0.029	0.024	0.045	<DL	0.024	0.018	<DL
Sr	0.01	0.72	0.20	0.35	0.35	0.46	0.19	0.70	3.3	0.18	0.31	0.70
Y	0.01	<DL	0.015	<DL	0.012	<DL	0.016	<DL	0.043	0.063	<DL	<DL
Zr	0.01	4.3	5.4	0.031	2.8	3.1	3.9	5.5	8.2	8.0	0.20	1.2
Nb	0.01	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.012	<DL	<DL	<DL
Mo	0.01	0.093	0.078	0.082	0.24	0.13	0.046	0.15	0.15	0.058	0.12	0.061
Cd	0.01	0.024	<DL	0.017	<DL	<DL	0.013	<DL	<DL	0.045	0.012	0.015
In	0.01	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.010	<DL	<DL
Sn	0.04	<DL	<DL	0.049	0.064	<DL	<DL	<DL	<DL	0.16	<DL	<DL
Sb	0.01	<DL	<DL	0.58	0.51	<DL	0.088	<DL	1.2	0.048	<DL	<DL
Cs	0.01	0.024	<DL	<DL	<DL	0.025	0.021	0.043	<DL	<DL	<DL	<DL
Ba	0.01	0.12	0.23	0.41	1.02	0.23	0.59	0.34	1.3	0.91	7.4	0.40

Element	DL	Colour no.										
		Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Grey
		2	3	11	12	42	23	30	43	51	58	10
La	0.01	<DL	4.8	1.1	0.91	<DL	1.1	<DL	2.7	0.024	0.39	<DL
Ce	0.01	<DL	0.26	0.11	0.049	<DL	0.22	<DL	0.86	0.039	0.062	<DL
Pr	0.01	<DL	0.027	0.012	<DL	<DL	<DL	<DL	0.024	<DL	<DL	<DL
Nd	0.01	<DL	0.033	0.022	<DL	<DL	<DL	<DL	0.042	<DL	0.011	<DL
Sm	0.01	<DL	0.023	0.017	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Gd	0.01	<DL	<DL	0.010	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Er	0.01	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.010	<DL	<DL
Yb	0.01	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.013	<DL	<DL
Lu	0.01	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Hf	0.01	0.058	0.092	<DL	0.050	0.042	0.064	0.083	0.17	0.20	<DL	<DL
W	0.01	<DL	<DL	<DL	0.098	<DL	0.048	<DL	0.031	0.021	<DL	0.21
Tl	0.01	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.039	<DL	<DL
Pb	0.01	0.029	1.5	0.035	0.045	0.017	0.052	0.024	0.033	1.5	0.056	0.13
Bi	0.01	<DL	<DL	0.12	<DL	<DL	<DL	<DL	<DL	0.10	<DL	0.28
Th	0.01	<DL	0.016	0.78	<DL	<DL	0.015	<DL	0.024	0.12	<DL	<DL

Be, Ta, Ru, Pd, Ag, Te, Eu, Td, Dy, Ho, Tm, Os, Ir, Pt, Au, Hg and U were not demonstrated in the black tattoo inks.

Table 2 Result of ICP/MS screening analysis for red tattoo inks, µg/g

Element	DL	Colour no.											
		Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red
		1	5	17	18 *	24 *	33	34	39	48 *	49 *	53 *	63
Li	0.04	2.2	0.45	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.063	0.059
B	1	2.9	1.6	<DL	<DL	<DL	<DL	<DL	<DL	2.0	<DL	<DL	<DL
Na	1	920	590	64	58	200	150	99	160	310	210	500	52
Mg	0.1	67	19	23	23	16	1300	19	6.7	760	660	100	1700
Al	0.2	2300	2600	3.0	590	710	270	120	220	61	52	23	210
Si	0.4	240	300	20	97	170	490	130	30	100	120	39	210
P	0.2	65	50	46	33	76	13	21	12	110	120	26	13
K	1	300	22	28	24	16	31	14	7.5	45	12	19	26
Ca	1	96	300	75	72	76	350	930	37	96	86	16000	130
Sc	0.01	0.13	0.18	<DL	0.053	0.043	0.19	0.062	0.016	0.038	0.044	0.038	0.078
Ti	0.02	7.0	30	0.31	100	57	43	21	45	2.2	6.6	1.5	5.6
V	0.1	0.18	0.81	<DL	0.26	0.35	<DL	<DL	<DL	<DL	0.26	<DL	0.50
Cr	0.04	11	1.6	0.53	0.63	3.8	<DL	3.6	6.3	1.8	6.9	6.4	3.9
Mn	0.01	2.5	0.33	0.26	0.25	0.41	0.97	0.42	0.31	0.47	4.6	2.6	0.91
Fe	1	180	100	10	9.5	23	52	23	16	27	25000	89	50
Co	0.01	0.15	0.019	<DL	0.011	<DL	<DL	0.012	<DL	<DL	0.21	0.042	0.018
Ni	0.02	7.3	0.11	0.13	0.31	0.28	0.21	0.24	0.18	0.18	1.4	3.4	0.85
Cu	0.02	1.2	0.40	0.17	0.61	0.68	11	2.1	1.5	0.69	3.8	0.33	8.0
Zn	0.2	3.1	1.8	2.0	1.6	2.6	53	1.8	1.5	8.1	8.9	4.8	1.5
Ga	0.01	0.79	0.64	<DL	0.051	0.098	0.051	0.030	0.043	0.018	0.25	0.014	0.045
As	0.04	0.94	0.72	0.044	0.041	0.12	0.35	0.13	0.027	0.051	0.86	0.096	0.11
Se	0.04	1.71	0.33	0.081	<DL	0.17	0.15	0.15	0.074	0.13	<DL	<DL	0.31
Rb	0.01	4.2	0.18	<DL	<DL	0.028	0.025	0.042	0.011	0.022	0.016	0.019	0.027
Sr	0.01	2.5	0.77	0.53	0.54	0.78	1.7	5.9	0.16	0.38	0.39	9.9	3.8
Y	0.01	4.1	0.11	<DL	<DL	0.016	<DL	0.013	<DL	0.017	0.042	0.18	0.14
Zr	0.01	70	2.4	0.89	110	18	0.83	1.9	21	4.7	9.5	5.8	49
Nb	0.01	0.10	<DL	<DL	<DL	<DL	<DL	<DL	0.031	<DL	0.11	<DL	0.000
Mo	0.01	1.6	0.020	0.016	<DL	<DL	<DL	0.016	0.036	0.082	0.52	0.059	0.088
Pd	0.01	<DL	<DL	<DL	<DL	0.14	<DL	<DL	0.18	<DL	<DL	0.017	0.36
Ag	0.01	0.016	<DL	<DL	0.025	<DL	<DL	<DL	0.013	<DL	0.015	<DL	0.000
Cd	0.01	0.026	0.015	0.041	0.021	0.036	0.042	0.051	0.040	0.058	0	0.017	0.051
Sn	0.04	0.53	<DL	<DL	0.065	<DL	<DL	<DL	<DL	<DL	0.41	<DL	<DL
Sb	0.01	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.039	0.014	<DL
Cs	0.01	0.43	0.028	<DL	<DL	<DL	0.041	<DL	<DL	<DL	<DL	<DL	<DL
Ba	0.01	4.5	1.1	21	19	29	140	100	0.53	0.89	4.6	25	180
La	0.01	0.25	0.16	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.015	0.057	0.012
Ce	0.01	0.62	0.28	<DL	<DL	0.044	0.027	0.016	2.8	0.041	0.094	0.068	0.025
Pr	0.01	0.079	0.037	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.012	<DL
Nd	0.01	0.31	0.12	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.051	0.012
Sm	0.01	0.087	0.023	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Eu	0.01	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.013
Gd	0.01	0.10	0.021	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.015	<DL

Element	DL	Colour no.											
		Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red
		1	5	17	18 *	24 *	33	34	39	48 *	49 *	53 *	63
Dy	0.01	0.082	0.014	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.018	0.011
Er	0.01	0.043	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.013	0.014
Yb	0.01	0.053	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.013	0.024
Hf	0.01	1.0	0.040	0.014	1.7	0.12	<DL	0.026	0.26	0.077	0.15	0.10	0.64
W	0.01	0.13	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.081	0.056	<DL	<DL
Pt	0.01	<DL	<DL	<DL	0.018	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Tl	0.01	0.019	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Pb	0.01	0.39	1.34	0.039	0.051	0.067	0.30	0.13	0.13	0.082	0.20	0.50	0.13
Bi	0.01	0.20	0.015	<DL	<DL	<DL	<DL	<DL	0.018	<DL	<DL	<DL	<DL
Th	0.01	1.3	1.5	<DL	<DL	0.15	0.054	0.088	0.014	0.043	0.10	0.11	0.36
U	0.04	<DL	0.14	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.11	0.062

'*' indicates tattoo inks where skin reactions have been observed.

Be, Ru, In, Te, Tb, Ho, Tm, Lu, Ta, Os, Ir, Au and Hg were not demonstrated in the red tattoo inks.

Table 3 Result of ICP/MS screening analysis for orange, peach, violet and brown tattoo inks, µg/g

Element	DL	Colour no.									
		Orange	Orange	Orange	Peach	Peach	Peach	Violet	Violet	Violet	Brown
		20	28	65	21	29	64	35 *	37 *	50	57 *
Li	0.04	0.81	<DL	1.7	0.28	<DL	0.16	<DL	<DL	<DL	<DL
B	1	1.7	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.2	<DL
Na	1	520	150	270	180	490	320	210	530	330	450
Mg	0.1	76	24	20	14	250	240	14	22	15	67
Al	0.2	1100	2600	1700	7100	9300	8400	1.8	1030	6.2	14
Si	0.4	190	170	200	170	17	66	17	130	9.2	15
P	0.2	33	2.9	2.9	<DL	3.6	4.1	100	18	210	9.2
K	1	55	26	130	20	46	80	13	19	60	18
Ca	1	120	2400	68	70	220	170	53	87	66	1000
Sc	0.01	0.12	0.12	0.13	0.27	0.33	0.39	0.013	0.083	<DL	0.014
Ti	0.02	8.2	140	8.6	400	370	400	1.5	43	1.1	0.64
V	0.1	1.7	0.45	0.40	0.21	0.31	0.44	<DL	0.16	<DL	<DL
Cr	0.04	31	3.6	2.3	0.96	1.5	0.77	1.3	1.2	1.5	1.5
Mn	0.01	42	0.47	1.5	0.90	2.8	2.4	0.16	0.37	0.25	0.30
Fe	1	14000	21	64	19	56	54	8.6	22	20	25
Co	0.01	2.2	0.015	0.075	0.029	0.048	0.040	<DL	0.068	<DL	0.019
Ni	0.02	18	0.65	0.45	1.3	1.7	1.4	0.18	0.44	0.19	1.0
Cu	0.02	100	1.1	0.64	3.4	0.68	1.2	1.0	1020	0.69	140
Zn	0.2	24	1.6	1.1	2.2	3.5	2.6	0.89	1.7	0.62	2.5
Ga	0.01	0.67	0.29	0.71	0.88	1.1	1.0	<DL	0.12	<DL	<DL
As	0.04	0.70	0.066	0.051	<DL	0.30	0.26	0.14	0.081	<DL	0.088
Se	0.04	<DL	0.21	0.14	0.041	0.90	0.91	0.18	0.23	0.093	0.14
Rb	0.01	0.10	0.055	0.038	0.018	0.30	0.21	0.017	0.014	0.018	0.017
Sr	0.01	1.19	6.7	0.79	0.70	0.94	0.62	0.51	2.7	0.32	1.9
Y	0.01	0.082	0.022	0.061	0.023	0.14	0.11	<DL	0.033	<DL	0.015
Zr	0.01	2.3	360	3.3	1300	1900	2200	2.3	220	1.7	2.1
Nb	0.01	0.039	<DL	<DL	0.012	0.015	0.022	<DL	<DL	<DL	<DL
Mo	0.01	2.1	<DL	0.049	<DL	0.014	<DL	0.056	0.023	0.063	0.23
Pd	0.01	<DL	<DL	<DL	11	15	16	<DL	<DL	<DL	<DL
Ag	0.01	0.021	0.066	<DL	0.22	0.31	0.33	<DL	0.044	<DL	<DL
Cd	0.01	0.035	0.086	0.043	0.19	0.055	0.27	<DL	<DL	<DL	0.025
Sn	0.04	4.13	<DL	0.10	0.069	0.26	0.24	<DL	0.14	<DL	<DL
Sb	0.01	0.14	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.039	0.012
Cs	0.01	0.011	<DL	<DL	<DL	0.019	<DL	<DL	<DL	<DL	<DL
Ba	0.01	43	250	11	1.3	3.7	1.4	8.8	44	0.49	11
La	0.01	0.61	0.013	0.30	0.059	0.18	0.079	<DL	<DL	<DL	<DL
Ce	0.01	1.2	0.038	0.57	0.10	0.36	0.14	<DL	<DL	0.020	<DL
Pr	0.01	0.073	<DL	0.059	0.010	0.064	0.020	<DL	<DL	<DL	<DL
Nd	0.01	0.21	<DL	0.21	0.032	0.18	0.073	<DL	<DL	<DL	<DL
Sm	0.01	0.038	<DL	0.032	<DL	0.025	0.017	<DL	<DL	<DL	<DL
Eu	0.01	<DL	0.018	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Gd	0.01	0.026	<DL	0.028	<DL	0.027	<DL	<DL	<DL	<DL	<DL

Element	DL	Colour no.									
		Orange	Orange	Orange	Peach	Peach	Peach	Violet	Violet	Violet	Brown
		20	28	65	21	29	64	35 *	37 *	50	57 *
Dy	0.01	0.016	<DL	0.018	<DL	0.018	<DL	<DL	0.012	<DL	<DL
Er	0.01	<DL	<DL	<DL	<DL	0.010	<DL	<DL	0.014	<DL	<DL
Yd	0.01	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.020	<DL	<DL
Hf	0.01	0.039	4.9	0.042	15	25	28	0.024	2.5	0.022	0.036
W	0.01	0.021	<DL	<DL	<DL	<DL	<DL	0.016	<DL	0.32	0.051
Pt	0.01	<DL	0.042	<DL	0.15	0.21	0.21	<DL	0.022	<DL	<DL
Au	0.01	<DL	<DL	<DL	0.020	0.028	0.030	<DL	<DL	<DL	<DL
Hg	0.04	<DL	<DL	<DL	<DL	0.11	<DL	<DL	<DL	<DL	<DL
Pb	0.01	1.6	0.21	0.36	0.11	0.19	0.11	0.016	0.092	0.024	0.21
Bi	0.01	0.044	<DL	0.011	<DL	0.012	<DL	<DL	<DL	<DL	<DL
Th	0.01	0.27	0.026	0.67	0.073	0.23	0.18	0.011	0.082	<DL	0.030
U	0.04	0.069	<DL	0.11	<DL	0.037	<DL	<DL	<DL	<DL	0.080

'*' indicates tatttoo inks where skin reactions have been observed.

Be, Ru, In, Te, Tb, Ho, Tm, Lu, Ta, Os, Ir and Tl were not demonstrated in orange, peach, violet or brown tattoo inks.

Table 4 Result of ICP/MS screening analysis for blue tattoo inks, µg/g

Element	DL	Colour no.						
		Blue	Blue	Pale blue	Blue	Pale blue	Blue	Blue
		8	15	25	32	38	45	62
Li	0.04	0.040	<DL	<DL	<DL	<DL	<DL	0.082
B	1	<DL	<DL	<DL	<DL	<DL	0.83	<DL
Na	1	370	54	120	210	130	240	250
Mg	0.1	12	11	12	15	15	5.6	200
Al	0.2	3300	1900	7400	390	3400	2400	6500
Si	0.4	110	60	54	74	30	84	37
P	0.2	400	5.2	2.5	2.3	44	6.1	3.2
K	1	120	33	11	14	7.5	5.3	53
Ca	1	61	52	56	58	110	32	330
Sc	0.01	0.048	0.098	0.21	0.028	0.016	0.097	0.31
Ti	0.02	180	190	120	57	93	150	98
V	0.1	0.76	0.87	0.68	<DL	0.61	0.39	0.44
Cr	0.04	1.2	0.43	1.7	0.31	0.67	1.3	3.1
Mn	0.01	0.55	0.26	2.9	0.25	0.11	1.3	2.1
Fe	1	32	13	250	6.9	8.0	68	70
Co	0.01	0.48	0.052	0.027	<DL	0.036	0.033	0.080
Ni	0.02	1.5	1.4	2.2	0.75	1.6	1.7	3.0
Cu	0.02	19000	20000	5300	12000	1800	16000	7200
Zn	0.2	2.9	1.4	1.7	2.9	3.0	3.9	2.3
Ga	0.01	0.62	0.27	0.82	0.067	0.42	0.26	0.87
As	0.04	0.35	0.049	<DL	0.095	<DL	0.042	0.17
Se	0.04	0.75	0.12	0.12	0.15	<DL	0.16	0.63
Rb	0.01	0.027	0.016	0.014	0.012	0.011	<DL	0.13
Sr	0.01	0.39	0.36	0.59	0.50	0.62	0.29	1.0
Y	0.01	0.14	0.077	0.023	<DL	<DL	<DL	0.10
Zr	0.01	2.3	680	1400	0.17	2.2	650	1501
Nb	0.01	0.18	<DL	0.012	<DL	0.043	<DL	0.012
Mo	0.01	0.13	2.4	0.028	0.047	0.016	1.1	0.016
Pd	0.01	0.043	5.3	<DL	<DL	<DL	5.0	10
Ag	0.01	0.026	0.14	11	0.018	0.017	0.16	0.23
Cd	0.01	0.011	0.018	0.24	0.023	0.015	0.058	0.022
Sn	0.04	<DL	0.14	<DL	<DL	<DL	<DL	0.065
Cs	0.01	<DL	<DL	<DL	0.073	<DL	<DL	<DL
Ba	0.01	1.4	1.69	10	0.22	1.3	0.58	2.3
La	0.01	0.014	<DL	0.015	<DL	0.011	<DL	0.051
Ce	0.01	0.026	0.012	0.027	<DL	0.011	<DL	0.096
Pr	0.01	<DL	<DL	<DL	<DL	<DL	<DL	0.013
Nd	0.01	<DL	<DL	0.009	<DL	<DL	<DL	0.052
Gd	0.01	<DL	<DL	<DL	<DL	<DL	<DL	0.011
Dy	0.01	0.014	<DL	<DL	<DL	<DL	<DL	<DL
Er	0.01	0.020	<DL	<DL	<DL	<DL	<DL	<DL

Element	DL	Colour no.						
		Blue	Blue	Pale blue	Blue	Pale blue	Blue	Blue
		8	15	25	32	38	45	62
Yb	0.01	0.032	<DL	<DL	<DL	<DL	<DL	<DL
Hf	0.01	0.028	10.0	21	<DL	0.025	8.2	23
Ta	0.01	0.013	<DL	<DL	<DL	<DL	<DL	<DL
Pt	0.01	<DL	0.090	0.18	<DL	<DL	0.079	0.17
Au	0.01	<DL	0.015	0.026	<DL	<DL	0.012	0.023
Hg	0.04	<DL	<DL	<DL	<DL	<DL	0.038	<DL
Pb	0.01	5.7	0.052	0.079	0.067	1.7	0.099	0.083
Th	0.01	0.10	<DL	0.017	<DL	0.094	<DL	0.17

Be, Ru, In, Sb, Te, Sm, Eu, Tb, Ho, Tm, Lu, W, Os, Ir, Tl, Bi and U were not demonstrated in the blue tattoo inks.

Table 5 Result of ICP/MS screening analysis for green tattoo inks, µg/g

Element	DL	Colour no.								
		Pale green	Green	Green	Pale green	Pale green	Green	Green	Pale green	Green
		6	7	13	16	26	31	41	44	60
Li	0.04	0.061	0.076	<DL	0.39	<DL	<DL	<DL	<DL	0.10
B	1	<DL	<DL	<DL	<DL	<DL	<DL	2.5	4.5	<DL
Na	1	620	280	150	150	300	60	650	180	430
Mg	0.1	15	130	64	22	58	7.2	20	29	95
Al	0.2	6100	1700	140	380	1300	17	1100	99	2500
Si	0.4	26	120	16	150	120	18	50	73	120
P	0.2	650	300	1.2	2.2	3.9	7.6	24	2.4	5.2
K	1	230	73	28	34	32	17	44	26	19
Ca	1	55	330	140	78	2900	360	92	83	110
Sc	0.01	0.022	0.060	0.016	0.11	0.084	0	0.023	0.040	0.19
Ti	0.02	240	81	1.01	5.2	72	12	45	2.2	120
V	0.1	1.0	0.66	0.080	0.23	0.70	<DL	0.33	<DL	0.28
Cr	0.04	0.35	1.3	2.01	0.84	12	<DL	0.56	4.2	0.77
Mn	0.01	0.24	1.4	1.3	0.23	1.5	0.089	1.4	1.9	1.7
Fe	1	4.7	66	28	29	67	6.1	13	86	57
Co	0.01	0.0088	0.28	0.036	0.13	0.16	<DL	0.53	3.6	0.021
Ni	0.02	0.89	0.87	1.6	0.49	1.2	0.043	0.50	1.2	0.65
Cu	0.02	2100	12000	17000	1500	5300	16	2600	10000	1.1
Zn	0.2	3.9	3.2	2.04	1.05	3.1	8.8	14	4.3	3.8
Ga	0.01	0.97	0.35	0.051	0.17	0.14	<DL	0.20	0.038	0.34
As	0.04	0.29	0.36	0.22	0.17	0.11	0.054	0.098	0.12	0.072
Se	0.04	0.66	0.92	0.70	0.54	0.26	0.12	0.18	0.33	0.17
Rb	0.01	0.042	0.10	0.010	0.032	0.069	<DL	0.024	0.014	0.078
Sr	0.01	0.36	0.65	1.8	0.75	11	0.54	0.50	0.45	0.58
Y	0.01	0.23	0.11	0.026	0.022	0.028	<DL	<DL	0.083	0.056
Zr	0.01	1.5	3.4	12	4.6	300	0.96	0.78	28	660
Nb	0.01	0.18	0.065	<DL	<DL	<DL	<DL	0.014	0.015	<DL
Mo	0.01	<DL	0.14	0.85	0.082	<DL	0.015	<DL	2.2	<DL
Pd	0.01	<DL	0.060	0.24	<DL	0.033	<DL	<DL	<DL	<DL
Ag	0.01	<DL	0.016	0.012	<DL	0.064	<DL	0.026	0.023	0.11
Cd	0.01	0.022	0.033	0.023	<DL	0.057	0.020	<DL	0.088	0.015
Sn	0.04	<DL	<DL	0.68	0.040	<DL	<DL	<DL	1.1	1.1
Sb	0.01	<DL	<DL	0.011	<DL	<DL	<DL	<DL	0.011	<DL
Ba	0.01	1.2	2.1	300	0.55	480	0.61	1.2	0.81	0.69
La	0.01	0.016	0.038	<DL	0.10	0.016	<DL	<DL	0.013	0.039
Ce	0.01	0.028	0.075	<DL	0.20	0.047	<DL	0.025	0.15	0.065
Pr	0.01	<DL	<DL	<DL	0.018	<DL	<DL	<DL	<DL	<DL
Nd	0.01	<DL	0.035	<DL	0.052	0.012	<DL	<DL	<DL	0.029
Eu	0.01	<DL	<DL	0.023	<DL	0.033	<DL	<DL	<DL	<DL
Dy	0.01	0.017	0.010	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Ho	0.01	<DL	0.000	<DL	<DL	<DL	<DL	<DL	<DL	<DL

Element	DL	Colour no.								
		Pale green	Green	Green	Pale green	Pale green	Green	Green	Pale green	Green
		6	7	13	16	26	31	41	44	60
Er	0.01	0.024	0.012	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Tm	0.01	<DL	0.000	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Yb	0.01	0.043	0.017	<DL	<DL	<DL	<DL	<DL	0.012	<DL
Lu	0.01	0.010	0.000	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Hf	0.01	0.016	0.033	0.19	0.066	4.5	<DL	<DL	0.38	9.3
Ta	0.01	0.013	<DL	<DL	0.016	<DL	<DL	<DL	<DL	<DL
W	0.01	<DL	<DL	0.009	0.022	<DL	<DL	<DL	<DL	<DL
Pt	0.01	<DL	<DL	<DL	<DL	0.043	<DL	<DL	<DL	0.069
Pb	0.01	9.3	3.2	0.28	0.19	0.29	0.11	0.64	0.93	0.085
Bi	0.01	<DL	0.096	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Th	0.01	0.15	0.088	0.023	0.17	0.025	<DL	0.041	0.023	0.13
U	0.04	<DL	0.053	<DL	<DL	<DL	<DL	<DL	<DL	<DL

Be, Ru, In, Te, Cs, Gd, Tb, Os, Ir, Au, Hg, Tl and Sm were not demonstrated in the green tattoo inks.

Table 6 Result of ICP/MS screening analysis for yellow tattoo inks, µg/g

Element	DL	Colour no.						
		Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
		9	19	27	36 *	40	47	61
Li	0.04	<DL	0.67	<DL	<DL	<DL	0.28	0.070
B	1	<DL	<DL	<DL	<DL	1.4	0.73	<DL
Na	1	52	120	140	120	1100	71	130
Mg	0.1	5.8	19	32	9.8	36	10	479
Al	0.2	2.3	1000	1400	960	1600	420	774
Si	0.4	8.5	240	1100	110	53	220	130
P	0.2	3.9	0.93	160	2.7	32	2.5	4.5
K	1	26	28	32	19	6.2	22	39
Ca	1	27	71	3800	43	180	46	2200
Sc	0.01	0.016	0.13	0.090	0.084	0.018	0.12	0.069
Ti	0.02	0.16	12	130	78	34	9.1	76
V	0.1	<DL	0.36	0.58	0.35	0.36	0.18	0.26
Cr	0.04	<DL	0.79	5.9	0.33	0.34	0.72	0.37
Mn	0.01	0.043	0.20	0.75	0.12	0.13	0.091	0.43
Fe	1	2.8	31	43	4.2	1.2	11	27
Co	0.01	<DL	0.058	0.024	<DL	<DL	0.025	0.033
Ni	0.02	0.025	0.29	0.51	0.27	0.11	0.15	0.26
Cu	0.02	0.46	0.36	7.8	1.2	0.45	13	0.79
Zn	0.2	2.4	1.9	5.3	2.1	1.2	0.76	1.9
Ga	0.01	<DL	0.32	0.12	0.10	0.19	0.14	0.111
As	0.04	<DL	0.069	0.19	0.083	0.042	0.11	<DL
Se	0.04	<DL	0.19	0.52	0.24	0.075	0.39	0.052
Rb	0.01	0.013	0.041	0.082	0.012	<DL	0.027	<DL
Sr	0.01	0.11	0.67	11	0.28	0.93	0.38	20
Y	0.01	<DL	0.023	0.028	<DL	<DL	<DL	0.065
Zr	0.01	0.12	0.89	240	240	0.81	1.6	190
Nb	0.01	<DL	<DL	<DL	<DL	0.027	<DL	<DL
Mo	0.01	<DL	0.015	<DL	<DL	<DL	0.052	<DL
Pd	0.01	<DL	<DL	0.013	<DL	<DL	<DL	0.015
Ag	0.01	<DL	<DL	0.050	0.048	<DL	<DL	0.034
Cd	0.01	0.022	0.043	0.020	<DL	<DL	<DL	0.038
Sn	0.04	<DL	<DL	<DL	0.87	<DL	<DL	0.16
Ba	0.01	0.21	0.68	430	0.33	1.5	0.26	1800
La	0.01	0.094	0.20	0.016	<DL	<DL	0.071	0.043
Ce	0.01	<DL	0.36	0.040	<DL	0.020	0.14	0.063
Pr	0.01	<DL	0.038	<DL	<DL	<DL	0.014	<DL
Nd	0.01	<DL	0.10	<DL	<DL	<DL	0.043	0.030
Sm	0.01	<DL	0.016	<DL	<DL	<DL	<DL	0.013
Eu	0.01	<DL	<DL	0.035	<DL	<DL	<DL	0.12
Hf	0.01	<DL	0.012	3.6	3.1	<DL	0.019	3.0
Pt	0.01	<DL	<DL	0.034	0.044	<DL	<DL	0.022

Element	DL	Colour no.						
		Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
		9	19	27	36 *	40	47	61
Pb	0.01	0.034	0.25	0.34	0.019	0.80	0.099	0.80
Th	0.01	<DL	0.31	0.023	<DL	0.048	0.15	0.058

* indicates tattoo inks that are registered in connection with skin reactions

Be, In, Ru, Sb, Te, Cs, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Ta, W, Os, Ir, Au, Hg, Tl, Bi and U were not demonstrated in the yelloe tattoo inks.

Table 7 Result of ICP/MS screening analysis for white tattoo inks, µg/g

Element	DL	Colour no.				
		White	White	White	White	White
		4	14	22	46	59
Li	0.04	<DL	<DL	<DL	<DL	0.12
B	1	3.7	<DL	<DL	1.7	<DL
Na	1	650	78	110	110	430
Mg	0.1	8.2	8.6	9.2	9.5	310
Al	0.2	6800	5200	7800	11000	9000
Si	0.4	100	30	100	33	21
P	0.2	710	1.5	<DL	<DL	2.0
K	1	240	11	14	12	21
Ca	1	48	53	48	62	280
Sc	0.01	0.058	0.22	0.22	0.37	0.54
Ti	0.02	960	130	140	460	330
V	0.1	1.3	0.50	0.46	0.66	0.82
Cr	0.04	0.25	0.17	0.13	0.24	0.71
Mn	0.01	0.16	0.37	0.36	1.5	2.3
Fe	1	9.3	3.6	3.8	3.7	51
Co	0.01	<DL	0.011	<DL	0.019	0.04
Ni	0.02	0.83	1.5	1.5	3.8	1.9
Cu	0.02	1.3	12	0.27	6.3	0.52
Zn	0.2	6.4	1.7	1.9	3.0	3.0
Ga	0.01	1.04	0.61	0.71	0.99	1.1
As	0.04	0.24	<DL	<DL	<DL	0.17
Se	0.04	0.26	0.035	0.044	0.053	0.50
Rb	0.01	0.043	0.015	0.018	0.024	0.20
Sr	0.01	0.35	0.39	0.32	0.43	0.80
Y	0.01	0.26	0.026	0.023	0.034	0.12
Zr	0.01	20	1700	1300	2800	2300
Nb	0.01	0.80	0.012	0.011	0.02	0.025
Mo	0.01	<DL	0.012	<DL	0.015	0.015
Pd	0.01	0.19	0.017	10	21	17
Ag	0.01	0.019	0.29	0.21	0.51	0.40
Cd	0.01	<DL	0.095	0.019	0.028	0.072
Sn	0.04	<DL	0.036	<DL	<DL	<DL
Cs	0.01	<DL	<DL	<DL	<DL	0.013
Ba	0.01	1.3	0.78	0.52	0.53	1.0
La	0.01	0.023	0.012	0.017	0.022	0.089
Ce	0.01	0.034	0.019	0.025	0.029	0.16
Pr	0.01	<DL	<DL	<DL	<DL	0.022
Nd	0.01	<DL	<DL	<DL	0.012	0.088
Sm	0.01	<DL	<DL	<DL	<DL	0.016
Gd	0.01	<DL	<DL	<DL	<DL	0.016
Dy	0.01	0.020	<DL	<DL	<DL	0.015

Element	DL	Colour no.				
		White	White	White	White	White
		4	14	22	46	59
Er	0.01	0.026	<DL	<DL	<DL	<DL
Yb	0.01	0.041	<DL	<DL	<DL	<DL
Hf	0.01	0.28	21	17	28	38
Ta	0.01	0.044	<DL	0.012	<DL	<DL
Pt	0.01	<DL	0.21	0.16	0.30	0.25
Au	0.01	<DL	0.032	0.022	0.045	0.035
Pb	0.01	10	0.054	0.049	0.087	0.099
Th	0.01	0.24	0.013	0.022	0.030	0.21

Be, Ru, In, Sb, Te, Eu, Tb, Ho, Tm, Lu, W, Os, Ir, Hg, Tl, Bi and U were not demonstrated in the white tattoo inks.

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