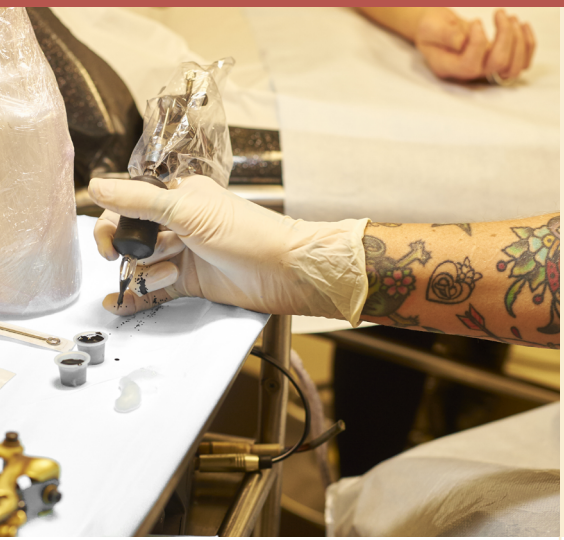
SAFER TATTOOING

Overview of current knowledge and challenges of toxicological assessment



Consumer Health Protection Committee (CD-P-SC)

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Foreword

Under the aegis of the Council of Europe's Consumer Health Protection Committee (CD-P-SC), this publication is based on the work of the *Ad hoc* Group on Safety of Tattoos and Permanent Make-up of the Committee of Experts on Cosmetic Products (P-SC-COS). The National Institute of Public Health and the Environment (RIVM) in the Netherlands had prepared an early draft on the same topic, following a request from the Dutch Ministry of Health, Welfare and Sport (VWS) and the Dutch Food and Consumer Product Safety Authority (NVWA).

Definitions

Tattooing

A practice by which a permanent skin marking or design ('tattoo') is administered by intradermal injection of inks containing colourants and auxiliary ingredients. The term as employed in this document also includes permanent make-up (PMU).

PMU

PMU consists of colourants and auxiliary ingredients that are injected via the intradermal route typically for enhancing facial contours.

Tattoo ink

A product or colouring mixture intended to create a mark on the surface of human body parts by intradermal injection.

Pigment

Pigments are coloured compounds consisting, in general, of solid particles sized in the nanometre and micrometre range. They are poorly soluble or insoluble in water and other aqueous application media. Unlike most dyes, pigments also have very low solubility in organic solvents.

Pigments are often coated which may modify the release of chemicals and the physical behaviour of the particles (e.g. tendency to form larger aggregates). Evidence available shows that pigments undergo very slow degradation in tissues with the formation of chemical cleavage products. The pigments themselves remain essentially in the solid state (including in living tissues).

Dye

Dyes are coloured organic compounds which are substantially soluble in some solvents.

Certain substances such as titanium dioxide (TiO_2) or barium sulphate can be used as carriers for dyes used in tattoos, thereby forming lakes that are insoluble in water.

Colourant

The commonly used generic term for the coloured materials pigments, lakes and dyes.

Cleavage products

Depending on the chemical structure, pigments can be cleaved (by light, metabolic enzymes or spontaneously) into sub-units that might differ in their chemical, physical and toxicological properties from the source molecule, and thus may need to be assessed separately.

Auxiliary ingredients

These are required to obtain ready-to-use tattooing products. They can be solvents, stabilisers, 'wetting agents', pH regulators, emollients and thickeners.

Glossary

BfR	Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment)
CMR	Carcinogenic, mutagenic and reprotoxic
CTFA	Personal Care Products Council
EMA	European Medicines Agency (previously EMEA)
FCA	Freund's complete adjuvant
GPMT	Magnusson–Kligman guinea pig maximisation test
ICH	International Conference on Harmonisation
LLNA	Local lymph node assay
MEC	molar extinction coefficient (also molar absorption coefficient or molar absorptivity)
MOS	margin of safety
NfG	Note for guidance
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PAA	primary aromatic amine

PAH	polycyclic aromatic hydrocarbons
P-SC-COS	Committee of Experts on Cosmetic Products
SCCS	European Union (EU) Scientific Committee on Consumer Safety
SED	systemic exposure dosage
SIS	skin-irritation studies
TIME	Tattoo Ink Manufacturers of Europe
TTC	Threshold of toxicological concern

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Introduction

Over the past 20 years, tattooing has become a fashion trend extending to large parts of the population, so that consumers with a tattoo no longer represent a small minority. However, the practice carries some risk to human health: cases are known of tattoo inks being subject to microbiological contamination or to the presence of other potentially dangerous substances.

For toxicological risk assessment, tattoo inks must be treated differently from cosmetic products because of differences in terms of their routes of exposure as well as their chemical and physical compositions.

Importantly, in the past, tattoo inks were often crude industrial products of largely unknown origin produced under no recognised good manufacturing practice. Even now, the legally responsible manufacturer, the site of manufacture, and the chemical content of inks are often unknown. Many chemicals, impurities and contaminants present in inks may exert harmful effects as single ingredients or by interaction. The final formulation might be simple (according to information from industry, a tattoo ink typically consists of ≈ 15 substances) but, given the number of substances to choose from, the problem of control is complex.

Council of Europe Resolution ResAP (2008) 1 – a step towards comprehensive regulation

Tattoo inks and the practice of tattooing are not yet covered by specific legislation at EU level. With a view to improving the level of health protection for consumers, the Council of Europe published Resolution ResAP (2008) 1 (Appendix 1), as adopted by the Committee of Ministers on 20 February, on requirements and criteria for the safety of tattoos and permanent make-up which includes, in particular, lists of hazardous substances that should not be used in tattoo products:

- Table 1 of ResAP (2008) 1 specifies 27 aromatic amines that should not be present in, or released from, azo colourants in tattoo inks.
- Table 2 of ResAP (2008) 1 lists 35 pigments classified as 'carcinogenic', 'mutagenic', 'reprotoxic' and/or having sensitising properties, that should not be present in tattoo inks.
- ResAP (2008) 1 prohibits the use in tattoo inks of compounds listed in Annex II to the Cosmetics Directive 76/768/EEC[1] as forbidden in cosmetic products.
- Compounds listed in columns 2–4 of Annex IV to Directive 76/768/EEC should not be present in tattoo inks. These are colourants and other ingredients with restricted use in cosmetics.
- Carcinogenic, mutagenic and reprotoxic (CMR) substances of categories 1, 2 or 3, which are classified under Directive 67/548/EEC,[2] should not be present in tattoo products. This directive is

1 Cosmetics Directive 76/768/EEC has been replaced by the Regulation (EC) No. 1223/2009 of the European Parliament and the Council of 30 November 2009 on cosmetic products.

2 Directive 67/548/EEC is now superseded by the new regulation with CMR classification into categories 1A, 1B, and 2 (Regulation (EC) No. 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No. 1907/2006).

now superseded by Regulation (EC) 1272/2008 on Classification, labelling and packaging of substances and mixtures, with CMR classifications 1A, 1B, and 2.

- Table 3 of ResAP (2008) 1 specifies maximum allowed concentrations of metal and polycyclic aromatic hydrocarbons (PAHs) impurities. Also, the minimum purity requirements for colourants used in foodstuffs and cosmetic products, as set out in Directive 95/45/EC laying down specific colours for use in foodstuffs, should be met.

Guidance on safety evaluation to supplement ResAP (2008) 1

The present document supplements Council of Europe Committee of Ministers' Resolution ResAP (2008) 1 on the requirements and criteria for the safety of tattoos and PMU. It is intended for manufacturers and persons or businesses responsible for marketing tattoo inks, to help them assess the specific risks of their products. It is also intended to facilitate the work of national authorities concerned with risk assessment. Here, data requirements are discussed to support the safety of using certain ingredients in tattoo and PMU inks. Appendix 2 presents pigments used in preparations on the European market between 2006 and 2013 in the Netherlands, Germany, Denmark, Norway and Switzerland.

The approach adopted in this document is based partly on established risk-assessment methods as addressed in the *Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation* produced by the European Union (EU) Scientific Committee on Consumer Safety (SCCS) (SCCS/1564/15). In addition, valuable input was provided by the international ISO standard for *Biological evaluation of medical devices*, as well as guidance concerning medicinal products for human use published by the European Medicines Agency (EMA), (ISO/FDIS 2009; EMA 1998, 2008, 2010).

Although the document deals with tattoos and PMU, the assessment strategy is identical for both. Hence, for simplicity, the text refers only to 'tattoos'.

It should be noted that approaches to risk assessment for nanoparticles are still under development. Consequently, no consideration is given here to the potential health risks of the pigments in tattoo inks resulting specifically from their nano- or micro-particulate state.

The challenge of establishing the safety of tattoo ingredients

Even though several countries now have national regulations, the question of how to assess pigments applied under the skin has yet to be answered.

The following questions need to be addressed:

- Are standard test methods, as used in chemical safety assessment for the different toxicological endpoints (application on the skin, oral studies) appropriate for tattoo application?
- Are special test protocols needed for tattoo inks, beyond existing standard methods such as the OECD guidelines for example? An additional question here is whether such test protocols are available or could become available in the near future.

Ingredients of tattoo inks differ in solubility. Mostly, the colourant in the tattoo ink is an insoluble pigment, but soluble ingredients of a tattoo ink comprise, for example, solvents, thickeners and preservatives. Risk assessment involves the quantitative assessment of toxicity and exposure, and these differences in solubility may necessitate different approaches.

For *soluble* components, a classical risk assessment can be carried out based on results obtained using the standard test methods for the various toxicological endpoints. Data from other areas such as cosmetics, pharmaceuticals or chemicals can be used (assuming that the bioavailability is 100 % for soluble chemicals when the skin barrier is passed). Data for preservatives, solvents, thickening agents and

contaminations should be readily available in many cases. If data are not available, they should be generated before the substance is used in tattoo inks.

The situation is more complex with regard to *insoluble* ingredients. For a very few pigments, data already exist which can be accessed (e.g. via PubMed) and used for risk assessment. In some instances, the particular properties of the pigment might exclude the use of established methods, but this must be decided on a case-by-case basis.

This document aims to give guidance for both soluble and insoluble ingredients. Accordingly, it covers test methods for a wide range of chemicals with different chemical and physical properties. Especially for insoluble pigments in tattoo inks, some of the methods outlined in this document are applicable only with caution. In the light of the above discussions, this document, including details in Appendix 4, should be considered a 'living' document that will need to be amended and revised as new information becomes available and new insights are gained on several crucial points.

It should also be emphasised that, for toxicological evaluation of the ingredients of tattoo inks, the nature of the tattooing process itself must be understood (see PART I).

Risk assessment of tattoo ingredients should be carried out by a qualified and experienced safety assessor who can exert sound reasoning and expert judgement in the evaluation of the different endpoints and the final assessment. This person should have a certificate attesting to basic knowledge in toxicology.

PART I – Tattooing process and biological response to tattooing

i. History, equipment and materials

The histopathology and history of the tattooing process have been reviewed widely since the end of the 1970s (Kluger *et al.*, 2008; Sperry, 1992; Lea, 1987; Mann *et al.*, 1981; Goldstein, 1979).

Tattooing is an invasive procedure during which a sharp object (needle, bone, hard wood, etc.) introduces various colourants through the epidermis into the dermis by repeated punctures. Four main methods exist: incrustation, burning, incision and injection (Adatto, 1993). Mechanical tattooing came about through an innovative machine invented by Thomas Edison in 1876 (US patent 196,747), called the Stencil-Pen. It was designed for use as an engraving tool, but in 1891 Samuel O'Reilly modified the device to inject ink into the skin. The device consisted of an electric motor driving a crankshaft similar to that of a sewing machine, which operated a metal stylus with a single needle or array of needles at its tip.

Nowadays, a similar device is the tool most commonly used to perform professional tattoos in the western world (Adatto *et al.* 2011). It is a handheld device with a low-voltage electricity supply, which is

controlled by a foot pedal that determines the speed of oscillation of the needle. Tattoo ink is spread onto the skin surface from an open reservoir and then repeatedly punctured into the dermis by the needle. The amount of ink deposited in the dermis is dependent upon the characteristics of the needle, ink, skin, puncture depth, and density of penetrations.

According to Vassivela *et al.*, (2007), large entomological needles, 36 mm in length and 0.36 mm or 0.41 mm in diameter, are used. The disposable tattoo needle is constructed of several integrated conical microneedles, which may be configured as a thin needle for line-work or a broad needle for filling in mottoes and for shading.

The angle between the skin surface and needle (10°–90°) is an additional variable. The depth of punctures has been reported to be 0.6–2.2 mm (Vassivela *et al.*, 2007). In practice, pigments are found at greatly varying depths in the dermis depending on the location of the tattoo on the body. The epidermis, epidermal-dermal junction and papillary dermis appear to be blurred immediately after pigment injection, and the pigment is distributed throughout the entire vertical depth penetrated by the needle.

In practice, pigments are found at greatly varying depths in the dermis depending on the location of the tattoo on the body.

Pigments

Particles of tattoo pigments are sized in the nanometre or micrometre range, as shown by degradation laser diffraction (Høgsberg *et al.*, 2011). Indeed, except for white pigments, the vast majority of tested tattoo inks contain significant numbers of nanoparticles [i.e. particles with at least one dimension between 1 nm and 100 nm (Høgsberg *et al.*, 2011)] and black pigments consist almost solely of nanoparticles.

Auxiliary ingredients

Besides pigments several soluble ingredients are used in tattoo inks, for example: to keep the pigment dispersed; to enhance the viscosity of the ink and for preservation purposes.

According to the tattoo industry, a typical tattoo ink comprises:

- up to 3 preservatives (which at present also include those not on the positive list of preservatives allowed in cosmetics);
- 1 astringent;
- up to 3 viscosity regulators;
- up to 3 solvents;
- water, and up to 6 pigments (added as powder).

The number of ingredients in a given ink is limited but there is a wide variety of substances to choose from to obtain the desired functions.

ii. Tissue response after tattoo application and localisation of tattoo pigments in the skin

Human data on tissue response after tattooing

Details of the initial, immediate response after tattoo application in humans have been presented by Gopee *et al.*, (2005) who, in the discussion section of their article on a hairless-mouse model, reviewed the relevant human data on this topic. In the first 4 days after tattoo application, peeling of epidermal cells occurs, together with the pigment present therein. Oedema is seen immediately after tattoo application, followed by erythema up to days 7–10. Electron microscopy of freshly tattooed human skin confirms this picture, showing extensive injury to the epidermis and dermis surrounded by an 'inflammatory halo'. After ≈ 1 week, the sites of punctured skin have healed. The tattoo pigment is then present within the deeper layers of the skin. Complete regeneration of the epidermis has been reported to occur in ≈ 2 weeks. Electron microscopy confirms that necrotic and inflammatory cells have disappeared from the epidermis after 30 days. Importantly, after the initial phase of inflammation, proteolytic neutrophils and phagocytic macrophages migrate to regional lymph nodes (Gopee *et al.*, 2005). The latter process may play a part in pigment migration to lymph nodes, a phenomenon that has been observed in animals and humans.

According to the description provided by Linsmeier Kilmer *et al.*, (2008) trans-epidermal elimination of particles from the ink occurs to some degree for ≤1 month after tattoo application, with ink particles present in scaled-off keratinocytes, macrophages and fibroblasts. Re-establishment of an intact basement membrane prevents further trans-epidermal loss.

If the tattoo is stable after a few months, pigment particles and aggregates visualised by histology are found in the connective tissue of the dermis, predominantly in the papillary dermis underneath the basement membrane zone and accumulated around vessels. The epidermis, which is renewed over a few weeks, is usually free of pigment. It is not known to what extent pigments are deposited in fibroblasts, macrophages or extracellular milieu in the collagen mesh of the dermis. Deposition may be dependent upon pigment type and tendency of the applied pigment to aggregate in tissue, which data indicate is highly variable. Thus, the diameter of ink granules in the dermis has been reported to be ≈ 0.5 to ≈ 8 µm, but here further studies may be needed: pigments and their cleavage products may be present in tissue and, due to their small size, fall beneath the resolution of light microscopy.

Depth and density of tattoo-ink deposits in the skin seem to be dependent upon the tattoo artist. In tattoos created by professional tattoo artists, tattoo ink tends to be deeper in the dermis, less ink is deposited, and ink granules are spatially more concentrated than in tattoos created by amateurs (Engel *et al.*, 2008). Data regarding this parameter, however, are limited and it cannot yet be concluded that there is a consistent and systematic difference between tattoos related to the level of professionalism of the tattoo artist. Anyway, a pigment inserted in the skin tends to migrate to a secondary position in the skin more or less independently of the exact depth of introduction into the skin.

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Linsmeier Kilmer *et al.*, (2008) cited a study of freshly implanted eyeliner tattoo ink that revealed

a particle size in the extracellular matrix of 0.1–1.0 μm, whereas the mean particle size before implantation was 0.25 μm. This finding suggests that a certain degree of agglomeration takes place in the tissue. As stated above, under light microscopy, aggregates of particles in skin vary in size from 0.5 μm to 8 μm. Pigment particles in stock ink, and small-particle fragments in tissue as viewed under electron microscopy are illustrated in Appendix 3.

There is some suggestion that 'photo-bleaching' of tattoos occurs after exposure to sunlight (Engel *et al.*, 2010). Tattooed individuals are advised by some tattoo artists to apply sunblock cream while sunbathing.

Animal data on tissue response after tattooing

Detailed information, specifically for the period immediately after tattoo application, has been provided by Gopee *et al.*, (2005), who studied the nature of this process in SKH-1 hairless mice. Animals were tattooed using commercial tattoo inks, or suspensions of TiO_2, cadmium sulfide or iron oxide that contained none of the additives commonly contained in commercial inks. A control group was treated with 10 % glycerol in water. The skin was examined 0.5, 1, 3, 4, 7 or 14 days later and healing was monitored by histological means and by evaluation of various specific biomarkers of inflammation and cell proliferation usually present after skin injury. Histological examination showed, in the first few days, dermal haemorrhage and acute inflammation as well as epidermal necrosis and hyperplasia, which decreased in severity afterwards. Chronic inflammation persisted in all tattooed mice from 3 days to 14 days after tattooing. Pigment was found in inguinal lymph nodes and, to a lesser degree, in axillary lymph nodes. Inguinal lymph nodes were most active, as shown by lymphoid hyperplasia and infiltration of polymorphonuclear cells. Inflammatory and proliferative biomarkers in skin increased up to day 4 but decreased to control levels by day 14. Surprisingly, the skin response in animals tattooed with the glycerol control was similar to that in pigment-treated groups. According to the study authors, these data demonstrate that mice recover substantially from the tattooing

process by day 14, with pigment remaining in the dermis and regional lymph nodes (Gopee *et al.*, 2005).

More recently, Engel *et al.*, (2010) used a hairless mouse model to study the influence of laser light and ultraviolet (UV) light on a tattoo in which azo pigment was present. Their study provides some information on the fate of tattoo pigments after tattoo application. After 42 days, only 68 % of the levels of pigment present initially in untreated tattoos was recovered. The authors suggest that transport into the mouse body (including to the lymph nodes) is the most important route of pigment loss. However, in this study, the initial destination of the pigment was not checked so it is not known if the pigment was injected into the dermis of mice or into deeper structures. The mice were almost devoid of a layer of fat, and pigments and ingredients are easily dosed into deeper structures such as the musculature.

After 42 days, only 68 % of the levels of pigment present initially in untreated tattoos was recovered.

Another notable finding in the study was marked degradation of the tattoo under daily UV light. Over 42 days, ≤ 60 % of the azo dye was lost (compared with 32 % under normal daylight). This finding suggests that substantial degradation of tattoos exposed to sunlight could occur in practice. However, pigment loss may also have been due (at least in part) to increased wash-out caused by greater vascularisation from heating of the skin by the source of UV light. The authors also pointed out that humans may not notice photo-bleaching of the azo pigments in their tattoos because these pigments have very high colour strength and retain visibility even after substantial degradation. However, mouse skin is thinner than that of humans, so the pigments contained in it may be more sensitive to photo-degradation. It should also be noted that the hairless mouse is not a validated model for the study of tattoo pigments, so the study must be treated with caution with respect to its applicability to humans.

PART II – **Data requirements for establishing safety**

Tattoo inks should be safe for consumers and have no adverse effects on human health.

They cannot be assessed as cosmetics, being different from cosmetic products in terms of their chemical and particulate composition as well as their route of exposure: cosmetics are applied topically, whereas tattoo inks are administered invasively and involve permanent marking of parts of the human body after intradermal injection. This invasive route of administration should be considered when assessing the relevant toxicological data.

According to Council of Europe Resolution ResAP (2008) 1 and some national regulations in member states, the manufacturer or importer is responsible for the safety of the products placed on the market. ResAP (2008) 1 presents a tentative outline of data requirements for the safety evaluation of ingredients for tattoo inks. Thus, the following information should be reviewed:

According to CoE Resolution ResAP (2008) 1, the manufacturer or importer is responsible for the safety of the products placed on the market.

- Data on physico-chemical properties, including:

  - purity
  - impurities (heavy metals, amines, etc.)
  - auxiliary ingredients
  - stability (UV, laser, enzymes, bacteria)
  - cleavage products (aromatic amines, etc.)
- Toxicological data on:
  - corrosion
  - irritation (skin, mucous membranes)
  - photo-toxicity
  - immuno-toxicity (sensitisation, photo-sensitisation, etc.)
  - genotoxicity in vitro, including testing of cleavage products
  - photo-genotoxicity on a case-by-case basis.

Additionally, it states that:

- further relevant data or tests should be agreed with competent authorities
- toxicological data for safety assessment should be obtained from test methods using guidelines wherever they exist (e.g. Organisation for Economic Co-operation and Development (OECD), EU).

Current testing limitations

As described below, significant gaps exist in our knowledge of the fate of ingredients of tattoo ink in the body and in the tools available to assess their safety. These problems apply especially to insoluble pigments in tattoo and PMU preparations.

Standard test methods, as used generally for testing of chemical safety, may need to be adapted for insoluble pigments or may even not be suitable at all. The strong colour of the inks, for instance, may make it technically difficult (or even impossible) to undertake certain tests. For pigments, the usefulness of the standard systems has to be decided on a

Significant gaps exist in our knowledge of the fate of ingredients of tattoo ink in the body and in the tools available to assess their safety.

case-by-case basis. With regard to risk assessment for pigments, the usual practice of calculating the margin of safety (MOS)[1] would require a study relevant to the tattooing situation of an insoluble pigment deposited in the dermis over a long period of time. Such studies are not available at present. Thus, for pigments, only limited guidance on conducting safety assessments can be given at the moment. The specific properties of the mixture may also affect the testing of ready-for-use tattoo inks in standard test systems.

The margin of safety (MOS) in chemical risk assessment equals the ratio between a toxicity point of departure and the estimated exposure level.

With respect to insoluble pigments, for the finished product, the usefulness of standard test systems is to be decided on a case-by-case basis. For soluble ingredients, no such problems are foreseen, and the results from the usual standard assays can be readily applied in risk assessment (including calculation of the MOS).

Appendix 4 presents a tentative list of toxicological test methods, applicable to the safety assessment of tattoo ingredients and products.

Over time, as more scientific information becomes available, this list will need revision.

i. Chemical and physical characterisation of inks

Required information on chemical and physical characteristics of inks, pigments and other substances used (solvents and auxiliary ingredients) includes:

- Chemical identity of substances present in the ink: chemical names, trade names and abbreviations, Chemical Abstracts Service (CAS) number, EC number;
- Composition of the ink (list of ingredients and their content);

1 The margin of safety (MOS) in chemical risk assessment equals the ratio between a toxicity point of departure (NOAEL or Benchmark Dose) and the estimated exposure level. MOS-calculation is the standard method used by for instance SCCS and EFSA.

- Molecular weight; melting point, vapour pressure or boiling point;
- Odour, pH, density, viscosity;
- Purity of pigments and of other substances in ink;
- Impurities (heavy metals, aromatic amines, etc.);
- Pigment particle size distribution;
- Solubility in water and other solvents (pigments, other substances used);
- Stability (UV, laser): taking into account exposure of a tattoo to the external environment (e.g. UV radiation from the sun), or removal of the tattoo by lasers, it is necessary to determine the stability of tattoo inks under UV light or laser conditions. Degradation products from decomposition should be identified.
- Further specifications (e.g. presence of coating on surfaces of particles).
- Stability during storage and stability after opening.[1]

Tattoo inks should be sterile and supplied in a container that maintains the sterility of the product until application (ideally in a packaging size appropriate for single use).

Preservatives may be added to ensure microbiological purity of the product after opening; their presence, however, will not compensate for any microbiological contamination during manufacture or for inadequate hygiene in tattooing practices.

The presence of preservatives will not compensate for any microbiological contamination during manufacture or for inadequate hygiene in tattooing practices.

1 Stability studies provide evidence on how the quality of ink varies under the influence of environmental factors such as temperature, humidity and light to establish a re-test period or a shelf-life for the ink as well as recommended storage conditions. For reference see for instance International Conference on Harmonisation (ICH) guidelines on stability Q1A to Q1F at www.ich.org/products/guidelines/quality/article/quality-guidelines.html.

ii. Genotoxicity

Ingredients of tattoo inks

The standard battery for genotoxicity testing of pharmaceuticals (ICH S2R1) can be used to assess the genotoxicity potential of tattooing inks. ICH S2R1 proposes the following test battery:

- One *in vitro* test on bacteria (Ames Test – OECD 471)
- One *in vitro* test on cell cultures (OECD 473, 476, 487)

If these tests indicate genotoxic potential, then *in vivo* testing should be carried out:

- One in vivo test in the bone marrow of a rodent (OECD 474)

An alternative to the *in vivo* bone marrow assay in rodents could be the *in vivo* comet assay. As stated by Brendler-Schwaab *et al.*, (2005), the *in vivo* comet assay is particularly useful for evaluation of local genotoxicity, especially for organs/cell types that cannot be evaluated easily with other standard tests.

In vitro testing should be done with and without metabolic activation. In some cases, chemical compounds with an aromatic amine structure are present in tattoo inks (e.g. 3,3′-dichlorobenzidine in some red and yellow pigments). The S9 hepatic fraction derived from hamsters is known to be more efficient for testing these chemicals than that of the rat because the S9 hepatic fraction from hamsters contains more N-acetyltransferase (Light *et al.*, 1987; Phillipson & Ioannides, 1983; Prival, 1984). For the Ames test, strains TA 1538 and TA 98 are known to be more sensitive for this type of mutagen (Reid *et al.*, 1984). Hence, in some cases, carrying out independent *in vitro* tests using two exogenous metabolic systems is important.

Tattoo inks are composed of several ingredients. In principle, the genotoxicity of tattoo inks should be assessed ingredient by ingredient. Nevertheless, ink composition can be complex such that assessment of the entire product may be required due to the

In principle, the genotoxicity of tattoo inks should be assessed ingredient by ingredient.

possibility of interactions of the ingredients that may result in additional genotoxic potential.

If a chemical is genotoxic *in vitro*, then the *in vivo* genotoxic potential should be explored. If a chemical testing positive *in vitro* does not show *in vivo* genotoxicity in an appropriate study, then its use in tattoo inks is acceptable. However, substances that are genotoxic *in vivo* cannot be considered to be safe for use in tattoos.

For insoluble pigments and finished products, the usefulness of standard tests may be compromised, for example, because of their strong colouring properties. This should be addressed on a case-by-case basis.

Identification of decomposition products

Photolytic cleavage of pigments and formation of toxic degradation products may occur during exposure of the site of tattooed skin to sunlight or under the influence of laser light during tattoo-removal treatments. An example of this phenomenon is the degradation of certain azo dyes to carcinogenic aromatic amines, as demonstrated *in vitro* by Vasold *et al.* (2004) and *in vivo* by Engel *et al.* (2010). Laser irradiation of two azo compounds, Pigment Red 9 (PR 9) and Pigment Red 22 (PR 22) resulted in the following degradation products:

- PR 9: 2,5-dichloroaniline (2,5-DCA) and 1,4-dichlorobenzene (1,4-DCB)
- PR 22: 2-methyl-5-nitroaniline (2,5-MNA), 4-nitro-toluene (4-NT).

As pointed out by the authors, 4-NT has been shown to be genotoxic in human lymphocytes, whereas 5-nitro-o-toluidine, which is also degraded to 2,5-MNA, may cause liver dysfunction. 2,5-MNA and other dinitrotoluene compounds show mutagenic activity toward *Salmonella typhimurium* YG tester strains. 1,4-DCB has been reported to cause tumours in the kidneys of male rats and in the liver of male and female mice, and 2,5-DCA

4-NT has been shown to be genotoxic in human lymphocytes, whereas 5-nitro-o-toluidine may cause liver dysfunction.

has been shown to induce nephrotoxicity in rats. The effects of UV radiation and natural sunlight on Pigment Red 22 have been shown to be responsible for the detection of naphthol AS (NAS) as a product of cleavage of the pigment and for the primary decomposition products 2-MNA and 6-NT.

Pigments should not be present if decomposition into toxic reaction products is possible by metabolic, photo- or laser-induced metabolisation.

Thus, genotoxicity tests should be considered for cleavage products formed during laser-removal treatment of tattoos or during exposure to UV radiation in natural sunlight.

Pigments should not be present if decomposition into toxic reaction products is possible by metabolic, photo- or laser-induced metabolisation.

Formation of potentially toxic photolytic degradation products should be evaluated on a case-by-case basis. Available data from the scientific literature or other reliable sources should be taken into account. If the chemical structure of the pigment molecule is such that cleavage into toxic products is likely, this phenomenon can be examined in an *in vitro* photolysis experiment using an appropriate light source.

Photolytic formation of genotoxic degradation products (as shown for certain azo dyes) would render a pigment unsuitable for use in tattoo inks.

iii. Local tolerance

Skin irritation

The outer layer of the skin, the stratum corneum or horny layer, consists of dead cells. Several other layers of cells are present underneath the stratum corneum: clear/translucent (stratum lucidum); granular (stratum granulosum), spinous (stratum spinosum); and basal/germinal (stratum basale/germinativum). Together, these layers form the epidermis. In typical skin-irritation studies (SISs), the test compound is applied topically to the skin surface (in older studies, it was also applied to abraded skin). As described in PART I, tattooing involves injection of an insoluble pigment (suspended in a carrier

fluid) into the skin, with the pigment subsequently settling in the area just below the epidermis (against the basal membrane). Initially, the pigments are in direct contact with epidermal/dermal cells of the tissue that is damaged by the tattooing process and the tissue presents erythema and oedema. Hence, because the skin is already damaged by the needle ('needle trauma'), the question is how much a tattoo ingredient with skin-irritating potential will exacerbate this needle damage and even interfere with healing. Irritant reactions also might increase the risk of infection.

To assess the effect of contact with damaged tissue, a typical SIS represents an incomplete model. Nevertheless, the results of typical SISs in animals may be useful because compounds showing irritative potential when applied topically to the skin might also be irritants upon intradermal application, and so should not be present in tattoo inks. Similarly, existing human data on dermal irritation should be taken into account.

Since tattoo inks are composed of many substances and particles which may have complex interactions, testing of the final product with respect to induction of irritation is important.

Tattoo inks with pH < 5 or > 8 should not be used because of expected irritant/corrosive effects.

Tattoo inks with pH < 5 or > 8 should not be used because of expected irritant/corrosive effects.

The Intra-cutaneous Reactivity Test is recommended for medical devices. If technically feasible, it is considered to be a suitable *in vivo* test to establish the irritation potential to the dermis of tattoo ingredients. This test is described in an International ISO/FDIS Standard (ISO/FDIS 2009) and involves intradermal injection of 0.2 ml of a test solution in an appropriate solvent to the clipped dorsal skin of rabbits. Animals are scored for erythema and oedema ≤ 72 h post-treatment. For strongly coloured pigments and inks, the test may not be applicable because the colour may interfere with the readout of the test (especially if the colour is red). Solubility of the test substance may also be an issue for pigments and finished products. The test method specifies

the scoring procedure. The requirements of the test are met (absence of significant irritation is shown) if the mean final test sample score is ≤ 1.0. If necessary, testing for irritation using this model might be combined with a photo-irritation test. Testing of irritancy may also be combined with testing for effects on wound healing (a combination which may be particularly relevant for tattooing). A sufficiently high concentration should be tested to determine the endpoints for any irritation caused by the ingredient.

Irritation of mucous membranes

Eye irritation is a potential risk when applying tattoo inks, especially for some PMU applications. Chemicals showing significant potential for eye irritation or having the capability of staining internal parts of the eye in *in vivo* tests are not suitable as ingredients in tattoo inks or PMU products.

Ingredients in PMU products can be tested for eye irritation using regular test models such as the OECD guideline 405.

PMU applied to lip borders can also be considered as a tattoo of a mucosal membrane. In fact, some individuals have a tattoo directly on the oral mucosa, most often on the inner side of the lower lips. Such tattoos are often limited to dark colours and basic designs due to the technical difficulties of tattooing such areas. Results of an eye-irritation test could be used to estimate the effects on the oral mucosa. However, in general, tattoos on the lip and cornea are not recommended.

In general, tattoos on the lip and cornea are not recommended.

For pigments and finished products, standard tests for irritation of mucous membranes may need to be adapted or may not be usable at all. This problem may be due, for instance, to the very strong colouring properties of the materials. Applicability of standard tests for insoluble pigments and finished products should be decided on a case-by-case basis. Such problems are not foreseen for colourless soluble ingredients.

Test for effect on wound healing

'Skin erosions' are superficial abrasions of the epidermis. 'Wounds' are deeper lesions reaching the deep dermal layers. Wound healing involves spontaneous repair of the epidermis and dermis. The epidermis heals quickly due to swift proliferation of keratinocytes in the basal cell layer. The dermis heals more slowly (over weeks or months) until full restitution is obtained with fibrosis and scar formation if the lesion goes beyond the mid-dermis. The biology of wound healing is reviewed in Rook's Textbook of Dermatology (Ferguson and Leigh, 1998).

The lesions made by tattoo needles are multiple, tiny puncture wounds. Combination of thousands of punctures and installation of tattoo inks holding chemicals and tiny foreign bodies (i.e. pigments) carries a potential risk of wound-healing problems with delayed healing, infections, and chronic sequelae (formation of abnormal scars; hypopigmentation; hyperpigmentation; altered skin sensations). Irritancy and corrosion due to tattoo inks may also interfere with healing.

Wound healing has been studied in animals and humans for decades and extensive literature is available dealing with *in vivo* and *in vitro* studies. Research is often designed to answer a specific clinical problem in relation to wound types (incisional, punch biopsy, burn, suction blister, infected, ischaemic, diabetic).

In vitro models and *in vivo* animal wound-healing models were reviewed by Sullivan *et al.*, (2001). They found better correlation of humans with pigs (78 %) than with small animals (53 %) in *in vitro* studies and therefore recommended that a study of wound healing be conducted in pigs (which also have a skin structure closer to that of humans).

No specific model is widely used with respect to wound healing and tattooing. The most suitable model would be assessment of epidermal and dermal healing in pigs in skin areas tattooed with the ingredient or tattoo ink (final) product in comparison with skin areas tattooed with the vehicle or another relevant control. This scenario would be followed for 4–12 weeks, with even longer observation if the risk of

abnormal scarring and pigment variation were to be addressed in a long-term perspective.

In conclusion, testing for effects on wound healing is identified as a relevant endpoint but, at present, acceptable *in vivo* or *in vitro* models for tattooing are not established.

Sensitisation and cutaneous allergy

Allergic reactions due to tattoos rank as probably the most common chronic complication of tattoos necessitating medical treatment (De Cuyper and Pérez-Cotapos, 2010). Red tattoos and mixtures of red most frequently lead to allergic reactions.

Red tattoos and mixtures of red most frequently lead to allergic reactions.

A skin sensitiser is an agent that can cause an allergic response in susceptible individuals. After exposure via the skin, the characteristic adverse health effects of allergic contact dermatitis may be provoked. A period of first exposure and sensitisation of typically a few weeks is required for allergy to develop. Once sensitised, the allergic response can be elicited in minutes, hours or a few days after relevant contact or exposure depending on the type of allergy. In tattooing, type-IV delayed allergy may intuitively appear to be of primary interest in the safety evaluation.

The most common *in vivo* test methods using laboratory animals to evaluate skin sensitisation are:

- Local lymph node assay (LLNA) [EC B.42, OECD Guideline 429][1]

1 The local lymph node assay (LLNA) [EC B.42, OECD Guideline 429] is based on the extent of proliferation of lymphocytes in the auricular lymph nodes draining the site of application of the test substance. The test substance is applied topically on three consecutive days to the dorsum of both ears in a suitable vehicle (Freund's complete adjuvant (FCA), as an immune enhancer causing local skin inflammation, is not used). After two days of rest, auricular lymph nodes are collected and proliferation of lymphocytes measured. The result is expressed as a stimulation index, which is the ratio of proliferation caused after applying the test substance in mice versus that in vehicle-treated control mice. Methodologically, the LLNA is a refinement (in the area of discomfort to the animal) compared with traditional guinea pig-based models, as described below.

- Magnusson–Kligman guinea pig maximisation test (GPMT)[1]
- Buehler test.[2]

In GPMT the compound is introduced by intradermal injections, which mimics the intradermal puncturing of tattooing. Freund's complete adjuvant (FCA) is added to activate the immune system. The results obtained with the GPMT are thus considered to be good predictors of the potential of the tested compound to induce dermal sensitisation upon intradermal application. Therefore, the GPMT is considered to be an acceptable test system for safety testing of tattoo ink ingredients. An adapted protocol is available for carrying out the GPMT with insoluble compounds (Maurer and Hess, 1989). The applicability of this protocol for insoluble tattoo pigments should be determined on a case-by-case basis.

Given the non-invasive nature of the Buehler test, it is considered of low relevance to tattooing. This is also the case for the standard LLNA to some degree because a negative response is not sufficient to establish absence of a sensitising potential of any tattoo ink ingredient. Thus, these tests provide sufficient evidence only if they show a positive result, thereby disqualifying the pigment for use in tattooing inks.

Clinical studies with patients allergic to their tattoo suggested complexities in causation of the disorder in at least some cases. The study showed that development of allergies took weeks, months or even years. Allergy patch testing with common standard allergens,

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1 The Magnusson–Kligman guinea pig maximisation test (GPMT) [EC B.6, OECD 406] is an adjuvant-type test. That is, the allergic response is potentiated by intradermal injection of the test substance with and without FCA. The GPMT is considered equal to the LLNA in terms of sensitivity. The test result is based on the challenge response to a non-irritant patch test with the test substance. Thus, the test mimics the 'real-life' development of allergic contact dermatitis. The method allows repeated challenges, cross-reactivity and vehicle–effect studies.

2 The Buehler test [EC B.6, OECD 406] is a non-adjuvant method that involves topical application only. The method is less sensitive compared with the GPMT. Scientific justification should be given if the Buehler test is used.

including para-phenylene-diamine (PPD, a primary aromatic amine (PAA)), a series of problem tattoo inks, and a series of disperse textile dyes (including several azo dyes) was negative even where there were severe allergic reactions to the tattoo.

Negative outcome of the allergy patch test using allergens from the European baseline series suggests allergen(s) are generated in the skin through very slow haptenisation of ink contents after processing in tissue (most likely more complex than simple chemical dissociation), together with tissue substances comprising the epitope or allergen causing the allergic reaction. This phenomenon is seen particularly in red tattoos (Serup and Carlsen, 2014). That finding was in agreement with studies of allergy to disperse textile dyes, in which patch testing of individual PAAs was also negative (Malinauskiena, 2009).

In conclusion, human studies suggest that at least in some (or possibly many) cases of allergic reactions to tattoos, the allergen is not directly present in the tattoo ink product but probably is formed slowly within the dermis from some unidentified precursor in the ink. For such cases, the predictive value of standard animal models is uncertain and seems limited. The standard designs of animal models match only traditional contact allergens and type-IV allergy, and do not take into account the different timeframes in at least some allergic reactions to tattoos. This may represent an important limitation to the usefulness of the standard animal models mentioned above. Without further information and evaluation, a firm conclusion cannot be drawn on this point. At present, it seems reasonable to retain standard animal models to provide relevant information for tattooing until further notice. Clearly, there is an urgent need for further research and evaluation of this issue.

At least in some cases of allergic reactions to tattoos, the allergen is formed slowly within the dermis from some unidentified precursor in the ink.

Photo-toxicity

According to two recent studies, ≈ 20 % of tattooed persons complain about skin reactions at the site of their tattoos if exposed to the

sun. Thus, photo-sensitivity is an important toxicological endpoint. Photo-sensitivity reactions may develop in minutes or days, and are likely to involve various mechanisms, for instance, immediate reactions with induction of reactive oxygen species and delayed reactions with cellular mechanisms. Clinical experience suggests that dark coloured tattoos are more prone to sun-induced reactions (Høgsberg *et al.*, 2013; Hutton *et al.*, 2013).

Dark coloured tattoos are more prone to sun-induced reactions.

In general, compounds that contain suitable chromophores (moieties capable of absorbing UV light or visible light in the range 290–700 nm, such as those with extended conjugation of double bonds or aromatic rings in the molecular structure) may be activated photo-chemically by UV light or visible radiation. These photo-activated structures may alter biological systems and, at sufficiently high exposure, may produce photo-toxicity (i.e. photo-irritation, -sensitisation, -genotoxicity or -carcinogenicity).

Photo-toxicity assessments may be made following the steps described below:

Absorption of UV radiation

As a first step, the necessity for photo-toxicity testing should be determined. According to the *Note for guidance (NfG) on photosafety testing for human medicinal products* (CPMP/SWP/398/01), as published by the EMA in 2002, only those chemicals that absorb light in the 290–700-nm range of the electromagnetic spectrum and reach the skin or eyes need to be tested for photo-toxicity. Since publication of that NfG, accumulated data and experience have shown shortcomings in the guideline. This situation prompted the EMA to publish a clarifying document (EMA/CHMP/SWP/336670/2010), in which a refinement was introduced specifying that, when determining light absorption, the 'molar extinction coefficient' (MEC) could be used as a threshold below which further photo-toxicity testing would not be needed. The MEC (also called 'molar absorptivity', ε) is a constant for any given compound under a specific set of conditions (e.g. solvent, temperature, wavelength) and reflects the efficiency with which a

molecule can absorb one photon of light. The method is described more fully in OECD Guideline 101 (the MEC is called 'molar absorption coefficient' by the OECD). The EMA clarifying document also states that recently published data clearly indicate that compounds with MEC < 1 000 L mol^{-1} cm^{-1} are of sufficiently low concern with regard to photo-safety issues, that this level can be accepted as an appropriate threshold below which further photo-safety testing is not warranted.

Pigments, given their strong colouring properties, will have high absorption in at least part of the relevant range of wavelengths. Their insolubility, however, may prevent measurement of the MEC according to OECD guideline 101. Some pigments may be soluble in specific solvents, which would allow measurement of the MEC. This parameter must be determined on a case-by-case basis.

Assessment of the phototoxicity potential by the 3T3 neutral red uptake phototoxicity test (3T3 NRU-PT)

In its guidelines for photosafety testing, the EMA recommends the *in vitro* 3T3 NRU-PT, which is based on comparison of the cytotoxicity of a chemical tested in the presence and absence of exposure to a non-cytotoxic dose of UV/visible light. The EMA recommends its use as an initial test in a tiered approach. If the result of the 3T3 NRU-PT shows an absence of phototoxic potential, then further tests for photogenotoxicity and/or photoallergenicity are not needed (EMA/CHMP/SWP/336670/2010).

Assessment of the phototoxicity potential in human keratinocytes

If other *in vitro* phototoxicity methods are validated, these methods could be used to determine the phototoxicity of tattoo inks. If the compound is photo-toxic in the 3T3 NRU-PT, this potential could be assessed further in a more elaborate model of the human epidermis.

If the compound is photo-toxic in the 3T3 NRU-PT, this potential could be assessed further in a more elaborate model of the human epidermis.

Although formal validation is lacking for such models, the tiered approach suggested by the EMA could also be used to cover the photoirritation induced by pigments or other ingredients used in tattooing. Photoirritants, as determined by an appropriate (*in vitro*) test, should be excluded from use in tattoo inks.

Photosensitisation and photoallergy

As indicated in section 'Photo-toxicity', testing for photoabsorption should be used as a trigger for determining the need for further testing of phototoxicity endpoints, including dermal photo-sensitisation. For chemicals showing light absorption above the recommended threshold of a MEC of 1 000 L mol^{-1} cm^{-1}, phototoxicity testing is required. As suggested in section 'Photo-toxicity', a tiered approach is possible using the *in vitro* 3T3 NRU-PT as the initial test. Only if this test is positive is further photo-sensitisation testing warranted. This follows the recommendations given by the EMA (2008, 2010) and is underlined by the Scientific Committee on Consumer Safety (SCCS, 9th revision Notes for Guidance), which indicates that chemicals showing photo-allergic properties are likely to give positive reactions in the 3T3 NRU-PT [2000/33/EC].

For tattooing inks, photo-sensitisation could be tested using the *in vivo* method in guinea pigs described by Ichikawa *et al.*, (1981). This method includes intradermal injection of FCA, with subsequent topical application of the test substance (topical application in induction phase and challenge phases). The method meets or exceeds requirements described in the CTFA Safety Testing Guidelines (CTFA, 1991). The test outlined by Ichikawa *et al.*, is one of the few methods capable of identifying musk ambrette (a well-known photosensitiser) and is regarded as the most relevant method for identifying photosensitisers. In this method, the test chemical is applied topically to immuno-potentiated skin, providing for some partial penetration of skin. In addition, this test includes pre-screening for photoirritation which may provide *in vivo* results for this endpoint that are supplementary to the *in vitro* 3T3 NRU-PT.

The test outlined by Ichikawa *et al.*, is one of the few methods capable of identifying musk ambrette.

The limitation of this test for tattooing, however, is that the chemical is applied to the skin surface only. This is a limitation even for soluble ingredients (which of course are applied intra-dermally in tattoos). For insoluble pigments and finished products, the test is unlikely to be valid for the same reason.

In conclusion, there is no method suitable for testing of the photosensitisation of tattoo inks, pigments and ingredients. Data for soluble ingredients, obtained using the method described by Ichikawa *et al.*, (1981) are relevant as supporting information but, given the limitations indicated, they cannot definitively establish the safety of any ingredients for this endpoint.

There is no method suitable for testing of the photosensitisation of tattoo inks, pigments and ingredients.

Photo-genotoxicity

For this endpoint for tattoo inks, it is recommended to follow the guidance of the EMA (Q&A, 2010).

Initially in its *Note for guidance (NfG) on photosafety testing for human medicinal products* (CPMP/SWP/398/01) from 2002, the EMEA recommended use of a photo-clastogenicity study (chromosomal aberration or micronucleus test) in mammalian cells *in vitro*. However, in its draft paper entitled *Question and answers on the Note for guidance on photosafety testing* of 24 June 2010 (EMA/CHMP/SWP/336670/2010), the EMA points out that experiences with these models in regulatory testing since 2002 suggest that they are substantially over-sensitive and that incidences of pseudo-photo-clastogenicity have been reported. Therefore, the EMA no longer recommends *in vitro* photo-clastogenicity tests for regulatory photo-genotoxicity testing. Because of the uncertainty surrounding the value of photo-genotoxicity results for determining possible photo-carcinogenicity, the EMA recommends excluding photo-genotoxicity testing as a routine part of the standard photo-safety testing programme. Thus, no photo-genotoxic test is required for tattooing products.

Photo-carcinogenicity

Carcinogenesis of the skin in human populations is driven by exposure to external light (mainly sunlight). Inks and pigments are colourful and their presence in the skin strongly influences skin optics, with absorption of a broad spectrum of light or narrower bands of the spectrum depending on the colour. Pigments interfere with incident light and influence backscattering in the skin and carcinogenic exposure of proliferating keratinocytes (from which basal cell and squamous cell carcinomas are generated). This interference may have negative or positive impact, since light absorption by pigments may diminish the carcinogenic effects of incident light. Interaction of light and chemical carcinogens present in skin, however, is difficult to predict.

According to EMEA (2002), the most widely used animal model for testing photo-carcinogenicity is the SHK1 (hr/hr) albino hairless mouse. Using this model, it was shown that mice develop squamous cell carcinomas over time, induced by experimental exposure to UV light. Tattoo-related photo-carcinogenicity may be studied in mice over months using tattooed mice versus relevant controls. In one study with hairless mice, it was found that mice tattooed with a preparation containing high levels of polycyclic aromatic hydrocarbons (measured as benzo(a)pyrene), which are known dermal carcinogens in mice, developed fewer tumours than did light-exposed controls (Lerche *et al.*, 2015).

iv. Biokinetics

As pointed out by the German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung, BfR) (2009), toxico-kinetic data are needed for safety evaluations.

Soluble compounds in the tattoo ink such as preservatives and conditioners presumably readily migrate into bodily fluids, undergoing metabolism and eventual excretion from the body. For these

Soluble compounds in the tattoo ink such as preservatives and conditioners presumably readily migrate into bodily fluids

compounds, existing toxico-kinetic data (for intravenous, oral and inhalation exposure routes) are relevant. Hence, it may not be strictly necessary to conduct special studies for tattoo applications. For soluble compounds, a high rate of absorption into the systemic circulation is expected, with only slight trans-epidermal loss.

However, the fate of the insoluble pigment(s) used in tattoo inks differs from that of the carrier fluid. As described in PART I, insoluble pigments predominantly end up in the dermis but also, to a certain degree, in lymph nodes and possibly other compartments. Pigments can form aggregates during transfer to these locations. Assessment of exposure and risk is highly complex and cannot rely on one simple experiment or one model for risk prediction. There is no pharmacological model by which absorption in the dermis from tattoo-ink pigments can be predicted. Fick's first law (which is usually employed for cutaneous applications) is not applicable to tattoo inks and substances. The biokinetics of insoluble tattoo pigments have been studied only in mice using Pigment Red 22 and, systematic knowledge on dose exposure and biokinetics in pigs and humans is absent.

With respect to the fate of insoluble pigments after application in tattoos, an important aspect is the degree of leakage (actively via phagocytosis or passively) from the tattooed skin site. Transport of pigment to lymph nodes occurs immediately after application and over the longer term. As noted in PART I section 'Animal data on tissue response after tattooing', Engel *et al.*, (2010) reported a loss of about 32 % of pigment over a 42-day post-tattooing period in their study in hairless mice. In part, this loss occurs trans-epidermally but, presumably, some pigment also ends up in the blood stream and could cause systemic toxicity. For better understanding of the leakage of insoluble pigments, further information is required, which may be obtained by conducting bio-kinetic studies for at least several additional pigments, representative of different structural classes, in a relevant experimental model. Information on the degree of pigment

Transport of pigment to lymph nodes occurs immediately after application and over the longer term.

loss from the tattooed site, and the time period over which this loss occurs, is needed to estimate systemic exposure to tattoo products.

Whether tattoo pigments, compounds or degradation products may be released beyond the lymph nodes and accumulate in other organs of the body remains the subject of speculation. Currently, there are no clinical data supporting systemic toxicity related to tattoo inks. Lehner *et al*., (2011) attempted to estimate the extent of decomposition and transportation of tattoo pigments by measuring the decrease in pigment concentration in human skin *in vivo*. Based on high-performance liquid chromatography analyses of tattooed skin samples from five deceased individuals, the authors calculated a substantial decrease in the concentration of Pigment Red 22 (≈ 87-99 %). However, those results are open to criticism because the ages of the tattoos at the time of analyses were not known. In addition, the authors of that study relied on an established value of 2.53 mg/cm² of pigment introduced during the tattooing procedure (Engel *et al*., 2008), which could also be contested. Hence this study does not provide unequivocal evidence of the fate of tattoo pigments in terms of UV degradation, skin degradation, generation of new by-products, or migration to the lymph nodes.

The mini-pig *in vivo* model is considered to be the most suitable option for investigating the biokinetics of several representative pigments applied in tattoos. Based on the results of biokinetic studies in mini-pigs, the degree of trans-dermal loss of pigments after tattooing can be established, as also can the timeframe of migration of pigments to the lymph nodes and other body compartments. In addition, the timeframe of leakage of pigments to blood vessels can be determined. To determine systemic availability (i.e. to what extent leakage and metabolism of pigments as well as degradation and excretion are processes lasting days, weeks, months or even years), a limited number of mini pigs can be tattooed, for example, using radiolabelled pigments and then the radiolabel can be measured in blood, urine or faeces. At the end of the test, the animals can be examined internally, and levels of radioactivity remaining at the tattooed skin

site and other selected sites determined. The chemical structure of the various residues found can also be elucidated.

As an extension, the envisaged biokinetics studies may include determination of systemic toxic effects.

Pigs are recommended because they closely resemble humans in terms of anatomy, physiology and biochemistry (Bode *et al.*, 2010). Pig skin is known to resemble human skin structurally and physiologically (see, for instance, Sullivan *et al.*, 2001) and applying tattoos to pig skin is technically feasible. Given the general aim of reducing the use of test animals, tests should be carried out in accordance with the 'reduce, refine, replace' (3Rs) principle, if possible, according to Directive 2010/63/EU on the protection of animals used for scientific purposes. In terms of 'refinement', pigs are considered the most predictive animal species for extrapolation to humans. With respect to 'reduction', the mini-pig is considered to be the best model for humans, so its use should contribute to reducing the conduct of irrelevant assays in inappropriate animal models. However, currently, an approved testing protocol for tattoo applications in mini pigs is not available. Development of such protocols is strongly recommended.

Given the general aim of reducing the use of test animals, tests should be carried out in accordance with the 'reduce, refine, replace' (3Rs) principle.

Alternatively, other appropriate analytical methods may be used to determine the pigments and their metabolites present in different body fluids and tissues.

Thus, a study of mass-balance would be useful for addressing different classes of pigments in order to determine their fate in the body, the metabolism and excretion.

v. Repeated dose toxicity

To evaluate systemic exposure to soluble tattoo substances and/or contaminants present in the formulation through migration from tattooed skin, a repeated dose toxicity study is needed. The objective

of this study would be to determine a 'no observed adverse effect level' (NOAEL), which can be used for the calculation of the MOS. Soluble ingredients are assumed to be released over days to weeks. For these ingredients a sub-acute study most likely will be suitable for selecting the NOAEL. If an adequate sub-acute study is not available, a sub-chronic study could be used. For further discussion of MOS calculations, see section 'i. Chemical and physical characterisation of inks'.

As an indication, some potential tests are:

- OECD 407 (Repeated Dose (28 days) Toxicity (oral))
- OECD 408 (Sub-chronic Oral Toxicity Test: Repeated Dose 90-day Oral Toxicity study in Rodents)
- OECD 409 (Sub-Chronic Oral Toxicity Test: Repeated Dose 90-day Oral Toxicity Study in Non-rodents).

For insoluble compounds the kinetics of pigment leakage in the systemic circulation should be determined based on the results of toxico-kinetic studies (see section 'iv. Biokinetics') and an appropriate toxicity study should then be selected. A priori, standard toxicity studies using oral or dermal applications seem of low relevance to insoluble pigments present in the dermis (as in tattoos).

In conclusion, for soluble ingredients in tattoo inks, a sub-acute toxicity study provides an acceptable basis for risk assessment for systemic toxicity. However, for insoluble pigments, no test system for systemic toxicity can be recommended at present because not enough is known about the kinetics of pigment leakage into the systemic circulation.

For insoluble pigments, no test system for systemic toxicity can be recommended at present.

vi. Threshold of Toxicological Concern (TTC)

The TTC approach, in general, is considered scientifically acceptable for human health risk assessment of systemic toxic effects caused by chemicals present at very low levels of exposure. If the systemic exposure after tattooing is below the TTC value for the appropriate class, then systemic toxicity is not likely.

Using the TTC approach for the safety assessment of soluble ingredients in tattoo inks would involve assigning the chemicals to a Cramer class based on their chemical structures. Some chemicals (e.g. metals) are excluded from the TTC approach. However, a problem in applying the TTC approach is its relation to the exposure pattern over time. That is, the TTC is calculated as a daily exposure level (µg/person/day), so it does not equate to the pattern of (possible) systemic exposure after tattoo application.

Finally, the joint SCCS, SCHER and SCENIHR (SCCP/1171/08) concluded that the TTC approach was not applicable to materials insoluble in water (< 1 mg/L; i.e. to most pigments used in tattoo inks). Hence, based on current knowledge, the TTC is not applicable for tattoo inks.

Based on current knowledge, the TTC is not applicable for tattoo inks.

vii. Carcinogenicity and reprotoxicity

Carcinogenicity

Clinical observation and evaluation of case reports from the literature suggests that cancer caused by tattoo-ink pigment is a rare occurrence. The few reported cases are considered coincidental (Kluger and Koljonen 2012). It is remarkable that no malignancy in regional lymph nodes due to tattoos has been reported because these nodes are often a secondary site of deposition following the migration of pigment through the skin and thus, for pigment particles, represent a first-pass organ.

The dermis has few proliferating cells whereas lymph nodes contain rapidly dividing cells, which have a higher risk of DNA damage. However, distant-organ cancers associated with tattoos and genotoxicity in distant tissues have not been reported.

This absence of reports is remarkable given that several million people around the world have been exposed, for periods lasting up to several decades, to tattoo-pigment formulations frequently containing chemicals or contaminants which, based on registered data (*in vitro*

and in experimental animals) have been classified as 'unsafe'. Thus, up to now, these well-recognised hazards are not matched by problems observed in the clinic. While this apparent discrepancy remains unresolved, it remains prudent to consider the presence of demonstrated genotoxic carcinogens in tattoo formulations as unacceptable.

As stated in the introduction, ResAP (2008) 1 excludes carcinogenic substances of classification categories 1, 2 and 3 (or now 1A, 1B and 2, respectively), from use in tattooing products.

For non-classified substances, given the strong link between genotoxicity and carcinogenicity, the requirement that genotoxic substances are not used in tattoo inks excludes substances that are carcinogenic and genotoxic. For non-genotoxic (epigenetic) carcinogens, in general, an action threshold will exist. For such substances, precursor effects (pre-neoplastic effects) will precede tumour formation. These effects can, provided presumptions are available, be evaluated using an NOAEL and by calculating the MOS (see section 'ix. Exposure assessment and MOS calculation').

With regard to skin cancer, in human populations the primary cause is known to be exposure to sunlight. Basal cell carcinomas and squamous cell carcinomas are seen in sunlight-exposed skin and malignant melanomas are especially related to sunburn episodes (MacKie, 1989). Skin is transparent and pigments (especially dark pigments) strongly absorb light, so reduction in the backscattering of light towards proliferating keratinocytes may occur. Thus, the tattoo pigments deposited in the outer dermis may reduce the effective exposure to light, which would be expected to be associated with a lower risk of skin cancer. This phenomenon may explain why cancers arising in tattoos seem to be so rare.

Reproductive toxicity

Whether there is a reproductive hazard from tattooing has not been studied. Nevertheless, there is a need for a general warning that women planning pregnancy and women in their first trimester of pregnancy (when the risk of major developmental hazards is greater)

should not undergo tattooing. Similar warnings may also be relevant for second and third trimesters and during breastfeeding (Kluger, 2012).

Women planning pregnancy and women in their first trimester of pregnancy should not undergo tattooing.

The risk of an effect on reproduction and development may be related to the soluble ingredients in the tattoo ink. Insoluble pigments may carry no risk if they remain strictly at the application site. At present, no conclusion can be drawn on this point because relevant data are lacking. Presence of known reproductive toxicants is unacceptable in principle, unless it can be shown by risk assessment that there is no reproductive and developmental risk of the ingredient in question.

As stated in the introduction, ResAP (2008) 1 excludes reprotoxic substances of classification categories 1, 2 and 3 (or now 1A, 1B and 2, respectively) from use in tattooing products. However, it is generally accepted that thresholds are applied for this endpoint. The presence of substances in concentrations below the specified thresholds would not pose a risk to the consumer.

For assessment of unclassified chemicals, a teratogenicity study (e.g. OECD TG 414) should be carried out. If warning signs of reprotoxicity are detectable in repeated toxicity studies (e.g. 28-day or 90-day studies or screening studies OECD TG 412 or OECD TG 422) on reproductive organs and if disruption of endocrine activity is detectable, specific reprotoxicity studies should be carried out (e.g. EOGRTS, OECD TG 443). With regard to other endpoints, special attention may be needed in testing insoluble pigments with the exposure route mimicking as far as possible the tattoo scenario. For insoluble pigments, where data are available only for standard routes of exposure (oral, dermal, inhalation), these may still provide useful supporting information. In using this information, a precautionary approach should be chosen, taking as relevant any adverse effects seen for tattoos unless convincing evidence to the contrary is available.

viii. Acute toxicity

Testing for acute toxicity is used to determine the LD50 value of a compound. The LD50 is the basis of a classification of a substance with respect to acute toxicity according to the chemical legislation.

Three alternative test methods have replaced the old method (OECD 401): the fixed dose method (OECD 420), the acute toxic class method (OECD 423) and the up-and-down-procedure (OECD 425). These alternative methods are in accordance with the 3R concept (reduce, refine, replace) for the protection of animals in research and testing.

ix. Exposure assessment and MOS calculation

Possible systemic toxic exposure via tattoos arises from their single application, with subsequent leakage from the skin site. To assess the likelihood of systemic effects, an MOS calculation can be made [with NOAEL and systemic exposure dosage (SED) values]. Again special attention is needed for both the insoluble pigments and soluble ingredients in the tattoo ink taking account of their expected different biokinetics.

To calculate the MOS, the expected level of exposure must be known. The following factors are needed for estimation of the exposure to a tattoo pigment:

- amount of ink present per cm^2 of treated skin.
- size of the tattoo (cm^2 per person).

Exposure to pigments

The amount of pigment per cm^2 of tattoed skin can be estimated based on the work by Engel *et al.*, (2008, 2010). The figures presented by Engel *et al.*, (2008) on the amount of tattoo product present in tattooed skin are given in mg of pigment per cm^2. This work involved tattooed human skin or pig skin *in vitro* using the azo dye Pigment Red 22 at 10 % w/v or 25 % w/v. Subsequently, they excised the tattoo and extracted the pigment quantitatively. A broad range of 0.6 mg (original suspension: 10 % w/v) to 9.42 mg (original suspension: 25 %

w/v) pigment/cm² skin was recovered (mean, 2.53 mg/cm²). In a series in which the tattoos were applied by a professional tattoo artist (the other series was applied by the investigators), the amount present was the lowest. Hence, the mean value of 2.53 mg/cm² (as opposed to the maximum value) might be a tentative estimate of the amount of pigment present in a tattoo.

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The degree of loss of pigment from tattooed skin sites over time is not known. A highly tentative estimate can be derived from the results reported by Engel *et al.*, (2010). They reported high loss of pigment from a tattoo site of 32 % over a 42-day period (the loss over intervening time intervals was not measured) in hairless mice *in vivo*. This mouse model is limited in its ability to mimic human skin and (as indicated in PART I section 'Animal data on tissue response after tattooing') some uncertainties remain around that study. Nevertheless, this figure can be used as a first approximation of a MOS. This approximation would probably be a worst-case scenario because part of the 32 % will have been lost trans-epidermally in the study. Using these figures can represent only an approximation because of the many limitations of the studies (only one pigment was tested in *in vitro* or hairless mice models). If further data become available, the figures may need to be adjusted.

Size of tattoos

The size of tattoos was addressed by the BfR (Opinion No. 044/2011) which introduced two scenarios that may be useful for risk assessment: normal case with 0.6 mg pigment/cm² and 600 cm² tattooed skin; worst-case scenario with 5 mg pigment/cm² and 4 500 cm² tattooed skin. For the risk assessment, a tattoo ink needs to be safe even in the worst case.

In an expert report *Chemical substances in tattoo ink* (No. 116, 2012), the Danish Environmental Protection Agency indicated that the mean area tattooed in humans was estimated to be 454 cm² (corresponding to 2.5 % of the total skin surface area).

NOAEL for soluble ingredients

An appropriate NOAEL is needed for MOS calculations. An intravenous NOAEL would be preferable, but an oral value could also be used. Effects observed after oral administration (and the basis for the NOAEL calculation) may have been caused by only a fraction of the administered dose and therefore, oral-absorption data of the substances is essential when comparing intravenous-exposure values with oral NOAEL values. The SCCS's Notes of Guidance also state that whenever oral absorption data are available, these should be included in the calculations. If they are not available, a conservative oral absorption factor should be used.

Use of an oral study may not be appropriate for chemicals with high first-pass metabolism in the liver.

The NOAEL used must be derived from a study of appropriate duration based upon the time period during which leakage from the tattooed skin site occurs. For soluble ingredients present in tattoo inks (carrier fluids, preservatives, etc.), ready migration from the tattoo site from days up to 1 week is assumed. As indicated above, a sub-acute toxicity study is considered appropriate for soluble ingredients and can be used for MOS calculation. In the absence of such a study, a sub-chronic study might be used. The selected study should have been well-conducted and, preferably, according to the appropriate guidelines.

Systemic exposure dosage (soluble ingredients)

The amount of tattoo ink per cm^2 can be calculated according to Engel *et al.* (2008). The authors, based on a study in mice and under the assumption that the studied pigment was recovered completely in their study, suggested an exposure of 0.025 mL/cm^2 (assuming a 10 % w/v suspension). High migration into the circulation can be assumed for the soluble ingredients present in the tattoo fluids (carrier fluids, preservatives, etc.), with only some collateral trans-epidermal loss.

For calculation of the MOS, this figure of 0.025 ml/cm^2 could be used tentatively in combination with the concentration employed in the

tattoo preparation to estimate the total body dose of the ingredient in mg/kg body weight (bw).

NOAEL for pigments

At present, no test system for deriving a NOAEL can be recommended for insoluble pigments because not enough is known about the kinetics of pigment leakage into the systemic circulation. Thus, for insoluble pigments, an MOS calculation is not possible at present.

An MOS calculation is not possible at present for insoluble pigments.

Conclusions

Publication by the Council of Europe of ResAP (2008) 1 gave an input to member states that have since passed various national legislations on tattoos and PMU as well as on the practice of tattooing. Among the first measures implemented by member states were market surveys, the results of which show that tattoo inks with substances that should not be present according to ResAP (2008) 1 are still found on the market even in those countries that adopted the resolution.

Tattoo inks comprise water-insoluble pigments and various auxiliary ingredients, including solvents, emulsifiers and preservatives. For risk assessment, insoluble pigments should be distinguished from soluble ingredients because their biokinetics are expected to be completely different. Especially for insoluble pigments, crucial knowledge gaps currently exist that preclude full risk assessment for such chemicals. To establish an appropriate method for safety evaluations of the insoluble pigments used in tattoo inks, more insight into the fate of these pigments within the body is needed. Studies of the toxico-kinetics of a selected set of pigments after intra-cutaneous application in mini pig skin *in vivo* could provide the requisite information. In particular, the degree of pigment leakage from the tattooed skin site into the blood stream and transport of pigments to draining lymph nodes or other compartments must be elucidated. Also important to

determine are the degree of trans-epidermal loss of pigments and the timescale of pigment leakage to systemic circulation.

For insoluble pigments, no test system for deriving an appropriate NOAEL for MOS calculation can be recommended at present. For many toxicological endpoints, use of standard systems for chemical safety testing of insoluble tattoo pigments may require adaptations, and some systems may not be applicable at all. Only if more information is available about the kinetics of pigment leakage into the systemic circulation can selection and/or development of a test system for systemic toxicity by insoluble pigments become possible.

For soluble auxiliary ingredients present in tattoo inks (carrier fluids, preservatives, etc.), high absorption into the systemic circulation is expected, with only slight trans-epidermal loss. For these compounds, ready migration from the tattooed skin site is assumed (i.e. 100 % absorption into the body). For MOS calculations of soluble auxiliary ingredients, a sub-acute NOAEL for oral or intravenous routes can be used against exposure, as estimated for a pre-defined standard scenario.

In defining a standard scenario for making an MOS calculation for soluble ingredients (i.e. comparing the SED with the NOAEL), the figure of 0.025 ml/cm² as derived from the study reported by Engel *et al.* (2008) can be used tentatively in combination with the concentration used in the tattoo ink to estimate total exposure as a body dose in mg/kg bw. This value can be used to input into the MOS calculation. A further item needed for a standard MOS calculation is the surface area of the tattoo in cm². In practice, this factor is highly variable across consumers. Following the approach taken by the BfR, a normal case can be defined as a 600 cm² tattooed skin surface and a realistic worst-case scenario of 4 500 cm² tattooed skin. In general, the data used for the standard MOS calculation must be well-founded. Estimation of exposure for insoluble pigments requires study of the toxico-kinetics of a selected set of pigments after intra-cutaneous application in mini-pig skin *in vivo*.

From insoluble pigments, toxic photo-degradation products may be formed. Formation of potentially toxic degradation products should be evaluated on a case-by-case basis taking into account the chemical structure of the pigment and available data from the scientific literature. Any toxic photo-degradation products identified may need to be submitted to a separate risk assessment.

Finally, given the incomplete current knowledge on tattoo toxicological risk assessment, this document, and in particular Appendix 4 (which presents a tentative list of data requirements for safety evaluation), should be considered living documents that must be revised as new information becomes available and new insights are gained.

Bibliography

Adatto M (1993), Lebendige Haut – Schmucktatowierungen und Dermatologie. Editiones Roche, Basel, 1993.

Adatto MA, Halachimi S, Lapidoth M (2011), Tattoo removal. *Curr Probl Dermatol* 42: 97-110.

BfR (2009) Anforderungen für eine Sicherheitsbewertung von Tätowiermitteln. *Stellungnahme* Nr. 012/2009 des BfR vom 27. January 2009. www.bfr.bund.de/cm/206/anforderungen_fuer_eine_sicherheitsbewertung_von_taetowiermitteln.pdf (retrieved on 07-10-2015).

BfR (2011) Opinion number 044/2011 from 1 July 2011. Some tattoo colours contain carcinogenic PAH. www.bfr.bund.de/cm/349/some-tattoo-colours-contain-carcinogenic-pah.pdf (Retrieved on 07-10-2015).

Bode G, Clausing P, Gervais F, Loegsted J, Luft J, Nogues V, Sims J (2010), The utility of the minipig as an animal model in regulatory toxicology. *J Pharmacol Toxicol Methods* 62: 196–220.

Brendler-Schwaab S, Hartmann A, Pfuhler S, Speit G (2005), The *in vivo* comet assay: use and status in genotoxicity testing. *Mutagenesis* 20: 245–254.

CEN (2014), CEN creates new project committee on tattooing services. www.cen.eu/news/brief-news/Pages/NEWS-2014-019.aspx (retrieved on 15-09-2014).

Commission Directive 95/45/EC of 26 July 1995 laying down specific purity criteria concerning colours for use in foodstuffs. *Official Journal* L 226, 22/09/1995 p. 1.

Council of Europe (2009), Resolution ResAP (2008) 1 on requirements and criteria for the safety of tattoos and permanent make-up (superseding Resolution ResAP (2003) 2 on tattoos and permanent make-up) (Adopted by the Committee of Ministers on 20 February 2008 at the 1018th meeting of the Ministers' Deputies). PA/PH/COS (09) 2, Strasbourg, July 2009.

CTFA (1991), CTFA safety testing guidelines. Cosmetic, Toiletry and Fragrance Association, Inc. Washington, DC, 20036. Guidelines for Evaluating Photodermatitis.

Danish Environmental Protection Agency (2012), Chemical substances in tattoo ink, no. 116. Available at www.mst.dk (retrieved on 16-04-2014).

De Cuyper, C, Pérez-Cotapos ML (eds), Dermatological complications with body art: tattoos, piercing and permanent make-up. Springer Verlag, Heidelberg, 2010.

EMA (2010), Question and answers on the 'Note for guidance on photosafety testing'. European Medicines Agency, 24 June 2010. Committee for Medicinal Products for Human Use (CHMP). Document reference EMA/CHMP/SWP/336670/2010.

EMEA (1998), Photostability testing of new active substances and medicinal products (ICH Q1B) CPMP/ICH/279/95-ICH Q1B. www.ema.europa.eu/docs/en_GB/document_library/

Scientific_guideline/2009/09/WC500002647.pdf (retrieved on 07-10-2015).

EMEA (2002), Note for guidance on phototoxicity testing. European Agency for the Evaluation of Medicinal Products. www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003353.pdf (retrieved on 12-09-2014)

EMEA (2008), Concept paper on the need for revision of the Note for Guidance on Photosafety Testing (CPMP/SWP/398/01). Committee for Medicinal Products for Human Use (CHMP). Document reference EMEA/CHMP/SWP/534549/2007.

Engel E, Santarelli F, Vasold R, Maisch T, Ulrich H, Prantl L, König B, Landthaler M, Bäumler W (2008), Modern tattoos cause high concentrations of hazardous pigments in skin. *Contact Dermat* 58: 228–233.

Engel E, Vasold R, Santarelli F, Maisch T, Gopee NV, Howard PC, Landthaler M, Bäumler W (2010), Tattooing of skin results in transportation and light-induced decomposition of tattoo pigments – a first quantification *in vivo* using a mouse model. *Exp Dermatol* 19: 54–60.

Ferguson MWJ, Leigh IM, Wound healing. In: Textbook of dermatology, 6th edn (Champion RH, Burton JL, Burns DA, Breatnach SM, eds), Blackwell Science, London, 1998, pp 337–356.

Goldstein AP (1979), VII. Histologic reactions in tattoos. *J Dermatol Surg Oncol* 5: 896–900.

Gopee NV, Cui Y, Olson G, Warbritton AR, Miller BJ, Couch LH, Wamer WG, Howard PC (2005), Response of mouse skin to tattooing: use of SKH-1 mice as a surrogate model for human tattooing. *Toxicol Appl Pharmacol* 209: 145–158.

Høgsberg T, Hutton Carlsen K, Serup J (2013), High prevalence of minor symptoms in tattoos among a young population

tattooed with carbon black and organic pigments. *J Eur Acad Dermatol Venereol* 27: 846–852.

Høgsberg T, Loeschner K, Löf D, Serup J (2011), Tattoo inks of general usage contain nanoparticles. *Br J Dermatol* 165: 1210–1218.

Hutton Carlsen K, Serup J (2013), Photosensitivity and photodynamic events in black, red and blue tattoos are common: A "beach study". *J Eur Acad Dermatol Venereol* (doi 10.1111/jdv.121093).

Ichikawa H, Armstrong RB, Harber LC (1981), Photoallergic contact dermatitis in guinea pigs: improved induction technique using Freund's complete adjuvant. *J Invest Dermatol* 76: 498–501.

ICH S2R1: Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals intended for Human Use; Nov 2011 ICH.

ISO/FDIS (2009), Biological evaluation of medical devices – Part 10: tests for irritation and skin sensitization. International Standard ISO/FDIS 10993-10. Final draft, 2009.

JRC (Joint Research Centre) Science for policy report: Safety of tattoos and permanent make-up – Final report. Paola Piccinini, Sazan Pakalin *et al.* JRC 101601, EUR 27947 EN (2016) Available at: https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/safety-tattoos-and-permanent-make-final-report.

Kluger N (2008), Tatouages permanents: état de l'art en 2008 [Tattooing practices: state of the art in 2008]. *Images en Dermatologie* 1: 38–42.

Kluger N, Koljonen V (2012), Tattoos, inks and cancer. *Lancet Oncol* 13: 161–168.

Lea PJ, Pawlowski A (1987), Human tattoos. Electron microscopic assessment of epidermis, epidermal–dermal junction, and dermis. *Int J Dermatol* 26: 453–458.

Lehner K, Santarelli F, Penning R, Vasold R, Engel E, Maisch T, Gastl K, König B, Landthaler M, Bäumler W (2011), The decrease of pigment concentration in red tattooed skin years after tattooing. *J Eur Acad Dermatol Venereol* 25: 1340–1345.

Lerche CM, Sepehri M, Serup J, Poulsen T, Wulf HC (2015), Black tattoos protect against UVR-induced skin cancer in mice. *Photodermatol Photoimmunol Photomed* 31 (5).

Light HC, Hubert JJ, Josephy PD, Mattano SS, Weber WW (1987), Benzidine activation in the Ames test: roles of hepatic N-acetyltransferase and other cytosolic and microsomal factors. *Carcinogenesis* 8: 139–143.

Linsmeier Kilmer A, Fitzpatrick RE, Goldman MP, *et al.* (2008), Tattoo lasers. Online publication, updated 25 June 2008. Department of Dermatology/Department of Internal Medicine. University of California, Davis/San Diego. http://emedicine.medscape.com/article/1121212-overview (retrieved on 07-10-2015).

MacKie R (ed) (1989), Skin Cancer: an illustrated guide to the aetiology, clinical features, pathology and management of malignant cutaneous tumours. Martin Dunitz, Chicago, 1989.

Malinauskiena L, Contact allergy to textile dyes, clinical and experimental studies on disperse azo dyes. Thesis, Lund University, Sweden 2009.

Mann R, Klingmüller G (1981), Electron-microscopic investigation of tattoos in rabbit skin. *Arch Dermatol Res* 271: 367–372.

Maurer T, Hess R (1989), The maximization test for skin sensitization potential – updating the standard protocol and validation of a modified protocol. *Food Chem Toxicol* 27: 807–811.

OECD Guidelines for the Testing of Chemicals. Section 4: Health Effects. Each Test Guideline is available separately online: www.oecd-ilibrary.org/environment/

oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788 (retrieved on 24-08-2010).

Papameletiou, D., *et al.*, Recommendations for regulatory action in the EU on the safety of tattoos, body piercing and of related practices in the EU, 2003: JRC Final Report. p. 1–31.

Phillipson CE, Ionnanides C (1983), Activation of aromatic amines to mutagens by various animal species including man. *Mutat Res* 124 (3–4), 325–336.

Prival MJ, Bell SJ, Mitchell VD, Peiperl MD, Vaughan VL (1984), Mutagenicity of benzidine and benzidine-congener dyes and selected monazo dyes in a modified Salmonella assay. *Mutat Res* 136: 33–47.

Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No. 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Skin sensitisation, EC B.6. *Official Journal* L 142, 31/05/2008, p. 202.

Regulation (EC) No 1272/2008 of the European Parliament and the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. *Official Journal* L 353, 31/12/2008 p. 1.

Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast). *Official Journal* L 342, 22/12/2009 p. 59.

Reid TM, Wang CY, King CM, Morton KC (1984) Mutagenicity of some benzidine congeners and their N-acetylated and N,N′-diacetylated derivatives in different strains of Salmonella typhimurium. *Environ Mutagen* 6: 145–151.

SCCS (Scientific Committee on Consumer Safety), SCHER (Scientific Committee on Health and Environmental Risks),

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks) (2012), Opinion on use of the threshold of toxicological concern (TTC) approach for human safety assessment of chemical substances with focus on cosmetics and consumer products, 8 June 2012. SCCP/1171/08. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_092.pdf (retrieved on 07-10-2015)

SCCS (2016), The SCCS Notes of Guidance for Testing of Cosmetic Ingredients and Their Safety Evaluation, 9th revision. Adopted by the SCCS at its 11th plenary meeting of 29 September 2015. SCCS/1564/15. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_190.pdf.

Serup J, Carlsen KH (2014), Patch test study of 90 patients with tattoo reactions: negative outcome of allergy patch test to baseline batteries and culprit inks suggests allergen(s) are generated in the skin through haptenization. *Contact Dermat* (doi: 10.1111/cod.12271).

Sperry K (1992), Tattoos and tattooing. Part II: gross pathology, histopathology, medical complications, and applications. *Am J Forensic Med Pathol* 13: 7–17.

Sullivan TP, Eaglstein WH, Davis SC, Mertz P (2001), The pig as a model for human wound healing. *Wound Repair Regen* 9: 66–76.

Vasold R, Naarmann N, Ulrich H, Fischer D, König B, Landthaler M, Bäumler W (2004), Tattoo pigments are cleaved by laser light – the chemical analysis *in vitro* provide evidence for hazardous compounds. Photochem Photobiol 80: 185–190.

Vassileva S, Hristakieva E (2007), Medical applications of tattooing. *Clin Dermatol* 25: 367–374.

Appendix 1. **Resolution ResAP (2008) 1**

on requirements and criteria for the safety of tattoos and permanent make-up (superseding Resolution ResAP (2003) 2 on tattoos and permanent make-up)

Adopted by the Committee of Ministers on 20 February 2008 at the 1018th meeting of the Ministers' Deputies

The Committee of Ministers, in its composition restricted to the representatives of Austria, Belgium, Bulgaria, Cyprus, Finland, France, Germany, Ireland, the Netherlands, Norway, Portugal, Slovenia, Spain, Sweden, Switzerland and the United Kingdom, member states of the Partial Agreement in the Social and Public Health Field,

Recalling Resolution (59) 23 of 16 November 1959, on the extension of the activities of the Council of Europe in the social and cultural fields;

Having regard to Resolution (96) 35 of 2 October 1996 revising the above-mentioned partial agreement, whereby it revised the structures of the Partial Agreement and resolved to continue, on the basis of revised rules replacing those set out in Resolution (59) 23, the activities

hitherto carried out and developed by virtue of that resolution, these being aimed in particular at:

a. raising the level of health protection of consumers in its widest sense, including the making of a constant contribution to harmonising – in the field of products having a direct or indirect impact on the human food chain as well as in the fields of pesticides, pharmaceuticals and cosmetics – legislation, regulations and practices governing, on the one hand, quality, efficiency and safety controls for products, and, on the other hand, the safe use of toxic or noxious products;

b. integrating people with disabilities into the community; defining and contributing to the implementation, at a European level, of a model of coherent policy for people with disabilities, which takes account simultaneously of the principles of full citizenship and independent living; contributing to the elimination of barriers to people's integration whatever their nature, whether psychological, educational, family-related, cultural, social, professional, financial or architectural;

Having regard to the action carried out for several years for the purpose of harmonising their legislation, in particular with a view to promoting consumer health as regards the use of cosmetic products;

Considering the increasing popularity of body adornment through tattoos or permanent make-up (PMU);

Considering that tattoos and PMU may pose a risk to human health due to microbiological contamination and/or the presence of harmful substances in the products used for tattoos and PMU and/or the possibility of being tattooed under questionable hygienic conditions;

Considering that colorants not restricted by this resolution have not been evaluated for safe use in tattoos and PMU by an independent scientific body;

Considering that risk assessment is an essential part of the decision-making process on preventive measures aimed at protecting public health;

Taking into account the fact that in most member states tattoos, tattooing and PMU are covered neither by specific national nor European Community regulations;

Aware of the need to fill this gap in legislation and thus to adopt specific legislation on the composition of the products used for tattoos and PMU and the assessment of their safety, including in particular the harmonisation of methods for the analytical determination of possibly harmful substances in colorants, and ensuring that practices for tattoos and permanent make-up are carried out under appropriate hygienic conditions;

Considering the fact that implementing specific legislation on tattoos and PMU may have a substantial positive impact on health risks related to product quality;

Taking the view that each member state, faced with the need to introduce regulations governing this matter, would find it beneficial for such regulations to be harmonised at European level;

Considering that this resolution follows a negative list approach by listing the substances which must not be used in tattooing products and PMU, based on current knowledge in this field;

Considering further that using a negative list-approach is only a first step towards ensuring that hazardous substances are avoided,

Recommends that the governments of the member states of the Partial Agreement in the Social and Public Health Field:

- take into account in their national laws and regulations on tattoos and PMU the principles set out thereafter in the appendix to this resolution, in particular on the composition of tattoos and PMU, and modes and criteria of the safety assessment with a view to public health protection;
- regulate the use of substances in tattoos and PMU by taking steps towards establishing – on the basis of safety assessments carried out by the competent bodies and harmonised at European level – an exhaustive list of substances proved safe for this use under certain conditions ("positive list").

Each government remains free to impose stricter regulations.

Appendix to Resolution ResAP (2008) 1

1. Field of application

This resolution applies to:

- the composition and labelling of products used for tattoos and PMU;
- the risk evaluation required before products used for tattoos and PMU are placed on the market;
- the conditions of the application of tattoos and PMU;
- the obligation to inform the public and the consumer of the health risks of tattoos and PMU and tattooing practices.

2. Definitions

Tattooing is a practice whereby a permanent skin marking or design (a "tattoo") is administered by intradermal injection of products consisting of colorants and auxiliary ingredients.

"Colorant" is the commonly used denomination for pigments, lakes and dyes that are coloured molecules. Pigments are in general very poorly soluble in water and application media, and unlike most dyes, they have low solubility in organic solvents. For this reason they remain essentially in the solid state, including in live tissues. Dyes are organic molecules that are soluble in general. Certain substances like titanium dioxide (TiO_2) or barium sulphate ($BaSO_4$) can be used as carriers for dyes used in tattoos, thereby forming "lakes" which are insoluble in water.

Auxiliary ingredients are necessary to obtain ready-to-use tattooing products. They are of different kinds like solvents, stabilisers, "wetting agents", pH-regulators, emollients and thickeners.

A permanent make-up (PMU) consists of colorants and auxiliary ingredients which are injected intradermally for the purposes of enhancing the contours of the face.

"Sterile" in this context means the absence of viable organisms, including viruses.

3. Specifications

3.1. When applied and used as intended, tattoo and PMU products must not endanger the health or safety of persons or the environment. To this end, the manufacturer or person responsible for placing the product on the market should perform a risk evaluation based on recent toxicological data and knowledge. This evaluation should be set out in a file which is readily available to the competent authorities.

3.2. Notwithstanding, and in addition to the requirements set out in paragraph 3.1, tattoo and PMU products must only be used if they comply with all the following requirements:

- they do not contain or release the aromatic amines listed in Table 1 of this appendix in concentrations that are technically avoidable according to good manufacturing procedures; the presence or release of these aromatic amines should be determined by using appropriate test methods which should be harmonised across the member states in order to ensure comparable health protection of the consumer and to avoid divergent enforcement, drawing on existing methods which can serve as models (see Tables 4.a-c);
- they do not contain the substances listed in Table 2 of this appendix;
- they do not contain substances listed in Directive 76/768/EEC (Annex II);
- they do not contain substances specified in Directive 76/768/EEC (Annex IV, columns 2 to 4);
- they do not contain carcinogenic, mutagenic and reprotoxic substances of categories 1, 2 or 3 which are classified under Directive 67/548/EEC;
- they comply with maximum allowed concentrations of impurities listed in Table 3 and the minimum requirements for further

organic impurities for colorants used in foodstuffs and cosmetic products as set out in Directive 95/45/EEC;

- they are sterile and supplied in a container which maintains the sterility of the product until application, preferably in a packaging size appropriate for single use. In case multi-use containers are used, their design should ensure that the contents will not be contaminated during the period of use;
- preservatives should only be used to ensure the preservation of the product after opening and by no means as a correction of insufficient microbiologic purity in the course of manufacture and of inadequate hygiene in tattooing and PMU practice;
- preservatives should only be used after a safety assessment and in the lowest effective concentration.

3.3. Tattoo and PMU products should contain the following information on the packaging:

- the name and address of the manufacturer or the person responsible for placing the product on the market;
- the date of minimum durability ;
- the conditions of use and warnings;
- the batch number or other reference used by the manufacturer for batch identification;
- the list of ingredients according to their International Union of Pure and Applied Chemistry (IUPAC) name, CAS number (Chemical Abstract Service of the American Chemical Society) or Colour Index (CI) number;
- the guarantee of sterility of the contents.

3.4. Tattooing and the application of PMU – including treatment and maintenance of the instruments, in particular their sterilisation and disinfection – must be carried out by the tattooist in conformity with the hygiene regulations laid down by national public health services.

4. Data for the safety assessment of substances which are used in tattoos and PMU

In order to ensure the use of only safe substances in tattoos and PMU, the competent authorities should evaluate specific safety data as set out below, with a view to excluding the use of harmful substances and to establishing gradually and publishing a list of substances shown to be safe in use. Priority should be given to the evaluation of colorants.

In doing so, the competent authorities may use amongst other sources the files which manufacturers are required to keep readily available to the authorities in accordance with paragraph 3.1 of this appendix and should exchange relevant data and conclusions.

Manufacturers should be encouraged to make data on the composition of the product and on the toxicology of the substances available to the competent authorities.

The competent authorities should continuously take steps towards establishing an exhaustive positive list of safe substances with a view to replacing negative lists of harmful substances. Pending the achievement of this goal, authorities should set up and publish non-exhaustive lists of substances shown to be safe in use.

Pigments forbidden in tattoos and PMU included in Table 2 of this appendix or Annex IV, columns 2 to 4 of Directive 76/768/EEC, but relevant for producers may be included in national positive lists if their safety is proven on the basis of additional data obtained under conditions of use in tattoos and PMU.

Safety data required for the assessment of substances used in tattoos and PMU

- Data on physico-chemical properties:
  - purity;
  - impurities (heavy metals, amines, etc.);
  - auxiliary ingredients;
  - stability (UV, laser, enzymes, bacteria);

– cleavage products (aromatic amines, etc.).

– Toxicological data:

– corrosion;

– irritation (skin, mucous membranes);

– phototoxicity;

– immunotoxicity (sensitisation, photo-sensitisation, etc.);

– genotoxicity in vitro including test of cleavage products; photo-genotoxicity.

Additionally:

– further relevant data or tests in agreement with competent authorities.

Toxicological data for safety assessment should be obtained from test methods using guidelines whenever they exist (for example, Organisation for Economic Co-operation and Development, European Union).

5. Public information

5.1. Governments should issue regulations constituting the legal basis for the information obligations incumbent upon the various players concerned. In this context, the tattooist should necessarily provide the consumer with complete, reliable and comprehensible information on the risks entailed by those practices, including the potential occurrence of sensitisation, care following the application of a tattoo, reversibility and removal of tattoos, and the advice of consulting a physician in case of medical complications.

5.2. Potential consumers should be provided with reliable and evidence-based information about the risks of tattooing or PMU by all appropriate means, for example, through mass information campaigns or via the Internet.

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

CAS number*	EC-number	Substances
293733-21-8		6-amino-2-ethoxynaphthaline
		4-amino-3-fluorophenol
60-09-3		4-aminoazobenzene
97-56-3	202-591-2	o-aminoazotoluene
90-04-4	201-963-1	o-anisidine
92-87-5	202-199-1	Benzidine
92-67-1	202-177-1	Biphenyl-4-ylamine
106-47-8	203-401-0	4-chloroaniline
95-69-2	202-411-6	4-chloro-o-toluidine
91-94-1	202-109-0	3,3'-d-dichlorobenzidine
119-90-4	204-355-4	3,3'-dimethoxybenzidine
119-93-7	204-358-0	3,3'-dimethylbenzidine
120-71-8	204-419-1	6-methoxy-m-toluidine
615-05-4	210-406-1	4-methoxy-m-phenylenediamine
101-14-4	202-918-9	4,4'-methylenebis(2-chloroaniline)
101-77-9	202-974-4	4,4'-methylenedianiline
838-88-0	212-658-8	4,4'-methylenedi-o-toluidine
95-80-7	202-453-1	4-methyl-m-phenylenediamine
91-59-8	202-080-4	2-naphtylamine
99-55-8	202-765-8	5-nitro-o-toluidine

* Chemical Abstract Service of the American Chemical Society.

CAS number*	EC-number	Substances
Other substances classified as carcinogens in Categories 1, 2, and 3 by the European Commission and mentioned in Council Directive 1967/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances		
101-80-4	202-977-0	4,4′-oxydianiline
106-50-3	2003-404-7	Para-phenylenediamine
139-65-1	205-370-9	4,4′-thiodianiline
95-53-4	202-429-0	o-toluidine
137-17-7	205-282-0	2,4,5-trimethylaniline
87-62-7		2,6-xylidine
95-68-1		2,4-xylidine

* Chemical Abstract Service of the American Chemical Society.

Table 2. Non-exhaustive list of substances, particularly with regard to their carcinogenic, mutagenic, reprotoxic and/or sensitising properties, which tattoo and PMU products should not contain (BC/CEN/97/29.11)

CI Name*	CAS Number †	CI Number
Acid Green 16	12768-78-4	44025
Acid Red 26	3761-53-3	16150
Acid Violet 17	4129-84-4	42650
Acid Violet 49	1694-09-3	42640
Acid Yellow 36	587-98-4	13065
Basic Blue 7	2390-60-5	42595
Basic Green 1	633-03-4	42040
Basic Red 1	989-38-8	45160
Basic Red 9	569-61-9	42500

* Colour index.
† Chemical Abstract Service of the American Chemical Society.

CI Name*	CAS Number †	CI Number
Basic Violet 1	8004-87-3	42535
Basic Violet 10	81-88-9	45170
Basic Violet 3	548-62-9	42555
Disperse Blue 1	2475-45-8	64500
Disperse Blue 106	12223-01-7	
Disperse Blue 124	61951-51-7	
Disperse Blue 3	2475-46-9	61505
Disperse Blue 35	12222-75-2	
Disperse Orange 3	730-40-5	11005
Disperse Orange 37	12223-33-5	
Disperse Red 1	2872-52-8	11110
Disperse Red 17	3179-89-3	11210
Disperse Yellow 3	2832-40-8	11855
Disperse Yellow 9	6373-73-5	10375
Pigment Orange 5	3468-63-1	12075
Pigment Red 53	2092-56-0	15585
Pigment Violet 3	1325-82-2	42535:2
Pigment Violet 39	64070-98-0	42555:2
Solvent Blue 35	17354-14-2	61554
Solvent Orange 7	3118-97-6	12140
Solvent Red 24	85-83-6	26105
Solvent Red 49	509-34-2	45170:1
Solvent Violet 9	467-63-0	42555:1
Solvent Yellow 1	60-09-3	11000
Solvent Yellow 2	60-11-7	11020
Solvent Yellow 3	97-56-3	11160

* Colour index.
† Chemical Abstract Service of the American Chemical Society.

Table 3. Maximum allowed concentrations of impurities in products for tattoos and PMU

Element or compound	ppm	ppb
Arsenic (As)	2	
Barium (Ba)	50	
Cadmium (Cd)	0.2	
Cobalt (Co)	25	
Chromium (Cr) (VI)*	0.2	
Copper (Cu) soluble †	25	
Mercury (Hg)	0.2	
Nickel (Ni) ‡	As low as technically achievable	
Lead (Pb)	2	
Selenium (Se)	2	
Antimony (Sb)	2	
Tin (Sn)	50	
Zinc (Zn)	50	
Policyclic aromatic hydrocarbons (PAH)	0.5	
Benzene-a-pyrene (BaP)		5

* The presence of traces of chromium (VI) in products for tattoos and PMU should be mentioned on the package together with a warning (for example, "Contains chromium. Can cause allergic reactions.").

† Soluble copper should be determined after extraction to an aqueous solution with pH 5.5.

‡ The presence of traces of nickel in products for tattoos and PMU should be mentioned on the package together with a warning (for example, "Contains nickel. Can cause allergic reactions.").

Tables 4.a-c. Methods which can serve as models for harmonising test methods

1. Summary of the method provided by the Dutch Food and Consumer Product Safety Authority

Table 4a. Determination of aromatic amines in tattoos and permanent make-up using GC-MS (SIG01-ND428)

1. Principle	This procedure describes a method* for determination of aromatic amines in tattoo and permanent make-up. It is derived from method EN 14362-1 for textile products. The method is validated for aniline, o-toluidine, o-anisidine, p-chloraniline, 4-chloro-o-toluidine, 2,4-diaminotoluene, 2-naphtylamine, 2-amino-4-nitrotoluene and 3,3'-dichloro-benzidine. Azo-dyes are characterised by a structure containing an azo-unit (-N=N-) which splits off aromatic amines. In this method, azo-dyes are reduced to release primary aromatic amines using sodium dithionite. The aromatic amines are then extracted with t-butylmethylether and analysed with GC-MS.
2. Operating procedures	
2.1. Preparation	Tattoo colorants and PMUs: homogenise the sample by shaking or mixing with a spatula.
2.2. Extraction	Weigh 500 mg sample in a tube. Add 5 ml dithionite solution (5 %) in phosphate buffer. Mix with a vortex mixer for 20 seconds. Place the tubes in a water bath at 70 °C for 90 minutes. After 30 minutes, mix the solution again with a vortex mixer. Cool the solution to room temperature. Add 5 ml internal standard solution. Mix the extract for 20 seconds with a vortex mixer. Centrifuge the tube at 2 500 g for 15 minutes. Filtrate the upper layer using a microfilter and put the extract in a vial.

* Report of the Dutch Food and Consumer Product Safety Authority.

2.3. Screening and quantification	Perform a screening with GC-MS by comparing the spectra of the peaks in the extract with a library. Positive samples are quantified in SIM-mode using calibration standards. For calculation an internal standard is used.
3. Validation	
Overview of validation data	See Analysis of aromatic amines in tattoos and permanent make-up by GC-MS in Table 4.b.

* Report of the Dutch Food and Consumer Product Safety Authority.

Table 4.b – Analysis of aromatic amines in tattoos and PMU by GC-MS (Matrix: tattoo products)

Component	Aniline	o-anisidine	4-chloro-o-toluidine	2,4-diamino-toluene	2-naphtyl-amine	2-amino-nitro-toluene	3,3'-dichlor-benzidine	o-toluidine	p-chlor-aniline	Benzidine
$C_{Detection\ limit}$ (mg/kg)	1.5	1.8	2.5	1.6	2.6	1.7	1.4	0.9	2.0	1.5
$C_{Determination\ limit}$ (mg/kg)	3.0	3.6	5.0	3.2	5.2	3.4	2.8	1.8	4.0	3.0
Working range of the method (mg/kg)	0-250	0-250	0-250	0-250	0-250	0-250	0-250	0-250	0-250	50-750
Recovery (%)	97.5	96.4	108.5	65	114.2	101.1	100.8	102.0	111.1	91.6
RSDr within working range (n=--)	5.2	5.8	9.1	3.5	5.6	5.6	4 6	3 1	7.5	9.4

2. Summary of the method provided by the Swiss Federal Office of Public Health included in the report on analysis of tattoo and PMU inks collected on the Swiss market in 2005

Table 4.c – Determination of aromatic amines in tattoos and PMU with LC/MS

1. Principle	The method is based on EN 71-7:2002.* The azo-compounds are reduced to release primary aromatic amines using sodium dithionite.
2. Operating procedures	
2.1. Sample preparation for aromatic amines as impurities	50 µl of tattoo ink are weighed into a HPLC vial. 1 ml of 0.07 M hydrochloric acid are added and the solution vortexed thoroughly for one minute. The sample solution is then sonicated for 15 minutes in an ultrasonic bath at room temperature and filtered through a 0.2 µm syringe filter into an HPLC glass vial. 5 µl of this solution are injected.
2.2. Sample preparation for aromatic amines after reductive cleavage	Reductive cleavage is performed according to EN 71-7:200211 with sodium dithionite. Instead of 1 g of sample, only 50 mg are used. Amounts of reagents are adapted proportionally. After reductive cleavage, samples are diluted with methanol and sonicated for 15 minutes. Afterwards extracts are filtered through a 0.2 µm syringe filter and 2 µl are injected without further clean-up.
2.3. HPLC analysis	For aromatic amines: HPLC/MS analysis is performed according to note †.
3. Additional information	Additional information is included in Hauri *et al.*, 2005.

* EN 71-7: 2002 Safety of toys – Part 7: Finger paints – requirements and test methods.

† Hauri U., Lütolf, B., Schlegel U. and Hohl C., Determination of carcinogenic aromatic amines in dyes cosmetics, finger paints and inks for pens and tattoos with LC/MS. *Mitt. Lebensm. Hyg.* 2005; 06: 321-335.

Appendix 2. **Pigments found on the market in Europe between 2006 and 2013**

Summary of pigments reported to be present in ready-to-use preparations on the European market between 2006 and 2013 are presented on the following pages. The table displays data collected from surveillance activities in the Netherlands, Germany, Denmark, Norway and Switzerland.

Pigments listed with a shaded background are prohibited according to Council of Europe Resolution ResAP (2008) 1.

CI Number*	CAS-Number	CI Name	Chemical Family	Presence in the market				
				NL[a]	D[b]	DK[c]	NO[d]	CH[e]
11680	2512-29-0	Pigment yellow 1	Mono-azo	5	Yes	0	0	8
11710	6486-23-3	Pigment Yellow 3	Mono-azo	1	No	0	0	5
11740	6528-34-3	Pigment Yellow 65	Mono-azo	0	No	4	2	6
11741	6358-31-2	Pigment Yellow 74	Mono-azo	3	Yes	2	6	24
11767	12225-18-2	Pigment Yellow 97	Mono-azo	37	Yes	0	0	18
12075	3468-63-1	Pigment Orange 5	Mono-azo	0	No	2	2	1
12085	2814-77-9	Pigment Red 4	ß-Naphthol	25	Yes	0	0	4
12315	6448-95-9	Pigment Red 22 (naphthol red)	Naphthol AS	0	No	0	0	1
12370	6535-46-2	Pigment Red 112	Naphthol AS	1	Yes	0	2	5
12385	6410-32-8	Pigment Red 12	Mono-azo	–	–	–	–	3
12390	655-84-1	Pigment Red 17	Mono-azo	0	No	1	0	–
12466	67990-05-0	Pigment Red 269	Mono-azo	5	Yes	0	3	–

* CI Number – colour index according to the reference database of Colour Index International for colourants.
a The Netherlands, 2006. Number of products containing pigment out of 402 tested products.
b Gemany (Berlin) 2006/2007. Indication of the presence (Yes) and non-presence (No) of the pigment on the market.
c Denmark, 2012. Number of products containing pigment out of 49 tested products
d Norway, 2013. Number of products containing pigment out of 51 tested products.
e Switzerland, 2008/2012: Number of products containing pigment out of 416 tested products.

CI Number*	CAS-Number	CI Name	Chemical Family	Presence in the market				
				NL[a]	D[b]	DK[c]	NO[d]	CH[e]
12470	6358-48-1	Pigment Orange 22	Naphthol AS	2	No	0	0	–
12475	2786-76-7	Pigment Red 170/120	Naphthol AS	27	Yes	2	2	30
12477	61932-63-6	Pigment Red 210	Naphthol AS	3	No	7	3	10
12485	5280-68-2	Pigment Red 146	Naphthol AS	1	No	2	0	3
12490	6410-41-9	Pigment Red 5	Naphthol AS	81	Yes	2	0	17
12510	6992-11-6	Pigment Brown 25	Benzimidazolone	4	Yes	0	0	3
13980	31837-42-0 61036-28-0	Pigment Yellow 151	Benzimidazolone	3	No	2	6	7
15580	5850-87-3	Pigment Red 51	Mono-azo	–	–	–	–	1
15585	2092-56-0	Pigment Red 53	beta-Naphthol	–	–	–	–	3
15630	1248-18-8	Pigment Red 49	beta-naphthol, Na	0	Yes	0	0	–
15850:1	5281-04-9	Pigment Red 57:1	Mono-azo	24	Yes	0	0	3
15860	17852-99-2	Pigment Red 52:1	BONA, Ca	0	Yes	0	0	–

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CI Number*	CAS-Number	CI Name	Chemical Family	Presence in the market				
				NL[a]	D[b]	DK[c]	NO[d]	CH[e]
15880	6417-83-0	Pigment Red 63:1	Mono-azo	0	No	1	0	–
16035	25956-17-6	Food Red 17	Mono-azo	25	Yes	0	0	–
19140	1934-21-0	Acid Yellow 23	Mono-azo	0	Yes	0	0	–
20195	5850-16-8	Acid Brown 14	Di-azo	0	No	2	0	–
21090	6358-85-6	Pigment Yellow 12	Diarylide Y	–	–	–	–	4
21095	5468-75-7	Pigment Yellow 14	Diarylide Y	21	No	2	3	11
21108	5567-15-7	Pigment Yellow 83	Diarylide Y	11	Yes	2	4	3
21110	3520-72-7	Pigment Orange 13	Disazopyrazolone	18	Yes	3	4	14
21115	15793-73-4	Pigment Orange 34	Disazopyrazolone	0	No	0	0	2
21160	6505-28-8	Pigment Orange 16	Diarylide Y	7	Yes	2	4	3
21290	77804-81-0	Pigment Yellow 180	Benzimidazolone	26	No	0	0	–
22095	6375-58-2	Direct Red 53	Di-azo	1	No	0	0	–

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CI Number*	CAS-Number	CI Name	Chemical Family	Presence in the market				
				NL[a]	D[b]	DK[c]	NO[d]	CH[e]
42090	3844-45-9	Acid Blue 9	Triphenylmethane	3	No	0	0	–
45000	2465-29-4	Acridine red	Xanthene	0	Yes	0		–
45160	989-38-8	Basic Red 1	Xanthene	–	–	–	–	1
45170	81-88-9	Basic Violet 10	Xanthene	0	No	0	0	1
47005	8004-92-0	Acid Yellow 3	Quinoline	3	Yes	0	0	1
51319	6358-30-1	Pigment Violet 23	Oxazine	14	Yes	0	1	21
51345	17741-63-8	Pigment Violet 37	Oxazine	–	–	–	–	3
56110	84632-65-5	Pigment Red 254	Diketopyrrolopyrrol	–	–	–	–	13
56117:0	84632-59-7	Pigment Orange 73	Pyrrole	18	Yes	0	0	19
56300	30125-47-4	Pigment Yellow 138	Quinolone	–	–	–	–	8
61570	4403-90-1	Acid Green 25	Anthraquinone	3	No	0	0	–
71105	4424-06-0	Pigment Orange 43	Perinone	3	No	0	0	–

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CI Number*	CAS-Number	CI Name	Chemical Family	Presence in the market				
				NL[a]	D[b]	DK[c]	NO[d]	CH[e]
73015	860-22-0	Acid Blue 74	Indigoid	14	No	0	0	–
73360	2379-74-0	Pigment Red 181	Thioindigo	0	No	0	0	6
73900	1047-16-1	Pigment Violet 19	Quinacridone	5	No	1	1	4
73907	3089-17-6	Pigment Red 202	Quinacridone	–	–	–	–	3
73915	16043-40-6 980-26-7	Pigment Red 122	Quinacridone	14	Yes	2	5	23
74160	147-14-8	Pigment Blue 15	Cu Phthalocyanine	95	Yes	7	15	66
74180	1330-38-7	Direct Blue 86	Cu Phthalocyanine	0	Yes	0	0	0
74260	1328-53-6	Pigment Green 7	Cu Phthalocyanine	34	Yes	6	2	30
74265	14302-13-7	Pigment Green 36	Cu Phthalocyanine	19	Yes	0	5	8
75470	1390-65-4 1260-17-9	Natural Red 4	Natural dye	6	Yes	0	0	–
77007	1317-97-1	Pigment Blue 29	Inorganic	24	Yes	0	0	–

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CI Number*	CAS-Number	CI Name	Chemical Family	Presence in the market				
				NL[a]	D[b]	DK[c]	NO[d]	CH[e]
77266	7440-44-0	Pigment Black 6 & 7	Graphite	74	Yes	4	15	–
77267	8021-99-6	Pigment Black 9	Charcoal bone	1	No	0	0	–
77268:1	1339-82-8	Coke black	Carbon	0	Yes	0	0	–
77288	1308-38-9	Pigment Green 17	Chromium oxide	0	Yes	0	0	–
77489	1345-25-1	ferrous oxide black	Iron oxide	26	No	0	0	–
77491	1309-37-1	Pigment Red 101	Iron oxide	64	Yes	1	1	–
77492	51274-00-1	Pigment Yellow 42	Iron oxide	39	No	0	0	–
77499	12227-89-3	Pigment Black 11	Iron oxide	36	No	0	0	–
77742	10101-66-3	Pigment Violet 16	inorganic	24	Yes	0	0	–
77891	13463-67-4	Pigment White 6	Titanium dioxide	234	Yes	22	27	–

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c Denmark, 2012. Number of products containing pigment out of 49 tested products
d Norway, 2013. Number of products containing pigment out of 51 tested products.
e Switzerland, 2008/2012: Number of products containing pigment out of 416 tested products.

Appendix 3. **Electron microscopy of tattoo pigment particles in stock ink product and *in vivo* in a tattoo**

Figure 1. Stock ink product

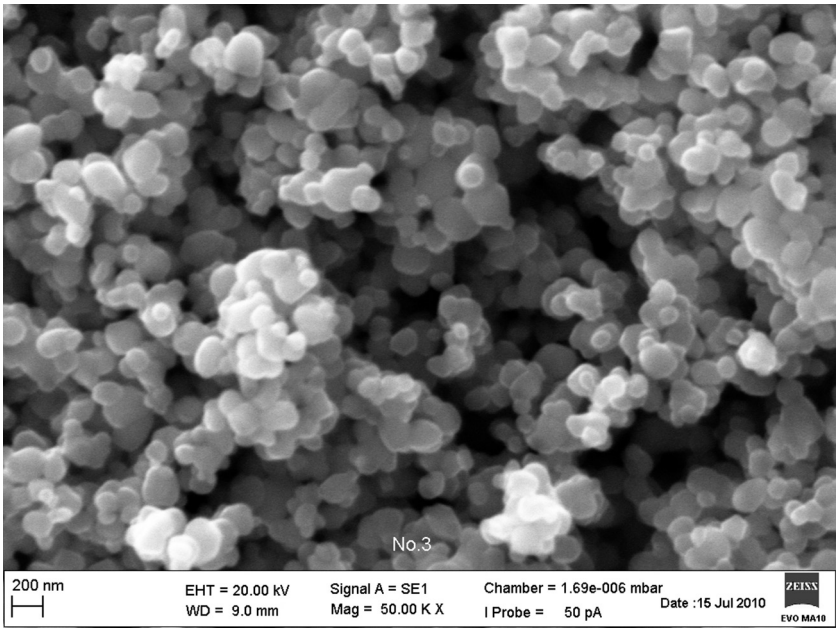
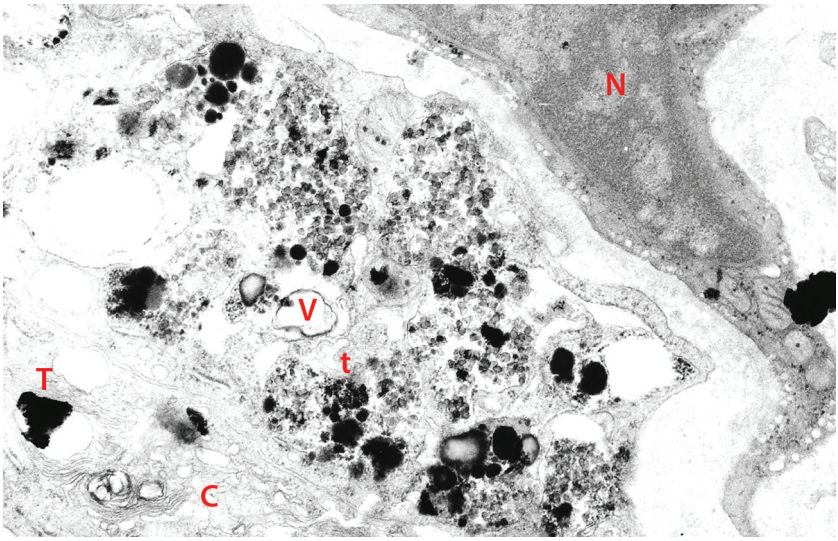


Figure 1 shows a scanning electron micrograph of stock ink showing pigment particles, with primary particles sized 100–200 nm, and spontaneous formation of aggregates of particles forming larger clusters. Dye chemicals in particles are not free molecular forms and not accessible for release and 'local metabolism and distribution'. The physical organisation of these particulate bodies is responsible for colour characteristics as well as robustness over time.

Courtesy of K. Ståhl, Danish Technical University, Department of Chemistry, Lundtofte, Denmark.

Figure 2. Tattoo pigment *in vivo*, sample from a tattooed human volunteer



V: vesicle
N: nucleus
C: cytoplasm
T: Black tattoo pigment
t: digested black tattoo pigment

Figure 2 shows a transmission electron micrograph from macrophages showing intracellular tattoo pigments of variable sizes and densities

located in relation to endoplasmic vesicles. The cell nucleus is seen in the upper right part of the image.

Courtesy of T. Kaobayasi, Bispebjerg University Hospital, Copenhagen, Denmark.

Appendix 4. **Toxicological testing methods applicable for safety evaluation of ingredients of tattooing colourants/products**

Toxicological endpoint	Method	Result	Conclusion
Mutagenicity/ genotoxicity (see 3.2)	OECD 471: Bacterial Reverse Mutation Test in combination with either OECD 473: *In vitro* Mammalian Chromosome Aberration Test, OECD 476: *In vitro* Mammalian Cell Gene Mutation Test or OECD 487: *In vitro* Mammalian Cell Micronucleus Test	negative	+
		positive	o
	OECD 474: *In vivo* Mammalian Erythrocyte Micronucleus Test	negative	+
		positive	–
Skin irritation* (see 3.3.1)	Intra-cutaneous Reactivity test (ISO/FDIS 2009)	negative	+
		positive	–
Irritation to mucous membranes* (see 3.3.2)	OECD 405: Acute Eye Irritation/Corrosion	negative	+
		positive	–

Toxicological endpoint	Method	Result	Conclusion
Sensitisation (see 3.3.4)	OECD 406: Skin Sensitisation, Guinea Pig Maximisation Test (GPMT) OECD 429: Skin Sensitisation, Local Lymph Node Assay (LLNA)	negative	+
		positive	–
Photo-toxicity † (see 3.3.5)	OECD 432: *In vitro* 3T3 NRU Photo-toxicity Test	negative	+
		positive	–
Carcinogenicity ‡ (see 3.7.1)	OECD 451: Carcinogenicity Studies OECD 453: Combined Chronic Toxicity/ Carcinogenicity Studies	negative	+
		positive	–
Reproductive toxicity (see 3.7.2)	OECD 414: Prenatal Development Toxicity Study OECD 416: Two-Generation Reproduction Toxicity	negative	+
		positive	–

Key

+ No concern for use in tattooing products with respect to the end point tested.

– Substance not recommended for use in tattooing products.

o Testing using recommended *in vivo* method is required for further evaluation.

* Substances with pH < 5 or > 9 are not recommended for tattooing products.
† For substances capable of absorbing UV or visible light in the range of 290-700 nm.
‡ Tests on carcinogenicity may be necessary in specific cases, especially for non-genotoxic carcinogens.

Other data generated from human studies or other validated methods may be considered in a risk assessment. If they show toxic substance properties, these data may suffice to support that the substance cannot be recommended for use in tattoo inks. Otherwise, the methods listed above have to be applied.

To evaluate systemic exposure to soluble tattoo substances and/or contaminants present in the formulation through migration from tattooed skin, the following studies are needed:

Toxicological endpoint	Method	Result
Repeated dose toxicity* (see 3.5)	OECD 407: Repeated Dose 28-day Oral Toxicity Study in Rodents	NOAEL
	OECD 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents,	
	OECD 409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents	
Acute toxicity † (see 3.8)	OECD 420: Acute Oral Toxicity – Fixed Dose Procedure	LD_{50}
	OECD 423: Acute Oral toxicity – Acute Toxic Class Method	
	OECD 425: Acute Oral Toxicity: Up-and-Down Procedure	

* It has to be considered that to date, no harmonised models exist for calculation of the MOS for ingredients of tattooing products.

† Substances classified fatal, toxic or harmful according to Globally Harmonized System of Classification and Labelling of Chemicals (GHS) should not be allowed in tattooing colourants.

While nowadays intradermal injection of inks for tattoos and permanent make-up (PMU) has become common among a considerable part of the population, the practice carries inherent risks. Cases of tattoo inks becoming contaminated microbiologically are well known and the toxicological risk assessment of tattoos and PMU presents further challenges, because of their specific routes of exposure and their chemical and physical compositions. Many substances, impurities and contaminants present in inks may have harmful effects on human health, either as single ingredients or by interaction. In addition, the fact that the typical ink may consist of up to 15 different substances makes the problem of control even more complex.

This scientific publication supplements Committee of Ministers Resolution ResAP (2008) 1 on requirements and criteria for the safety of tattoos and permanent make-up. It aims at facilitating the work of national authorities concerned with risk assessment and provides support to ink manufacturers in assessing the specific risks of their products.

ENG

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