Bundesinstitut für Risikobewertung

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Tattoo inks: minimum requirements and test methods

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Tattoo inks contain pigments and additives. According to the provisions of the German Food, Consumer Goods and Feed Code (Lebensmittel-, Bedarfsgegenstände- und Futtermittelgesetzbuch, LFGB), tattoo inks may not be used if there is any doubt as to their safety to health. Substances or mixtures for tattooing purposes are regulated in the REACH Regulation [entry 75 of Annex XVII of the REACH Regulation (Regulation (EC) No 1907/2006)]. However, there are as yet no binding criteria according to which a safety assessment of tattoo inks should be carried out. There is also a lack of suitable test methods and data for a health risk assessment. For example, little is known about adverse effects that may be associated with the injection of tattoo inks into the skin or about possible effects that may be induced in other organs. Therefore, the German Federal Institute for Risk Assessment (BfR) has developed minimum requirements for tattoo inks as well as test methods for manufacturers and distributors who are primarily responsible for the safety of their products.

Test methods are already available for the minimum toxicological and analytical requirements presented, so that they can be applied immediately. In addition, the BfR indicates requirements for which further research is necessary or methods would have to be developed, i.e., which are therefore not yet operable at present.

The BfR minimum requirements presented below are intended to mark the basis for a future health risk assessment of tattoo inks and the start of a comprehensive consultation process with all stakeholders from science, industry, politics and the public. As pigments form the main component of tattoo inks, they are particularly important for the health risk assessment for the protection of consumers. Therefore, the BfR has particularly considered pigments in this statement. The Institute expects that already by applying the operable minimum requirements described here, the level of protection of tattooed persons can be significantly increased compared to the current situation.

On the one hand, the minimum requirements are intended to help identify tattoo pigments that are not suitable for tattoos. On the other hand, the use of pigments that meet the minimum toxicological requirements will reduce potential health risks according to the current state of science and technology. However, due to a lack of data, the BfR does not currently make any recommendations for use.

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1 Introduction

Tattooing means the injection of formulations of colouring substances into the skin or mucous membranes to affect appearance. To protect consumers, tattooing substances are regulated. In addition to the provisions of the German tattoo inks Ordinance[1], the REACH Regulation [entry 75 of Annex XVII of the REACH Regulation (Regulation (EC) No 1907/2006)], abbreviated as REACH-VO[2], applies to substances in tattoo inks and permanent make-up at European level since 14 December 2020. This regulation restricts the use of substances with known and suspected adverse health effects. It sets maximum concentrations for these substances in tattoo inks.

[1] Ordinance on tattooing preparations including certain comparable substances and preparations of substances (Tattooing Preparations Ordinance), https://www.gesetze-im-internet.de/t_tov/BJNR221500008.html.
[2] COMMISSION REGULATION (EU) 2020/2081 of 14 December 2020 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards substances in tattooing ink or permanent make-up.

For the purpose of understanding, the BfR has defined the following terms:

Terms and explanations

- **Tattoo inks** are defined in Section 4(1)(3) of the German Foodstuffs, Commodities and Feedstuffs Code.
- **Tattoo pigments** are the coloring components in the tattoo ink.
- The specifications are designed for **tattoo inks** and apply accordingly to pigments and all auxiliary substances contained therein.
- The **minimum toxicological requirements** currently apply only to **tattoo pigments**.
- Tattoo pigments that meet the minimum toxicological requirements reduce possible health risks according to the **current state of science and technology**. However, no recommendations for use can be made at present.

In general, little is known about undesirable consequences that may be associated with the injection of tattooing agents into the human skin (dermal layer). It cannot be excluded that effects on organs other than the skin may occur at a later stage as a consequence of exposure to the ingredients of tattoo inks. Responsibility for the safety of Tattoo inks lies with the manufacturer and the person placing the product on the market. Defined criteria according to which a safety assessment should be carried out do currently not exist. The basis of a future risk assessment of tattoo inks should be their analytical and toxicological characterization. For this purpose, the German Federal Institute for Risk Assessment (BfR) has elaborated requirements in the following. A distinction is to be made between:

1) Minimum requirements for which test methods exist and are already operable today.
2) Requirements that need further research or method development; these are not yet operable.

By introducing minimum requirements (1), risks associated with tattooing can be reduced as far as possible according to the current state of science and technology. A full risk assessment of Tattoo inks requires a comprehensive set of data. It can only be carried out after further research. This means a comprehensive coordination process with all stakeholders from science, industry, politics and the public, and the establishment of suitable methods (2). The latter is not possible at present in the view of the BfR.

Pigments are the main component of tattoo inks and their risk assessment is considered to be of particular priority for consumer health protection. Therefore, pigments are considered in particular in this opinion. However, the purity requirements listed below are proposed for tattoo pigments as well as for other ingredients of tattoo inks (Chapter 2.1). The toxicological *in vitro tests* are designed for tattoo pigments (Chapter 2.2).

The analysis of pigments and their toxicologically relevant ingredients and impurities presents a particular challenge. Currently, there are no established routine methods for this and generally only very few laboratories can perform this analysis. In the future, however, these substances should be detected both in the formulation and examined with regard to potential degradation products formed in the body. The requirements, which are already feasible today, include a five-batch analysis of some ingredients with regard to primary aromatic amines (PAAs), metals and polycyclic aromatic hydrocarbons (PAHs), as well as an indication of the particle size distribution of the pigments. Tattoo pigments may be nanomaterials

(NMs) according to the EU definition, which requires appropriate consideration[3]. For pigments subject to registration, the information requirements under REACH Annex VI, 2.4.2-2.4.5 apply[4]. After adaptations of further test guidelines, these are to be considered in the future.

Toxicological *in vitro tests* may be required for the endpoints eye irritation/damage, skin irritation/corrosion, phototoxicity, skin sensitisation, genotoxicity and photogenotoxicity. Established OECD test guidelines are proposed for this purpose. These guidelines have already been used for a long time for the risk assessment of cosmetic products. The information requirements described here are to be fulfilled stepwise in a test strategy. Hazardous substance properties that conflict with use in tattoo inks (e.g. because they meet the criteria of the restriction of hazardous substances for use in Tattoo inks under REACH) terminate further testing and would finally justify non-use in tattoo inks. It is expected that already the application of the minimum operable requirements described here will significantly increase the level of protection compared to the current situation.

A full assessment of all health risks with *in vitro tests* is currently not possible. For example, an assessment of chronic toxic effects as well as carcinogenicity and reproductive toxicity is not possible (unless data are available that demonstrate e.g. chronic toxicity). No validated *in vitro test methods* are currently available for these endpoints. The suitability of established test methods with *in vivo study design* (e.g. the OECD test guideline programme), as they are anchored in the context of other regulatory areas (e.g. in the REACH Regulation), is not yet sufficiently proven scientifically. In order to mimic the process of tattooing, i.e. the intradermal application of pigments, BfR considers it necessary to further develop and adapt the existing *in vivo test methods* for the three endpoints mentioned. Therefore, no operable information requirements for the endpoints chronic toxicity, reproductive toxicity and carcinogenicity can currently be proposed.

Pigments are mainly poorly soluble particulate substances. Very little is known about their fate and effect in the human body[5]. Numerous aspects such as the distribution of pigment particles in the body, their persistence in organs in the form of nanoparticles or microparticles, the degradation of injected substances during enzymatic digestion or their photostability when exposed to sunlight are insufficiently clarified. For this purpose, the use of human data and targeted epidemiological studies is essential, as only these can indicate the potential effects of lifelong human exposure.

The BfR is already working on obtaining human data. For example, the analysis of blood and urine samples from persons shortly after the tattooing process is planned. In addition, work is being done on the use of existing epidemiological databases, e.g. the LIFE study[6] and NAKO[7]. A short-term bioavailability study to determine the amount of tattooing agent introduced per skin area (per cm^2 of skin) is currently being prepared. The concept of *in vivo testing of* selected pigments in the form of a representative study with intradermal exposure is described in the context of this statement (Chapter 3.1). Basic information on toxicokinetics is

[3] https://ec.europa.eu/environment/chemicals/nanotech/faq/definition_en.htm
[4] https://euon.echa.europa.eu/reach-test-methods-for-nanomaterials
[5] Giulbudagian M et al, (2020) Safety of tattoos and permanent make-up: a regulatory view. Arch Toxicol 94:357-369. https://doi.org/10.1007/s00204-020-02655s
[6] Loeffler M et al, (2015) The LIFE-Adult-Study: objectives and design of a population-based cohort study with 10,000 deeply phenotyped adults in Germany. BMC Public Health 15(1):691
[7] https://nako.de/

to be obtained; however, this study is not to be seen as a standard requirement for all pigments. All experimentally obtained data should be considered for use in *in silico approaches* (such as mathematic modelling).

The aspects that appear necessary to develop a complete risk assessment are listed below:

Short-term	Medium-term	Long-term
1. Preparation of representative test materials and development of methods for the detection and quantification of pigments. 2. Definition of the extraction and analysis procedure for PAHs from Tattoo inks. Harmonization of the method. Establishment of a reproducible and robust method for reductive cleavage of azo pigments.	1. Conducting and evaluating human biomonitoring studies, collecting and documenting human data. 2. Planning and carrying out representative toxicokinetic studies to analyse organ burden and systemic effects. Develop analytical methods for the quantification of pigments and their degradation products in organs and tissues. 3. Develop grouping approaches for testing and assessment strategies.	1. Establishment of appropriate *in vitro/ex vivo* methods and/or *in silico* models to test/assess the behavior of insoluble pigments after intradermal application.

PAHs: Polycyclic aromatic hydrocarbons, PBPK: Physiologically based pharmacokinetic (modelling), QSAR: Quantitative structure-activity relationship

In the case of cosmetic ingredients, the balancing of animal protection and consumer interests has led to a Europe-wide ban on animal testing for this type of use since 2013[8]. Although tattoo inks and cosmetic products cannot be equated in principle with regard to their application, the concept presented here largely refrains from proposing the use of animal testing. Clarification on further endpoints is still pending.

The fundamental decision on the use of *in vivo methods* for the safety testing of tattooing agents in animal models is outside the competence of the BfR.

Finally, the occupational health and safety of tattoo artists is also mentioned as an important aspect. However, these issues are not addressed here. These would have to be addressed separately, if necessary, with the involvement of the competent authorities. Furthermore, risks that could arise from the subsequent removal of the tattoo from the skin are not addressed in this framework, nor are medical applications of tattooing.

2 Operable minimum requirements and test methods

2.1 Necessary specifications on ingredients of tattoo inks

The specifications of the ingredients of tattoo inks and the identification of the impurities present are essential for risk assessment. These data are also needed for the planning of toxicological studies and their evaluation. Analytical methods must be suitable for the reproducible determination of substances within the concentration limits specified under the REACH restriction on substances in tattoo inks and permanent make-up. The analysis must be performed in compliance with Good Laboratory Practice (GLP), DIN EN ISO/IEC 17025[9] or an equivalent other quality assurance system. It must include intentionally added ingredients

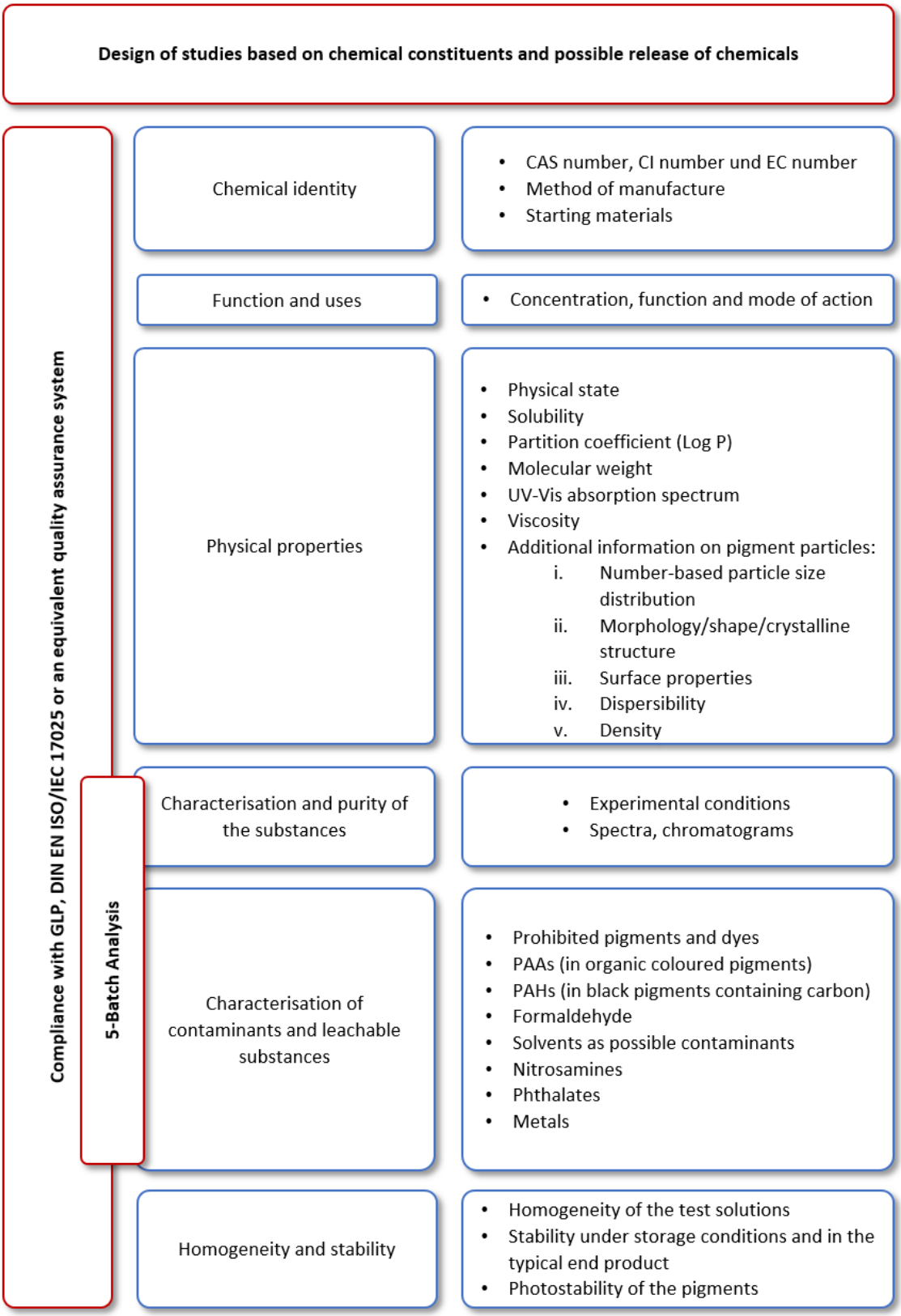
[8] See Regulation 1223/2009/EU on cosmetic products.
[9] General requirements for the competence of testing and calibration laboratories (ISO/IEC 17025:2017).

and the impurities present. The use of accredited methods is desirable. If the method in question is not evidently accredited, has not been validated by an interlaboratory comparison or does not conform to a specific harmonised procedure (e.g. OECD, ISO standard), laboratory methods may be used under certain conditions. The suitability of the methods used should be demonstrated by providing the validation documentation and the detailed validation parameters.

The specifications of the tested ingredients or tattoo inks or the technical equivalence of an ingredient/tattoo ink should be derived and confirmed by a five-batch analysis. This concerns the chemical composition of the intentionally added substances and the impurities present. The five-batch analysis should be presented in the report together with the respective measurement uncertainty. Here it is referred to the specification of technical active substances in the authorisation procedure for plant protection products[10]. Small changes in the composition of some impurities may alter the overall toxicity of the product. In general, the results of toxicity studies with a given substance are only relevant if they relate to a specific purity and impurity profile. The scientific validity and transferability of toxicological studies performed on batches of the substance with a different impurity profile require careful consideration. It must therefore be ensured that neither other impurities nor increased levels of impurities are present in the representative product. For this purpose, the reproducibility of the synthesis process, including any purification steps, is important. The synthesis process of the pigments and the substances and conditions used in this process must be specified. A change in this process requires a careful re-evaluation of the impurities, even if the purity remains unchanged. The standard requirements for the chemical characterisation of tattoo inks are summarised in **Fehler! Verweisquelle konnte nicht gefunden werden.**

[10] Specification of technical active substances in the approval procedure for plant protection products, https://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/zul_dok_wirkstoffspezifikation.html

Figure 1: Standard requirements for the chemical characterisation of tattoo inks



PAAs: Primary aromatic amines, PAHs: Polycyclic aromatic hydrocarbons, CAS number: Chemical Abstracts Service number, CI number: Colour Index number, EC number: European Community number

The required specifications for ingredients of tattoo inks are listed below. Any deviation from these requirements requires a scientifically based justification.

2.1.1 Chemical identity

Precise information on the structural formulae and chemical properties of all ingredients intentionally added during formulation and, where possible, of the cleavage products. CAS number (Chemical Abstracts Service number) and CI number (Colour Index number) - if available - and EC number (European Community number) shall be provided. If a substance cannot be clearly identified from its structural formula, sufficient information on the method of manufacture and the starting material should be provided to allow the likely structure and activity to be inferred. The safety data sheets of all ingredients should be included.

For the safety assessment of complex mixtures (e.g. extracts or natural products) as ingredients of Tattoo inks, complete information on the origin of the starting material, the available extraction methods and any additional procedures and/or purification steps should be provided in order to recognise a standardised material as representative of the mixture present in commercial products.

Indication of the manufacturing processes of the pigments and their formulation in the tattoo inks:

It is important to consider the effect of manufacturing conditions on pigments and the presence of impurities. Where differences in impurities occur due to a change in manufacturing conditions/location, a scientific statement is required as to why the chemical differences are not relevant to the safety of the pigment. Information that should be provided includes:

1. Non-intentionally added substances (NIAS) (e.g. process residues or impurities)
2. Intentionally added additives incl. processing aids
3. Possible degradation products

2.1.2 Function and uses

Indication of the concentration, function and mode of action of the substance under evaluation in the marketed final product.

2.1.3 Physical properties

a. Description of physical state (solid or liquid) and form (powder, gel, etc.).

b. Solubility and dissolution

Indication of the solubility [EC A.6][11] of the substance in water and/or other relevant organic solvents (in g/l at °C). Some substances (e.g. pigments) are poorly soluble or insoluble in

[11] Water solubility Council 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). Official Journal L 142, 31/05/2008, p.57.

aqueous medium. Nevertheless, it is essential to estimate their solubility and dissolution. Substances that can leach out should be identified. This can be done in water, but also in physiological media, appropriate simulants (sweat, blood, lymph) or skin homogenates to simulate the environment of the pigment after tattooing. Since insoluble and partially soluble pigments are likely to form suspensions in the solvent media, it is important that all suspended particles are completely removed from the suspension by ultracentrifugation and/or ultrafiltration before the chemical analyses are performed to avoid overestimation of the actual dissolved amounts of the substance. For this purpose, the "Guidance document 318 for the testing of dissolution and dispersion stability of nanomaterials, and the use of the data for further environmental testing and assessment" [12]developed for environmental media can be used. In addition, the ISO standard TR19057:2017 "Nanotechnologies - Use and application of acellular *in vitro* tests and methodologies to assess nanomaterial biodurability" can be used[13]. Further guidance documents relevant to health risk assessment are currently under development.

c. Partition coefficient (Log P)

Where applicable, the n-octanol/water partition coefficient [EC A.8][14] with specification of pH and temperature shall be indicated. In case of calculation of the coefficient; specification of the method.

d. Other relevant physical and chemical specifications, as applicable:

- Molecular weight: to be given in Daltons; for mixtures, to be provided for each individual substance. For polymer ingredients, the molecular weight distribution should be indicated.
- Organoleptic properties (colour, odour (if relevant)).
- Physical properties depending on the state of aggregation:

  Liquids: Boiling point [EC A.2][15], flash point [EC A.9][16], relative density [EC A.3][17](at ..°C) and bulk density (DIN 66133/ISO 15901-1), pKa (at ..°C), viscosity (at ..°C), vapour pressure [EC A.4][18] (at ..°C).

  Solids: crystallinity (crystalline - mention crystal structure, non-crystalline,

[12] Guidance Document for the Testing of Dissolution and Dispersion Stability of Nanomaterials and the Use of the Data for further Environmental Testing and Assessment Strategies, No. 318.
[13] https://www.iso.org/standard/63836.html
[14] Partition coefficient Council 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). Official Journal L 142, 31/05/2008, p.67.
[15] Boiling temperature Council 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). Official Journal L 142, 31/05/2008, p.14.
[16] Flash-point Council 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). Official Journal L 142, 31/05/2008, p.80.
[17] Relative density Council 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). Official Journal L 142, 31/05/2008, p.21.
[18] Vapour pressure Council 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). Official Journal L 142, 31/05/2008, p.26.

amorphous), melting temperature [EC A.1][19] , density, pKa (at ..°C), for UV-Vis absorbing ingredients: UV-Vis absorption spectrum.

Final product: viscosity (at ..°C) (OECD Test Guideline 114), UV-Vis absorption spectrum, pH (at ..°C) - in principle the pH of the final product should not be less than 5 and not more than 8.

Some information on the raw materials may be available in the safety data sheets or in the literature. These should be used to document and evaluate the safety of the tattoo inks.

e. Additional information on pigment particles:

Due to possible changes in the physicochemical properties, the state of the pigments in the final product should be determined at various stages. The characterization of the pigments used should be carried out both in the pristine (original) state and after addition to the final formulation. The information on the pristine materials can also be requested from the manufacturer. The parameters required or to be determined are summarized in Table 1and are based on the requirements of the Cosmetics Regulation[20] (Regulation (EC) No 1223/2009). Depending on the pigment or state (original or suspended in the formulation), not all parameters are always relevant. In this case, parameters can be omitted in individual cases by providing a justification. Further information can be found in the "Guidance on the safety assessment of NMs in cosmetics".[21]

Any analytical method used should fit for purpose and be reliable. Ideally, the method should be validated in terms of performance parameters (e.g. specificity, selectivity, robustness, recovery, repeatability and reproducibility) and should report detection/quantification limits and measurement uncertainties. The results should be tabulated, indicating the test method chosen and the testing laboratory. Alternatively, the parameters declared by the manufacturer should be provided, together with the test certificate. These may be number-based, graphical or pictorial, depending on the method used.

Table 1. Parameters and methods for characterizing particle properties of pigments for use in Tattoo inks, following SCCS/1602/18 and SCCS/1611/19, respectively

Parameter	Description	Proposed methods (other methods possible if equally suitable)
Number-based particle size distribution	a) Mean and median size [nm] and graphical plots of size distribution for primary and secondary (agglomerates and aggregates) particle size (number and mass based). (b) The number of particles in the size range 1-100 nm.	FFF, HDC, HPLC, analytical ultracentrifugation, CLS, disc centrifugation, TEM, SEM, AFM, DMA, PTA/NTA The suitability of the measurement technique for the respective pigment can be assessed with the help of the method

[19] Melting / freezing temperature Council 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). Official Journal L 142, 31/05/2008, p.4.
[20] REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30 November 2009 on cosmetic products.
[21] Guidance on the Safety Assessment of Nanomaterials in Cosmetics of the SCCS (SCCS/1611/19).

		manual prepared within the framework of NanoDefine[22]. OECD WNT Project 1.4: New test guideline on particle size and size distribution of manufactured nanomaterials - under Development
Morphology/shape/crystalline structure	(a) Imaging and graphical evidence of physical shape and form (particle, tube, rod or fibre shape, porosity). Aggregation/agglomeration state (primary particles or agglomerates/aggregates), crystalline structure. (b) Indication of the preparation (powder, solution, suspension or dispersion).	a) TEM, SEM, AFM NMR, XRD (b) Descriptive
Surface	Graphical and number-based (in $^{m2/g}$) indication of the specific surface area of the pigments.	BET OECD WNT Project 1.3: New test guideline on determination of the (volume) specific surface area of manufactured nanomaterials ongoing, under finalization, s. a. ISO/TR 14187
Surface properties	a) Qualitative, if possible quantitative information on the components bound to the surface, presence of any functional groups (e.g. carboxy-, amino-, hydroxy-). b) Indication of the surface charge (zeta potential in mV)	a) XPS, MS, RS, SERS, FTIR, NMR, GE, NanoSIMS b) Phase Analysis Light Scattering, DLS
Reactivity and photoreactivity	Information on the chemical reactivity of the (possibly nanoscale) pigment or surface coating. Information on the photocatalytic activity and radical formation potential of the relevant materials.	ESR, FRAS, acellular DCFDA
Dispensability	For insoluble dispersible particles: Information on dispensability in the sense of a relative amount of particles that can be dispersed in a suspending medium.	OECD Test Guideline 318: Dispersion Stability of Nanomaterials in Simulated Environmental Media
Density and bulk density	Information on density (e.g. mg/g or g/cm3)	Methods described in ISO 697:1981, EN ISO 60:1977, DIN 66133/ISO 15901-1

AFM: Atomic Force Microscope, BET: Brunauer, Emmett, and Teller Method, CLS: Confocal Laser Scanning Microscopy, DCFDA: 2',7'-Dichlorodihydrofluorescein diacetate, DLS: Dynamic Light Scattering, DMA: Differential Mobility Analyser, ESR: Electron Spin Resonance, FFF: Field Flow Fractionation, FRAS: Free Radical Analytical System, FTIR: Fourier Transform Infrared Spectrometry, GE: Gel Electrophoresis, HDC: Hydrodynamic Chromatography, HPLC: High-Performance Liquid Chromatography, MS: Mass Spectrometry, NanoSIMS: Nano-Secondary Ion Mass Spectrometry, NMR: Nuclear Magnetic Resonance, PTA/NTA: Particle Tracking Analysis/Nanoparticle Tracking Analysis, SEM: Scanning Electron Microscope, RS: Raman Spectroscopy, SERS: Surface Enhanced Raman Spectroscopy, SPM: Scanning Probe Microscopy, TEM: Transmission Electron Microscopy, WNT: Working Group of National Coordinators of the Test Guidelines programme, XAS: X-ray Absorption Spectroscopy, XPS: X-ray Photoelectron Spectroscopy, XRD: X-ray Diffraction.

[22] Part 2: Mech et al, The NanoDefine Methods Manual. Part 2: Evaluation of methods, EUR 29876 EN, Publications Office of the European Union, Luxembourg, 2019, ISBN 978-92-76-11953-1, doi:10.2760/071877, JRC117501.

2.1.4 Characterization and purity of the chemical

Description of the experimental conditions of the techniques used to characterise the substance (UV-vis spectroscopy (ultraviolet), IR spectroscopy (infrared), NMR spectroscopy (nuclear magnetic resonance), MS (mass spectrometry), elemental analysis, etc.) and of the resulting spectra and chromatograms, indication of the degree of purity, demonstration of the validity of the method used. The substance used for testing must be the same as that used in the commercial product. The identity of the pigments and dyes used should be shown by means of the corresponding spectra and chromatograms.

2.1.5 Characterisation of contaminants and leachable substances

Substances covered by the REACH Regulation or other legislation should not be present in the tattooing product above the permitted concentrations. Therefore, all relevant impurities must be identified and quantified. For the common impurities listed in Table 2corresponding compliance data must be provided.

Tattoo pigments can decompose in a biological environment or during storage, producing degradation products. These may have different properties compared to the starting material. In addition, substances occurring as impurities and degradation products formed by decomposition of substances under the influence of light, enzymatic activity, etc. must be considered. For example, photostability issues are addressed in Chapter 2.1.6

Furthermore, ISO 10993-12[23] serves as a guide for the extraction of leachable constituents and ISO 10993-18[24] serves as a guide for the chemical characterization of materials and their leachable constituents. The design of the analytical test to detect possible impurities beyond those listed in Table 2could be carried out following these standards.

Table 2 . Ingredients and impurities to be fully characterised taking into account the recommended procedures listed

Substance	Concentration limits for the final product according to the restriction under REACH[25]	Notes on sample preparation and analysis
Currently operable		
PAAs (in organic coloured pigments)	Tattoo inks shall not contain azo pigments that may release one or more of the PAAs listed in Appendix 13 of the restriction. The limit value for free PAAs is 0.0005 % (5 ppm).	Free PAAs have to be analysed by LC-MS/MS according to the state of the art. LC-DAD is less sensitive and selective. According to the current status, there is no standardized norm method for PAAs in tattoo inks. However, according to the information provided by the state investigation offices, the in-house methods, which are mostly based on standard methods from other areas, provided very

[23] Biological evaluation of medical devices - Part 12: Sample preparation and reference materials (ISO 10993-12:2012); German version EN ISO 10993-12:2012

[24] Biological evaluation of medical devices - Part 18: Chemical characterization of materials for medical devices in the context of a risk management system (ISO/DIS 10993-18:2018); German and English version prEN ISO 10993-18:2018.

[25] COMMISSION REGULATION (EU) 2020/2081 of 14 December 2020 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards substances in tattooing ink or permanent make-up.

		good results for free PAAs (following DIN EN ISO 14362-1 or DIN EN 71-7).
PAHs (in black carbonaceous pigments, raw material)	Polycyclic aromatic hydrocarbons (PAHs), classified as carcinogenic or mutagenic substances of category 1A, 1B or 2 in Part 3 of Annex VI to Regulation (EC) No 1272/2008[26]: 0.00005 % (0.5 ppm) (individual concentrations): (a) Benzo[*a*]pyrene (BaP) CAS No 50-32-8. 0.0000005% (5 ppb) (b) Benzo[*e*]pyrene (BeP) CAS No 192-97-2 (c) Benzo[*a*]anthracene (BaA) CAS No 56-55-3 (d) Chrysene (CHR) CAS No 218-01-9 (e) Benzo[*b*]fluoranthene (BbFA) CAS No 205-99-2 (f) Benzo[*j*]fluoranthene (BjFA) CAS No 205-82-3 (g) Benzo[*k*]fluoranthene (BkFA) CAS No 207-08-9 (h) Dibenzo[*a*,*h*]anthracene (DBahA) CAS No 53-70-3	In carbon-containing pigments, PAHs must be determined analytically prior to their formulation in tattoo inks. Together with the content in the formulation of the tattoo ink, compliance with the limit values can thus be concluded. For this purpose, e.g. "ASTM D8143 - 17 - Standard Test Method for Determination of the EU-8 List of PAH Compounds in Carbon Black" can be used. For the analysis of the whole formulation there are no harmonized methods available so far - the yield depends strongly on the extraction conditions. Extraction of the homogenised sample material with toluene in an ultrasonic bath at 80 °C is considered suitable. The development of a standard method is necessary. If PAH contents in the final product are indicated, proof of the applicability of the method must be provided (see above).
Solvents as possible impurities from the production phase	Benzene (Carc 1A): 0.00005 % Toluene (Repr. 2): 0.001 %. Ethylbenzene: not restricted Xylene (Skin Irrit. 2): 0.001 %. Other remaining solvents from production	Headspace gas chromatography with mass spectrometric detection (HS-GC-MS). No harmonisation necessary.
Nitrosodipropylamine	0.00005 % (0.5 ppm)	HPLC-MS/MS
Nitrosodimethylamine	0.00005 % (0.5 ppm)	
N-nitrosodiethanolamine (NDELA)	0.00005 % (0.5 ppm)	
Mercury	0.00005 % (0.5 ppm)	Pressure digestion according to K 84.00-29 and analysis according to K 84.00-33 Analysis of cosmetic products - Determination of mercury in cosmetic products and tattoo inks with atomic absorption spectrometry (AAS) - Cold vapour technique after pressure digestion.
Nickel	0.0005 % (5 ppm)	Measurement according to or based on: Pressure digestion according to K 84.00-29 and K 84.00-31 Investigation of cosmetic products - Determination of antimony, arsenic, barium, lead, cadmium and nickel in cosmetic products and tattoo inks by mass spectrometry with inductively coupled plasma (ICP-MS) after pressure digestion or DIN 11699:2020-10/K 84.00-32 Analysis of cosmetic products - Determination of barium, nickel and other elements in cosmetic products and tattoo inks by inductively coupled plasma optical emission spectrometry (ICP-OES) after pressure digestion.
Organometallic tin	0.00005 % (0.5 ppm)	
Antimony	0.00005 % (0.5 ppm)	
Arsenic	0.00005 % (0.5 ppm)	
Cadmium	0.00005 % (0.5 ppm)	
Cobalt	0.00005 % (0.5 ppm)	
Lead	0.00007 % (0.7 ppm)	

[26] 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

Selenium	0.0002 % (5 ppm)	
Chromium VI	0.00005 % (0.5 ppm)	For chromium (VI), validation parameters should pay special attention to stability in solution (pH & time dependent).
Not operable, method establishment/harmonization necessary		
Prohibited pigments and dyes	The content of the 44 pigments listed in Appendix 13 of the restriction shall not exceed 0.1 % (1000 ppm).	Identification and semi-quantification of the pigments: MALDI-MS, pyrolysis GC-MS, or for partially soluble dyes with LC-DAD/MS. Identification of "pure" pigments also possible via infrared/Raman spectroscopy and database matching. Quantitative analytical methods for determining the content of pigments are not yet available. Via the azo cleavage, prohibited azo pigments could possibly also be identified via the cleavage products (PAAs). However, current methods for reductive cleavage of pigments in tattoo inks are difficult to reproduce.
PAHs (in colorants containing black carbonaceous pigments)	Polycyclic aromatic hydrocarbons (PAHs), classified as carcinogenic or mutagenic substances of category 1A, 1B or 2 in Part 3 of Annex VI to Regulation (EC) No 1272/2008[27]: 0.00005 % (0.5 ppm) (individual concentrations): (a) Benzo[*a*]pyrene (BaP) CAS No 50-32-8. 0.0000005 % (5 ppb) (b) Benzo[*e*]pyrene (BeP) CAS No 192-97-2 (c) Benzo[*a*]anthracene (BaA) CAS No 56-55-3 (d) Chrysene (CHR) CAS No 218-01-9 (e) Benzo[*b*]fluoranthene (BbFA) CAS No 205-99-2 (f) Benzo[*j*]fluoranthene (BjFA) CAS No 205-82-3 (g) Benzo[*k*]fluoranthene (BkFA) CAS No 207-08-9 (h) Dibenzo[*a*,*h*]anthracene (DBahA) CAS No 53-70-3	For the analysis of the whole formulation there are no harmonized methods so far - the yield depends strongly on the extraction conditions. Extraction of the homogenised sample material with toluene in an ultrasonic bath at 80 °C is considered operable. The development of a standard method is necessary. If PAH contents in the final product are indicated, proof of the applicability of the method must be provided (see above).
Formaldehyde	0.00005 % (0.5 ppm)	HPLC-FLD with post-column derivatisation, achievement of the limit value currently still uncertain.
Dibutyl phthalate (DBP)	0.00005 % (0.5 ppm)	GC-MS, GC-MS/MS, LC-MS/MS, achievement of the limit value currently still uncertain.
Bis(2-ethylhexyl) phthalate (DEHP)	0.00005 % (0.5 ppm)	
Barium (soluble)	0.05 % (500 ppm)	Currently not operable, definition of the extraction parameters necessary.
Copper (soluble)	0.025 % (250 ppm)	Currently not operable, definition of the extraction parameters necessary.

[27] 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

Zinc (soluble)	0.2 % (2000 ppm)	Currently not operable, definition of the extraction parameters necessary.

GC-MS: Gas chromatography with mass spectrometry coupling, ICP-MS: Inductively coupled plasma mass spectrometry, ICP-OES: Inductively coupled plasma optical emission spectrometry, LC-DAD: Liquid chromatography - diode array detector, LC-MS/MS: Liquid chromatography with tandem mass spectrometry, MALDI-MS: Matrix-assisted laser desorption/ionization mass spectrometry, PAAs: Primary aromatic amines, PAHs: Polycyclic aromatic hydrocarbons.

2.1.6 Homogeneity and stability

The homogeneity of the test solutions with respect to the distribution of the test substance should be ensured. The stability of the test substance under different experimental conditions should be reported, as well as the stability under storage conditions and in the typical end product. General aspects for the determination of degradation products are given in Part 9 of ISO 10993: "Framework for the identification and quantification of possible degradation products".[28]

Pigments that decompose via metabolism or photo-induced cleavage into toxic products in harmful concentrations should not be used. Pigments based on the following chemical structures should not be used in tattoo inks: Benzidine (EC index number 611-024-00-1), *o-Dianisidine* (EC index number 611-029-00-9) and *o-Toluidine* (EC index number 611-030-00-4).

The stability of the test substances to UV light should be reported. If a molar extinction coefficient according to OECD Test Guideline 101[29] below 1000 L mol-1cm-1 is found, the photostability test may be omitted. This assumption does not apply to pigments due to their high light absorption properties in a wide spectral range. The photostability tests should take into account the stability of the pigments and the formation of degradation products both during storage and in the human body.

Photostability shall be tested in accordance with the Note for Guidance on the Photostability Testing of New Active Substances and Medical Products (CPMP/ICH/279/95[30]). This guideline is designed for the analysis of active substances in medical products and is applicable to tattoo pigments. For systematic testing, the pigment should be considered before formulation in the tattooing product. If possible, pigments should be tested in an aqueous suspension rather than in their dry state. A full spectrum lamp providing both UV-A and visible radiation should be used to produce a daylight spectrum of standard illuminant D65 (outdoor daylight). The light dose should be expressed in units of W h/m², with an indication of the wavelength. The dose should be at least 200 W h/m². At the end of the exposure period, samples should be examined for changes in physical properties (e.g. appearance, clarity or colour of the solution) and for degradation products by an appropriate method (see point 2.1.5Characterisation of contaminants and leachable substances). Decomposition products must be analysed according to the same specifications and with the same methods as contaminants. Ideally, mass balances and percentage balances of degradation products should be calculated relative to the original test substance. The concentration of the resulting degradation products should not exceed the limits given in Table 2

[28] DIN EN ISO 10993-9:2010-04 Biological evaluation of medical devices - Part 9: Framework for identification and quantification of potential degradation products (ISO 10993-9:2009); German version EN ISO 10993-9:2009
[29] OECD Guideline for Testing of Chemicals No. 101: UV-VIS Absorption Spectra (Spectrophotometric Method).
[30] Note for Guidance on the Photostability Testing of New Active Substances and Medicinal Products (CPMP/ICH/279/95).

If quantitative detection of the degradation products is not possible, the light fastness should be determined according to DIN ISO 12040:1998-01[31] light fastness shall be determined using the grey scale to evaluate the change in colour according to ISO 105-A02:1993-09.[32] A pigment with a light fastness below 6 is not suitable for use in tattoo inks.

2.2 Operable minimum toxicological requirements

This chapter compiles operable minimum requirements that are considered necessary and feasible for a toxicological evaluation of tattoo pigments according to the current state of science and technology. The test requirements and evaluation criteria described here represent an update of the BfR statement (No. 013/2013) of 2012[33]. They are based on the EDQM document 'Safer Tattooing' of 2017[34] and on the REACH and CLP Regulations. In this context, reference is made to the detailed guidance documents available for these Regulations on information requirements, chemical safety assessment[35] and evaluation criteria. Although the information requirements under REACH, which are tiered depending on the production volume, are not directly applicable to testing requirements for tattooing pigments, essential elements such as the evaluation of all available data on intrinsic substance properties, the use of *in vitro/in silico* testing strategies with extensive use of current harmonised OECD test guidelines, and the evaluation of substance properties following the CLP Regulation may still apply. Furthermore, tattoo pigments can be considered as nanomaterials according to the EU definition. Thus, nano-specific adaptations have to be taken into account.

When considering the conduct of new studies on a pigment, existing data should be taken into account. Like other substances, (tattoo) pigments are also subject to registration obligations (including information requirements) under the REACH Regulation[36] in the EU if they are manufactured or imported in quantities of more than one tonne per year. Furthermore, the CLP Regulation (Regulation (EC) No. 1272/2008) applies to pigments[37] and they must be classified in hazard classes (self and harmonised, if applicable) according to the CLP Regulation if they have hazard characteristics and the classification criteria according to the CLP Regulation are met.

If pigments are harmonised for certain substance properties (so-called "endpoints"), they may not be used in tattoo inks above certain concentration limits in accordance with the

[31] DIN ISO 12040:1998-01 Printing and reproduction techniques - Printing and printing inks - Determination of light fastness using filtered xenon arc light (ISO 12040:1997).
[32] ISO 105-A02:1993-09 Textiles - Tests for colour fastness - Part A02: Grey scale for assessing change in colour.
[33] https://www.bfr.bund.de/cm/343/anforderungen-an-taetowiermittel.pdf
[34] Safer Tattooing, Overview of current knowledge and challenges of toxicological assessment, Consumer Health Protection Committee, EDQM 1st Edition, 2017
[35] Compiled at: https://echa.europa.eu/ and https://echa.europa.eu/de/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[36] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (Text with EEA relevance).
[37] 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 (Text with EEA relevance)

REACH restriction for tattoo inks and permanent make-up[38]. Concentration limits apply here according to entry 75 of Annex XVII of REACH for substances with the following harmonised classifications: Carc./Muta. /Repr. (1A/B and 2 respectively), Skin Sens. (1, 1A/B), Skin Corr./Irrit. (1, 1A/B/C, 2) and Eye Dam./Irrit. (1, 2). Even if the pigments have substance properties that fulfil the criteria for classification for the endpoints mentioned and a self-classification or harmonised classification is not (yet) available, they should not be used in tattoo inks above the concentration limits indicated from the point of view of consumer protection[39]. It should be noted that the concentration limits specified in the REACH restriction are below 0.1 % by weight. As tattoo pigments are usually used in tattoo inks in higher percentages by weight, it is considered that the concentration limits indicated in the restriction for pigments with the above harmonised classifications imply a practical ban on their use as tattoo pigment.

For tattoo pigments, tests shall be carried out for the following endpoints:

- Eye irritation/eye damage
- Skin irritation/skin corrosion
- Phototoxicity
- Skin sensitization
- Genotoxicity
- Photogenotoxicity

For each of these endpoints, minimum requirements were defined and summarized in tables. Column 1 of the tables listed from Chapter 2.2.1lists the required standard test requirements. Column 2 describes rules according to which deviations from the requirements in column 1 can or should be made. In the case of deviations from the requirements in column 1 according to the rules in column 2, a detailed justification should be provided.

For each endpoint, the associated table also provides a field with corresponding assessment criteria. The assessment of the endpoints should generally be based on the CLP classification criteria. Substance properties that are relevant for classification as hazardous according to the CLP criteria (Carc./Muta. /Repr. (1A/B and 2 each), Skin Sens. (1, 1A/B), Skin Corr./Irrit. and Eye Dam./Irrit. (each 1, 1A/B/C, 2)) are sufficient, lead to a practical ban on use, taking into account the concentration limits specified in Annex XVII, entry 75 (REACH Regulation).

Test strategy

Studies shall be carried out for each tattoo pigment following the testing strategy outlined below. This testing strategy follows the general approach of first collecting and evaluating existing data and then providing missing information through *in vitro* testing. If existing data or results from *in vitro* studies show that relevant classification criteria as hazardous substances

[38] COMMISSION REGULATION (EU) 2020/2081 of 14 December 2020 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards substances in tattooing ink or permanent make-up
[39] Entry 75 of Annex XVII of the REACH Regulation only refers to harmonised classified substances. The extended use ban for tattoo inks proposed here is currently not legally binding in Europe.

according to the CLP Regulation are fulfilled, these represent an exclusion criterion for the use of the pigments in tattoo inks, as already mentioned above.

Prior to the toxicological evaluation, a characterization of the pigments, existing impurities and possible degradation products must be carried out (see chapter 2.1) and integrated into the test design. The physicochemical parameters of the substance investigated in the toxicological tests must comply with the defined specifications or lie within defined limits. The comprehensive analysis in terms of particle size and distribution, morphology and surface chemistry of the pigments must be taken into account for the assessment of the dose to be used and the expected effect. The design of the analysis must take into account that the properties and toxicological mechanism of the pigments do not depend solely on their molecular structure, but on the properties of the pigment particles, some of which are listed in Table 1this document.

The following step-by-step procedure should be followed in the health evaluation of tattoo pigments (Figure 2A):

Step 1: Collection of all available data

The applicant[40] is encouraged to consider all available information for the pigment. This shall include, but not be limited to:

- Literature search for all relevant information on the pigment
- Verification of data generated under other regulations (e.g. REACH Regulation)
- Examination of the applicability of *in silico* methods (e.g. QSARs (quantitative structure-activity relationship) and analogy/grouping approaches, "read-across") of other similar pigments
- Human data including epidemiological data (especially concerning tattooing, medical case reports etc.).

Available information should be documented in detail.

With regard to read-across, a case-by-case assessment is necessary, which is based on the specifications of the so-called "Read-across Assessment Framework"[41] of the European Chemicals Agency (ECHA). It is noted that a purely structural similarity alone is not sufficient to establish a valid read-across. Here, the application of the concept "Set of Similar Nanoforms" should be examined in order to be able to[42] carry out hazard assessment, exposure assessment and risk assessment of these pigments together, if necessary.

With regard to *in silico* methods, it should be noted that great progress has been made in recent years for individual endpoints. Information on modelling options for the individual endpoints can be found in the endpoint-specific guidance (REACH) on information requirements

[40] 'Applicant' includes manufacturers, importers or distributors intending to test and/or authorise a pigment for use in Tattoo inks. The intended tests and later, where appropriate, the intended approvals in a jurisdiction are intended to apply to manufacturers in the jurisdiction and the same requirements are intended to apply to importers or distributors of tattooing pigments or of Tattoo inks containing those pigments.
[41] ECHA (2017): Read-Across Assessment Framework (RAAF). No. ECHA-17-R-01-EN, ISBN 978-92-9495-758-0. European Chemicals Agency, Helsinki. DOI: 10.2823/619212; https://echa.europa.eu/documents/10162/13628/raaf_en.pdf
[42] Appendix for nanoforms applicable to the Guidance on Registration and substance identification, https://echa.europa.eu/documents/10162/23047722/appendix_nanoforms_draft_to_peg_en.pdf/bb84f0ca-7688-5293-604e-fb43982c7afd

and chemical safety assessment (Guidance on Information Requirements and Chemical Safety Assessment)[43], which is subject to continuous, albeit delayed, updating[44].

Step 2: Checking the existing data

The applicant is required to check whether, on the basis of all available information for the pigment, the requirements of column 1 (Chapter 2.2.12.2.6) are fulfilled, if necessary taking into account the deviations proposed in column 22.2.1In this case, further testing may not be necessary for the endpoint in question. In case of deviations from the requirements in column 1, a detailed justification should be provided according to the rules in column 2. It is important that only relevant, reliable and adequate data of good quality can fulfil the standard data requirements.

It is necessary to consider whether it can be concluded from the available data that the exclusion criteria[45] summarised in Box 1 are met, which preclude the use of the pigments in tattoo inks and thus necessitate termination of the evaluation.

BOX 1: Exclusion criteria for use as a tattoo pigment:

I. Compliance with CLP criteria or existing harmonised classification as:

CMR 1A. 1B, 2
Skin Sens. 1, 1A, 1B
Skin Corr. 1, 1A, 1B, 1C, Skin Irrit. 2
Eye Dam. 1, Eye Irrit. 2
or
STOT RE 1, 2
Acute Tox. 1, 2

Complies with VO 2020/2081 Restriction of substances in tattooing inks and PMU

II. Positive results in any of the OECD test guidelines listed in Step 3 for the endpoints: Skin irritation/corrosion, Eye irritation/damage, Skin sensitisation, Phototoxicity and Genotoxicity (Incl. Photogenotoxicity).

Step 3: Performance and evaluation of in vitro tests

If, after reviewing all available data, the applicant identifies information gaps for individual endpoints, it shall be assessed whether further *in vitro* testing is required and, if necessary, one or more new *in vitro* tests shall be performed. In this context, special attention must be paid to the fact that the selected *in vitro* tests must be feasible with the tattoo pigments, taking into account their physicochemical properties. *In vitro* tests are required for the endpoints eye irritation, skin irritation, skin sensitization, phototoxicity and genotoxicity, among others

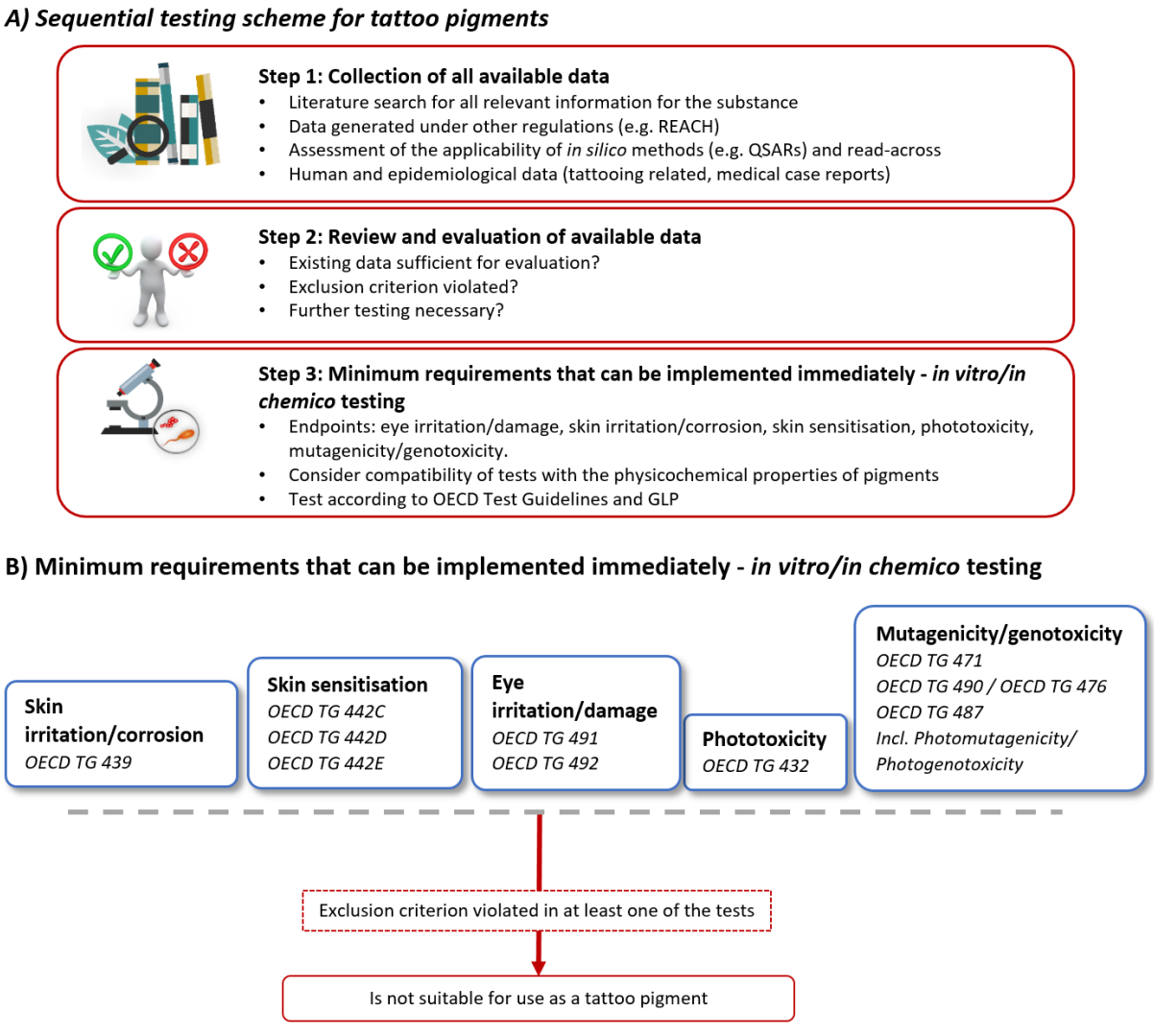
[43] ECHA: Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance; https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf/e4a2a18f-a2bd-4a04-ac6d-0ea425b2 a567f
[44] EURL ECVAM Database on Alternative Methods to Animal Experimentation (DB-ALM)
[45] Entry 75 of Annex XVII of the REACH Regulation only refers to harmonised classified substances as CMR (1A/B and 2), Skin Sens. (1, 1A/B) and Skin & Eye Irrit. (1, 1A/B/C, 2)). The extended ban on the use of tattoo inks proposed here is currently not legally binding in the EU.

(**Fehler! Verweisquelle konnte nicht gefunden werden.**). In some cases, there is insufficient experience available for the performance of these tests for the testing of pigments envisaged here. For the significance with regard to intradermal application, there are still uncertainties and possibly a need for adaptation of the epidermis models.

New tests should be performed according to the respective OECD test guidelines and GLP. Corresponding deviations are deposited in the tables in column 2.

After the *in vitro* tests have been performed, a re-evaluation of all available data is required. If for any of the endpoints there is evidence that the (classification) criteria of the CLP Regulation are met, animal studies with the pigment are not allowed for these endpoints in the context of the assessment for use as a tattooing pigment and the use of the pigment in tattoo inks is excluded according to the exclusion criteria (see BOX 1).

Figure 2: Sequential procedure for testing tattoo pigments; if necessary, after adjustment for (nano)particulate properties.



GLP: Good Laboratory Practice, QSARs: Quantitative structure-activity relationship, Pictures: cleanpng.org

Detailed description of the information requirements on the health-related endpoints

2.2.1 Endpoint Eye irritation/damage

2.2.1.1 Check request

2.2.1	Endpoint Eye irritation/damage	
	Column 1 Standard test requirement	Column 2 Rules for derogation from column 1
2.2.1.2.2.1.a	OECD Test Guideline 491 "Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage". ➢ Pigments can be suspended in PBS, 5 % DMSO or mineral oil (for 5 min) ➢ Measurement of viability via MTT formazan formation only via HPLC/UPLC analyses	2.2.1a and 2.2.1b need not be carried out if for the substance ➢ the CLP criteria for classification as CMR (1A/B and 2), Skin Sens (1, 1A/B), Skin Corr./Irrit. (1, 1A/B/C, 2, 3 (GHS only)), Eye Dam./Irrit. (1, 2, 2B (GHS only), STOT RE (1, 2), Acute Tox. (1, 2) are fulfilled and/or the substance is classified harmonized in these endpoints. ➢ data are already available showing that the substance has phototoxic properties according to OECD guidelines.
2.2.1.2.2.1.b	OECD Test Guideline 492 "Reconstructed human cornea-like epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage". ➢ Measurement of viability via MTT formazan formation only via HPLC/UPLC analyses	
Evaluation Criteria: Both tests allow a classification (according to CLP regulation) as "eye damaging" or "not eye irritating", but no categorization as "eye irritant". In case of positive results, the use of the pigments in tattoo inks should generally be avoided.		

PBS: Phosphate Buffered Saline, DMSO: Dimethyl Sulfoxide, MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide, HPLC: High Performance Liquid Chromatography, UPLC: Ultra Performance Liquid Chromatography, CMR: Carcinogenic, mutagenic and reprotoxic, Skin Sens: Skin Sensitization, Skin Corr./Irrit. Skin Corr./Irrit. : Skin corrosion/irritation, Eye Dam./Irrit. : Serious eye damage/eye irritation, STOT RE: Specific target organ toxicity (repeated exposure), Acute Tox: Acute toxicity

2.2.1.2 Standard test requirements

According to the REACH Regulation, eye-irritating or eye-damaging substances above a certain concentration limit may not be used in tattoo inks. It is assumed that eye-irritating or -damaging substances could also have this effect after intradermal application. Consequently, an evaluation of the eye-irritating or -damaging potential of a pigment must be carried out before use as a tattoo pigment.

This evaluation is also essential for tattoo pigments used for possible episcleral tattooing on the eye.

In order to investigate experimentally the eye-irritating or eye-damaging potential of pigments, *in vitro* tests have to be performed according to OECD Test Guideline 491 (2.2.1a) and OECD Test Guideline 492 (22.2.1.b). The method designed for soluble substances (OECD Test Guideline 460) is considered unsuitable for pigment analysis. OECD Test Guideline 437 "Bovine Corneal Opacity And Permeability Test Method" and OECD Test

Guideline 438 "Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage" were not included in the test requirements because a human test system is already available and the information gain of a second non-human test system is questionable.

2.2.1.3 Test strategy

If data are available that meet the criteria for classification according to the CLP Regulation[46] or indicate other undesirable properties (see 2.2.1.1, column 2), the standard test requirements according to 2.2.1.1column 1 do not need to be performed and the corresponding pigment should not be used as a tattoo pigment. If no corresponding data are available, the *in vitro* tests for eye irritant effects may be performed. It is recommended to start the tests with the less complex test system, i.e. OECD Test Guideline 491 (2.2.1a) (test system cell culture) first. If the results are not relevant for classification according to the criteria, OECD Test Guideline 492 (2.2.1.b) (test system reconstructed cornea) may be conducted subsequently. Reference is also made here to OECD GD 263 (IATA - Serious eye damage and eye irritation)[47], which describes methods and their combination in test strategies.

2.2.1.4 Evaluation criteria/evaluation strategy

If the results of the tests according to OECD Test Guideline 491 or OECD Test Guideline 492 show a classification-relevant eye-irritating or eye-damaging effect of the pigment, the exclusion criterion (see Box 1) for use as a tattoo pigment is fulfilled.

2.2.2 Endpoint Skin irritation/corrosion

2.2.2.1 Check request

2.2.2	Endpoint Skin irritation/corrosion	
	Column 1 Standard test requirement	Column 2 Rules for derogation from column 1
2.2.2.2.2.2.a	OECD Test Guideline 439 "In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method". ➢ Classification as "skin irritant/corrosive" (category 1 or 2) or "non-irritant".	2.2.2.2.2.2.a need not be performed if for the substance ➢ the CLP criteria for classification as CMR (1A/B and 2), Skin Sens (1, 1A/B), Skin Corr./Irrit. (1, 1A/B/C, 2, 3 (GHS only)), Eye Dam./Irrit. (1, 2, 2B (GHS only)), STOT RE (1, 2), Acute Tox (1, 2) are met and/or the substance is harmonised in these endpoints. ➢ data are already available showing that the substance has phototoxic properties according to OECD guidelines.
Evaluation Criteria: OECD Test Guideline 439 allows classification as "skin irritant/skin corrosive" (category 1 or 2) or "non-irritant". In case of positive results, the use of the pigments in tattoo inks should generally be avoided.		

[46] ECHA (2017) Guidance on the Application of the CLP Criteria, https://echa.europa.eu/documents/10162/23036412/clp_en.pdf

[47] Guidance Document no 263 on Integrated Approaches to Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation

CMR: Carcinogenic, mutagenic and reprotoxic, Skin Sens: Skin sensitization, Skin Corr./Irrit.: Skin corrosion/irritation, Eye Dam./Irrit.: Serious eye damage/eye irritation, STOT RE: Specific target organ toxicity (repeated exposure), Acute Tox: Acute Toxicity

2.2.2.2 Standard test requirements

According to the REACH restriction (entry 75), skin irritating and corrosive substances above a certain concentration limit should not be used in Tattoo inks, as it is assumed that these substances may also have a corrosive or irritant effect intradermally. Therefore, a skin irritant or corrosive potential for tattoo pigments must be necessarily eliminated. For this purpose, an approach is proposed which requires the performance of only one test according to OECD Test Guideline 439 - thus allowing classification in at least category 2. A differentiation of Cat. 1 and Cat. 2 is not necessary. Test Guidelines 431 (In Vitro Skin Corrosion: Reconstructed Human Epidermis (RhE) Test Method) and 430 (Transcutaneous Electrical Resistance Test Method) only allow the identification of Cat. 1 substances, negative results would require additional testing according to OECD Test Guideline 439. Alternatively, a "bottom-up approach" described in OECD GD 203 for skin irritation/corrosion can be followed[48].

By means of OECD Test Guideline 439 (2.2.2.2.a) the skin corrosive properties of the pigments can be assessed. An unambiguous test result according to the relevant OECD Test Guideline Requirements (with the deviations listed accordingly) must be provided to allow evaluation according to the classification criteria as "skin damaging" (corrosive or irritant) or "non-irritant". A further differentiation of the subcategories of category 1 is not necessary (see assessment criteria).

2.2.2.3 Test strategy

If data are available leading to a classification according to CLP (see 2.2.2.1, column 2), the required standard test requirements according to 2.2.2.1, column 1 do not need be performed and the corresponding pigment should not be used as a tattooing pigment (cf. BOX 1). If no such data are available, testing for skin irritant or corrosive effects should be performed. If there is reasonable doubt that OECD Test Guideline 439 can be applied or the negative results are not reliable, the corresponding pigment should be excluded from use in Tattoo inks, further information can be found in the "Guidance on the Application of the CLP Criteria"[49].

2.2.2.4 Evaluation criteria/evaluation strategy

If OECD Test Guideline 439 (2.2.2.a) shows a classification-relevant skin-damaging effect of the pigments, the tested pigment should not be used for tattooing, as an exclusion criterion for use as a tattooing pigment is then fulfilled (see Box 1).

[48] OECD (2017), Guidance Document on an Integrated Approach on Testing and Assessment (IATA) for Skin Corrosion and Irritation, OECD Series on Testing and Assessment, No. 203, OECD Publishing, Paris, https://doi.org/10.1787/9789264274693-en.

[49] ECHA (2017) Guidance on the Application of the CLP Criteria, https://echa.europa.eu/documents/10162/23036412/clp_en.pdf

2.2.3 Endpoint phototoxicity

2.2.3.1 Check request

2.2.3	Endpoint phototoxicity	
	Column 1 Standard test requirement	Column 2 Rules for derogation from column 1
2.2.3.2.2.3.a	OECD Test Guideline 432 "In Vitro 3T3 NRU Phototoxicity Test". ➢ The measurement of the neutral red cannot be done colorimetrically. Either HPLC-/UPLC spectrometry or also here the use of the MTT method with a formazan measurement via HPLC-/UPLC spectrometry.	2.2.3.2.2.3.a need not be carried out if for the substance ➢ the CLP criteria for classification as CMR (1A/B and 2), Skin Sens (1, 1A/B), Skin Corr./Irrit. (1, 1A/B/C, 2, 3 (GHS only)), Eye Dam./Irrit. (1, 2, 2B (GHS only)), STOT RE (1, 2), Acute Tox (1, 2) are met and/or the substance is harmonised in these endpoints. ➢ data are already available showing that the substance has phototoxic properties according to OECD guidelines.
Evaluation Criteria: The *in vitro* test allows the assessment of the phototoxic properties of pigments. If there is a positive test result here, the pigments should not be used in tattoo inks.		

HPLC: High Performance Liquid Chromatography, UPLC: Ultra Performance Liquid Chromatography, CMR: Carcinogenic, mutagenic and reprotoxic, Skin Sens: Skin sensitization, Skin Corr./Irrit.: Skin corrosion/irritation, Eye Dam./Irrit.: Serious eye damage/eye irritation, STOT RE: Specific target organ toxicity (repeated exposure), Acute Tox: Acute toxicity

2.2.3.2 Standard test requirements

Exposure of a tattoo to solar radiation may have harmful effects if ingredients of the tattoo ink are photolabile and possible cleavage products lead to immediate or delayed effects such as genotoxicity (see chapter 2.2.6) or skin sensitization (photosensitization). Therefore, tattoo pigments have to be examined for their phototoxic properties.

Beyond the endpoints of the CLP Regulation, the assessment of the phototoxic potential of a pigment is carried out according to the Note of Guidance on Photosafety Testing (CPMP/SWP/398/01) of the Committee for Proprietary Medical Products. The supporting information in the questions and answers to this guidance (EMA/CHMP/SWP/336670/2010) should be followed. This study should be performed on substances that absorb light in the range 290-700 nm. Since most pigments absorb light in this range, the phototoxicity test is essential. Pigments are expected to have a molar extinction coefficient above 1000 L $mol^{-1}cm^{-1}$ (threshold for performing phototoxicity tests).

Phototoxicity

The *in vitro* 3T3 NRU phototoxicity assay is a cytotoxicity assay performed in the presence and absence of a light dose. The evaluation of the results shall be performed by HPLC/UPLC spectroscopy. OECD Test Guideline 495 "ROS (Reactive Oxygen Species) Assay for Photoreactivity" determines the ROS formation of irradiated chemicals with simulated sunlight. However, the scope of the ROS assay is currently limited to only those chemicals that meet the solubility criteria described in the protocol. Insoluble chemicals are not suitable for the assay, but can be tested with the addition of solubility enhancers. In the ROS assay, singlet oxygen generation is detected by spectrophotometric measurement of *p*-nitrosodimethylaniline (RNO) quenching followed by measurement of the decrease in absorbance of RNO at 440

nm. Due to the broad absorption spectrum of tattoo pigments, this measurement is considered to be of limited suitability.

Photosensitization

The endpoint photosensitization is relevant for tattoo inks. However, no validated methods are currently available for this. The SCCS (*Scientific Committee on Consumer Safety*) assumes that the endpoint of photosensitization could also be covered with the help of OECD Test Guideline 432 (SCCS/1602/18). Nevertheless, various *in vitro* methods are also currently discussed in the SCCS to adequately cover the photosensitization endpoint (such as the photo-h-CLAT, an NCTC2455 method and a photo-SH/NH_2 test).

Photogenotoxicity (see chapter 2.2.6)

As soon as further OECD test guidelines are developed and validated for the use of pigments as test substances, the standard test requirements can be extended to include these methods.

2.2.3.3 Test strategy

The test for phototoxicity is preceded by a literature search. If there is no evidence of phototoxic effects, the test should be performed according to OECD Test Guideline 432 (2.2.3.a).

2.2.3.4 Evaluation criteria/evaluation strategy

Chemicals that exhibit phototoxic properties according to OECD test guidelines or for which there is other sufficient evidence of phototoxic activity are not suitable for use in tattoo inks.

2.2.4 Endpoint skin sensitisation

2.2.4.1 Check request

2.2.4	Endpoint skin sensitisation	
	Column 1 Standard test requirement	Column 2 Rules for derogation from column 1
2.2.4.2.2.4.a	OECD Test Guideline 442C "Key-event-based test guideline for In Chemico Skin Sensitisation assays addressing the Adverse Outcome Pathway Key Event on covalent binding to proteins". ➢ Classification (according to UN GHS) as "skin sensitizing" or "not skin sensitizing", no subcategorization (para. 7)	Re 2.2.4.2.2.4.a As described in OECD Test Guideline 442C (Annex I, para 3), the method is not applicable to pigments containing metal ions (e.g. titanium dioxide).
2.2.4.2.2.4.b	OECD Test Guideline 442D "In Vitro Skin Sensitisation assays addressing the AOP key event on keratinocate activation".	

2.2.4	Endpoint skin sensitisation	
	Column 1 Standard test requirement	Column 2 Rules for derogation from column 1
	➢ Classification (according to UN GHS) as "skin sensitizing" or "not skin sensitizing", no subcat-egorization (para. 9)	
2.2.4.2.2.4.c	OECD Test Guideline 442E "In Vitro Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome Pathway for Skin Sensitisation". ➢ Classification (according to UN GHS) as "skin sensitizing" or "not skin sensitizing", no subcat-egorization (para. 7)	Re 2.2.4.2.2.4.c a) Not applicable for fluorescent pigments which are used in the wavelength range of fluorescein isothiocyanate (FITC) or propidium io-dide (PI). For this, the antibodies must be appropri-ately coupled with other fluorophores to exclude in-terference. (b) Test substances with a log P greater than 3.5 tend to produce false negative results. Therefore, negative results with test substances with a log P greater than 3.5 should not be considered. 2.2.4a, 2.2.2.4b, and 2.2.4c need not be per-formed if for the substance ➢ the CLP criteria for classification as CMR (1A/B and 2), Skin Sens (1, 1A/B), Skin Corr./Irrit. (1, 1A/B/C, 2, 3 (GHS only)), Eye Dam./Irrit. (1, 2, 2B (GHS only)), STOT RE (1, 2), Acute Tox (1, 2) are met and/or the substance is harmonised in these endpoints. ➢ data are already available showing that the substance has phototoxic properties according to OECD guidelines.
Evaluation Criteria: The *in chemico/in vitro* tests allow a classification (according to CLP regulation) as "skin sensitizing" or "not skin sensitizing", but no sub-categorization. In this context, 2 of the 3 tests are sufficient to make a statement, as described in the Defined Approach "2-out-of-3". In case of positive results in one of the required *in chemico/in vitro* tests, the use of the pigments in tattoo inks should generally be avoided. Alternatively, the De-fined Approach: "Integrated Testing Strategy (ITS)" should be considered. Here, Key Events 1 and 3 (442C and 442E) are addressed and include *in silico* prediction of skin sensitization[50]. Both defined approaches are published as TG 497 by the OECD.		

CMR: Carcinogenic, mutagenic and reprotoxic, Skin Sens: Skin sensitization, Skin Corr./Irrit.: Skin corrosion/irritation, Eye Dam./Irrit.: Serious eye damage/eye irritation, STOT RE: Specific target organ toxicity (repeated exposure), Acute Tox: Acute Toxicity

2.2.4.2 Standard test requirements

According to the REACH Regulation, sensitising substances above a certain concentration limit may not be used in tattoo inks.

Skin sensitizing substances may cause allergic contact dermatitis when applied to the epi-dermis or injected into the skin. Therefore, skin sensitization testing is required for tattoo pig-ments.

For the evaluation of the skin sensitizing properties of pigments, *in chemico/in vitro* test methods (OECD Test Guidelines 442C, 442D, and 442E) should be used as a matter of pri-ority, as they are also described for the testing of chemicals under the REACH Regulation[51]. When testing pigments, the deviations mentioned in Table 2.2.4.1be taken into account. For pigments with high molecular weights, the *in vitro* methods listed here (see 2.2.4.1, column

[50] Draft OECD Guideline Defined Approaches for Skin Sensitisation, https://www.oecd.org/env/ehs/test-ing/GL%20DASS_22Sep2019v2.pdf

[51] Table R.7.3-2 of the Guidance on IR&CSA - Chapter R.7a

1) may presumably prove to be more suitable than corresponding *in vivo* methods, taking into account the conditions determined for pigments. The reason for this is that dermal exposure in *in vivo* tests is not described as suitable for higher molar masses (500 g mol^{-1})[52]. Existing negative *in vivo* data are therefore not meaningful. Since some pigments exceed this molar mass limit, the required skin penetration cannot be assumed here. In the case of intradermal application, this step is bypassed. Therefore, a substance applied dermally *in vivo* and classified as non-sensitizing may have a sensitizing effect when applied intradermally. Thus, it can be assumed that validated *in vitro* methods for tattoo pigments provide more reliable data.

The methodological approach of the OECD Test Guidelines is based on the OECD[53] Adverse Outcome Pathways (AOPs) for Skin Sensitisation. Among other pathways, haptenisation (OECD Test Guideline 442C (2.2.4a)) and the activation of keratinocytes (OECD Test Guideline 442D (2.2.4b)) and dendritic cells (OECD Test Guideline 442E (22.2.4c)) can be investigated in the presence of the pigments.

2.2.4.3 Test strategy

At least two of the above mentioned methods (OECD Test Guidelines 442C, 442D and 442E) should be used. In case of positive results in one of the required *in chemico/in vitro* tests, the use of the pigments in tattoo inks should generally be avoided. Alternatively, the Defined Approach: "Integrated Testing Strategy (ITS)" should be considered. Here, Key Events 1 and 3 (442C and 442D) are addressed and include *in silico* prediction of skin sensitization[54]. In the future, other defined approaches may be used if their suitability has been confirmed by the OECD. The suitability of the test guideline for testing pigments should be reviewed. If necessary, an adaptation with scientific justification should be made.

Existing *in vivo* data according to OECD Test Guideline 406 "Skin Sensitisation" (Guinea Pig Maximisation Test (GPMT)) with intradermal application can be included in the evaluation. This *in vivo* test has the advantage of best simulating the tattooing process through intradermal puncturing and thus producing reliable data. Whereas non-invasive, often used *in vivo* methods such as the OECD Test Guideline 429 (LLNA), the preferred *in vivo* method according to the REACH standard requirements, do not provide certainty that a pigment for tattooing purposes may not have a sensitising effect after application, even if the results are negative[55].

2.2.4.4 Evaluation criteria/evaluation strategy

If the tests carried out show a skin sensitizing potential of the pigment, then an exclusion criterion are fulfilled. The tested pigment should not be used as a tattoo pigment. A differentiation into subcategories according to CLP is not necessary, since according to the REACH

[52] Bos, J.D. and Meinardi, M.M.H.M. (2000), The 500 Dalton rule for the skin penetration of chemical compounds and drugs. Experimental Dermatology, 9: 165-169. https://doi.org/10.1034/j.1600-0625.2000.009003165.x
[53] OECD (2014), The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins, OECD Series on Testing and Assessment, No. 168, OECD Publishing, Paris, https://doi.org/10.1787/9789264221444-en.
[54] Draft OECD Guideline Defined Approaches for Skin Sensitisation, https://www.oecd.org/env/ehs/testing/GL%20DASS_22Sep2019v2.pdf
[55] European Directorate for the Quality of Medicines & HealthCare (EDQM),2017, "Safer tattooing-Overview of current knowledge and challenges of toxicological assessment", 1st Edition

Regulation sensitizing substances above 0.001 weight percent may not be used in tattoo inks, regardless of the subcategory (1, 1A, and 1B).

2.2.5 Genotoxicity

2.2.5.1 Testing requirements

2.2.5	Mutagenicity/genotoxicity	
	Column 1 Check request	Column 2 Rules for derogation from column 1
2.2.5.2.2.5.a	OECD Test Guideline 471 "In vitro Bacterial Reverse Mutation Test	Re 2.2.5a, 2.2.2.5b and 2.2.5c: Uptake of the substance into the bacteria and mammalian cells used must be individually verified and demonstrated for each *in vitro test system* performed.
2.2.5.2.2.5.b	OECD Test Guideline 490 or 476 "In vitro Mammalian Cell Gene Mutation Test".	2.2.5a and 2.2.5b need not be carried out, ➢ if an appropriate valid *in vivo* mammalian genotoxicity test (test for gene mutations, e.g. OECD TG 488 or OECD TG 489) is available.
2.2.5.2.2.5.c	OECD Test Guideline 487 "In vitro Mammalian Cell Micronucleus Assay with fluorescence in situ hybridisation or immunochemical labelling of kinetochores" (CREST)	2.2.5.2.2.5.c need not be carried out, ➢ if a valid *in vitro* OECD test guideline 473 test or ➢ if a valid *in vivo* mammalian genotoxicity test (cytogenicity test, e.g. OECD TG 474, OECD TG 475 or OECD TG 489) is available. 2.2.5a, 2.2.2.5b, and 2.2.5c need not be performed if for the substance ➢ the CLP criteria for classification as CMR (1A/B and 2), Skin Sens (1, 1A/B), Skin Corr./Irrit. (1, 1A/B/C, 2, 3 (GHS only)), Eye Dam./Irrit. (1, 2, 2B (GHS only)), STOT RE (1, 2), Acute Tox (1, 2) are met and/or the substance is harmonised in these endpoints. ➢ data are already available showing that the substance has phototoxic properties according to OECD guidelines.
Evaluation Criteria: In case of positive results in one of the three required in *vitro* tests, *the* use of the pigments in tattoo inks should generally be discouraged unless a valid unequivocal negative *in vivo* comet assay (OECD Test Guideline 489) is available.		

CMR: Carcinogenic, mutagenic and reprotoxic, Skin Sens: Skin sensitization, Skin Corr./Irrit.: Skin corrosion/irritation, Eye Dam./Irrit.: Serious eye damage/eye irritation, STOT RE: Specific target organ toxicity (repeated exposure), Acute Tox: Acute Toxicity

2.2.5.2 Standard test requirements

For the endpoint genotoxicity, the test strategy is based on the performance of three selected *in vitro* mutagenicity tests. These tests determine whether the tattoo pigment has the potential to induce *in vitro* gene mutations or chromosomal aberrations. In case of positive results in one of these *in vitro* tests, the use of the pigments in tattoo inks should generally be avoided.

All three *in vitro* studies mentioned in Table 2.2.5.1must be performed with the pigment. This approach ensures that the potential of the substance to induce genetic mutations in bacteria

and mammalian cell cultures and structural and numerical chromosomal aberrations in mammalian cell cultures is investigated simultaneously. Since many pigments are expected to be insoluble particles, it is essential that the applicant verify that the substance is taken up by the bacteria or mammalian cells used. If no uptake of the substance into the cells can be shown, the validity of the test result must be doubted and, if necessary, another cell type such as macrophages must be used.

The following provides information and specific testing requirements for the required *in vitro* tests:

In vitro Bacterial Reverse Mutation Test (OECD Test Guideline 471) (2.2.5a)
The "in vitro Bacterial Reverse Mutation Test" (Ames test) can be used to detect gene mutations.

The test must be performed according to OECD Test Guideline 471 and therefore with and without metabolic activation using the following five bacterial strains: *S. typhimurium* TA98; TA100; TA1535; TA1537 (or TA97a or TA97); and one of the following *S. typhimurium* strains TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101).

If the tattoo pigment is an azo pigment, the test must be performed with and without metabolic activation according to the 'Prival modification' (see section 16 in OECD Test Guideline 471).

In vitro Mammalian Gene Mutation Test (OECD Test Guideline 490 or OECD Test Guideline 476) (2.2.5b)
This *in vitro* test is required to investigate the potential of the test substance to induce gene mutations in mammalian cells. The two *in vitro* tests: *"in vitro* mammalian cell gene mutation tests using the hprt and xprt genes" (OECD Test Guideline 476) and *"in vitro* mammalian cell gene mutation tests using the thymidine kinase gene" (OECD Test Guideline 490) are considered suitable to fulfil this standard test requirement. For OECD Test Guidelines 490 and 476, adaptations for particulate substances are generally necessary. For example, cytochalasin B should not be applied simultaneously with the particles, contrary to the test guideline, as it inhibits particle uptake.

In vitro Mammalian Cell Micronucleus Test with fluorescence in situ hybridization or immunochemical labelling of kinetochores (CREST) (OECD Test Guideline 487) (2.2.5c)
The '*in vitro* Mammalian Cell Micronucleus Test with fluorescence in situ hybridization or immunochemical labelling of kinetochores (CREST)' allows the detection of structural and numerical chromosomal aberrations *in vitro.*

Alternatively, there is the '*in vitro* Mammalian Chromosomal Aberration Test' (OECD Test Guideline 473), with which chromosomal aberrations can also be detected. However, this test is not optimally suited for the detection of numerical chromosomal aberrations and is not routinely used for this purpose. If a valid test according to Test Guideline 473 is available, testing according to Test Guideline 487 is not necessary. In addition, the nanomaterial-specific test guideline "Guidance Document on the Adaptation of In Vitro Mammalian Cell Based Genotoxicity TGs for Testing of Manufactured Nanomaterials" is under development and will be considered in the future.

2.2.5.3 Test strategy

The proposed testing strategy requires first the evaluation of *in vitro* findings. The *in vitro* tests required here correspond as far as possible to the *in vitro* mutagenicity tests required under the REACH Regulation as a standard data requirement. Depending on the tonnage, these could therefore already be required or available for registered tattoo pigments under REACH (see ECHA Dissemination Website). An exception is the additional requirement for 'kinetochore labelling' in the *in vitro* micronucleus test. This is considered important as it offers the possibility to identify numerical chromosomal aberrations (aneugenicity).

Prohibiting the use as a tattoo pigment on the basis of positive *in vitro* genotoxicity findings can be seen as a conservative approach, since for many substances that are positive *in vitro,* no genotoxic effect can be detected *in vivo.*

Existing *in vivo* genotoxicity tests and deviations from column 1:

Where valid *in vivo* mammalian genotoxicity tests are available, the test requirement in column 1 may be waived. Such test systems may include, but are not limited to:

- In vivo Mammalian Bone Marrow Chromosome Aberration Test (In vivo CA Test, OECD Test Guideline 475)
- In vivo Mammalian Erythrocyte Micronucleus Test (In vivo MN Test, OECD Test Guideline 474)
- Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo (UDS, OECD Test Guideline 486)
- Transgenic Rodent (TGR) Somatic and Germ Cell Gene Mutation Assays (OECD Test Guideline 488)
- In vivo Mammalian Alkaline Comet Assay (OECD TG 489)

Here it is important to note:

- Only valid tests performed according to the respective OECD test guideline are accepted for derogation. Important validity criteria here include, for example, for the *in vivo* CA test and the *in vivo* MN test*,* that exposure of the bone marrow to the substance has been demonstrated.
- The *in vivo* MN test and *in vivo* CA test are accepted only as a derogation from the *in vitro* cytogenicity tests (detection of chromosomal aberrations) required in column 1, but not as gene mutation tests.
- An available valid TGR test is only accepted as a deviation from the *in vitro* gene mutation tests required in column 1.
- A negative UDS test is not considered relevant as a negative test alone is not considered sufficient evidence that the substance does not induce gene mutations (see ECHA Guidance IR & CSR, Chapter R.7a: Endpoint specific guidance, Version 6.0- July 2017).

Genotoxicity testing does not need to be carried out for tattooing pigments if the criteria for classification as CMR set out in the CLP Regulation are met, as in this case entry 75 of Annex XVII of REACH applies and these pigments cannot be used for the purpose of tattooing above the concentration limits mentioned.

2.2.5.4 Evaluation criteria/evaluation strategy

In the case of positive results in one of the three required *in vitro* tests, the use of the pigments in tattoo inks should generally be avoided unless a corresponding valid unequivocal

negative test is available which invalidates the respective positive finding (e.g. for the induction of gene mutations or chromosomal aberrations) (e.g. an unequivocal negative *in vivo* comet assay (OECD Test Guideline 489).

2.2.6 Photogenotoxicity

2.2.6.1 Testing requirements

2.2.6	Photomutagenicity/photogenotoxicity	
	Column 1 Check request	Column 2 Rules for derogation from column 1
	The following is a list of *in vitro* mutation studies to be performed with irradiated (according to CPMP/SWP/398/01) pigments	Re 2.2.6a, 2.2.2.6b and 2.2.6c: Uptake of the substance into the bacteria and mammalian cells used must be individually verified and demonstrated for each *in vitro* test system performed.
2.2.6.2.2.6.a	According to OECD Test Guideline 471 "Photo-In vitro Bacterial Reverse Mutation Test".	
2.2.6.2.2.6.b	According to OECD Test Guideline 490 or 476 "Photo-In vitro Mammalian Cell Gene Mutation Test".	2.2.6a and 2.2.6b need not be carried out, ➢ if a corresponding valid *in vivo* genotoxicity test in mammals (test for gene mutations) is available.
2.2.6.2.2.6.c	According to OECD Test Guideline 487 "Photo-In vitro Mammalian Cell Micronucleus Assay with fluorescence in situ hybridisation or immunochemical labelling of kinetochores" (CREST)	2.2.6.c need not be performed, ➢ if a valid *in vitro test* according to OECD Test Guideline 473 or ➢ if a valid *in vivo* mammalian genotoxicity test (cytogenicity test) is available.
		2.2.6a, 2.2.2.6b, and 2.2.6c need not be performed if for the substance ➢ the CLP criteria for classification as CMR (1A/B and 2), Skin Sens (1, 1A/B), Skin Corr./Irrit. (1, 1A/B/C, 2, 3 (GHS only)), Eye Dam./Irrit. (1, 2, 2B (GHS only)), STOT RE (1, 2), Acute Tox (1, 2) are met and/or the substance is harmonised in these endpoints. ➢ data are already available showing that the substance has phototoxic properties according to OECD guidelines.

CMR: Carcinogenic, mutagenic and reprotoxic, Skin Sens: Skin sensitization, Skin Corr./Irrit.: Skin corrosion/irritation, Eye Dam./Irrit.: Serious eye damage/eye irritation, STOT RE: Specific target organ toxicity (repeated exposure), Acute Tox: Acute Toxicity

For the testing of tattoo pigments, phototoxicity is considered relevant. Therefore, the applicant shall also perform photogenotoxicity tests.

The methods described by the Society for Environmental Mutation Research (GUM) Working Group also include the photo-ames test, the photo-HPRT/photo-mouse lymphoma test, the photo-micronucleus test and the photo-chromosome aberration test. In many cases, the concurrent use of irradiation in the conduct of a standard mutagenicity/genotoxicity study does not result in significant changes to the existing OECD protocol without irradiation. Therefore, the majority of photomutagenicity/photogenotoxicity tests described are considered valid (SCCS, 2019).

In general, a UV-Vis spectrum of the substance should be provided together with a molar extinction coefficient (MEC) determined by a validated method.

Photomutagenicity need not be investigated if a valid negative phototoxicity test is available or if the substance has a molar extinction coefficient < 1000 L $mol^{-1}cm^{-1}$.

The test strategy for photogenotoxicity is identical to the test strategy for genotoxicity described in Chapter 2.2.5and is not described again here. The tests are to be performed analogously to the OECD test guidelines, with simultaneous application of irradiation. Recommendations on irradiation can be found in the Note for Guidance on Photosafety testing, CPMP/SWP/398/01.

3 Toxicological testing requirements to be developed and assessment of exposure

3.1 Toxicological testing requirements to be developed - not yet operable

The intradermal route of application is not considered for the evaluation of the safety of chemicals under REACH (and CLP) and of cosmetic products. Beyond the requirements listed in Chapter 2it is therefore necessary to develop testing requirements that take into account the prediction of systemic exposure to tattooing pigments and their toxicity after intradermal application. The particulate form and insoluble nature (or low solubility) of pigments play an important role in the test guidelines to be developed.

3.1.1 Identification of leachable components from pigments and application of a threshold concept for soluble substances

Beyond the requirements listed in chapter 2, and in addition to the current maximum restriction concentrations under REACH, the determination of leachable components from pigments should be carried out in the future. For soluble substances, a threshold value concept may be considered.

Suitable *in vitro* tests, e.g. on tissues, homogenates or cells, can be used to analyse components that can be released from pigments (DIN EN ISO 10993-16:2018-02). Resulting particles and metabolites should be identified and quantified. Such an approach is also pursued for medical devices such as implants, as similar issues exist here. In order to simulate the characteristic milieu of skin, lymphatic fluid and blood, the specific adaptation of the analysis of the released components from tattoo pigments is planned. In the absence of toxicological data, the application of the "Threshold of Toxicological Concern" (TTC) concept will be tested to estimate the adverse health effects of these substances. The applicability of TTC has already been assessed for cosmetic products by the SCCS and is considered appropriate for substances within the scope[56]. The data obtained on the released substances will be used in *in silico* methods such as QSAR and PBPK models to predict toxicokinetic properties and in grouping approaches. For soluble substances, existing toxicologically derived No Observed Adverse Effect Levels (NOAELs) from studies with oral or intravenous application can be extrapolated. The margin of safety (MoS), i.e. the ratio between the NOAEL and the estimated exposure, can then be calculated. Pigments should be excluded from further use in tattoo inks if they show a release of harmful substances above relevant toxicological thresholds in

[56] https://ec.europa.eu/health/sites/default/files/scientific_committees/consumer_safety/docs/sccs_o_250.pdf

this analysis. Chemical identification of substances released from pigments must precede *in vivo* toxicokinetics testing.

3.1.2 Toxicokinetics of tattoo pigments

Toxicokinetics refers to the uptake, distribution, metabolism and excretion of a substance. While toxicokinetic studies after oral and dermal uptake or inhalation are already well established, the intradermal application relevant for the evaluation of tattoo pigments has so far been less well studied. There is currently a lack of information on the fate of pigments in the organism and on their occurrence in possible target organs. On the basis of toxicokinetic studies, it will be possible to select sensible dosages for *in vivo* studies and to transfer the results obtained in *in vitro* systems to the whole body situation as well as to parameterize *in silico* models (e.g. PBPK models).

Pigments are deposited in the skin after tattooing and can form local accumulations (aggregates). As a consequence, translocation is observed, i.e. the "migration" of pigments out of the tattoo. This can begin immediately after tattooing and occur over an extended period of time thereafter. According to a study by Engel et al (2010)[57], a reduction of 32% of an inserted pigment was observed over a period of 42 days. These processes are likely to be substance and particle form specific and could lead to systemic toxicity if the substances enter the blood and target organs. This particularly concerns the phase immediately after tattooing, when a considerably higher redistribution is to be expected than after healing of the tattooed skin area. At present, data on migration and metabolism are not sufficiently available. Therefore, several scenarios must currently be used for an exposure estimate.

A prerequisite for a validated *in silico* model is the availability of supporting *in vivo* data. The design of the toxicokinetic studies can be developed on the basis of OECD Test Guideline 417:2010 "Toxicokinetics". However, as this is not specifically designed for the testing of pigment particles, further aspects should be considered, which are described in various guidance documents. Validated extraction and analytical methods should be used. For the design of a toxicokinetic study, aspects of ISO/TR 22019:2019 "Nanotechnologies - Considerations for performing toxicokinetic studies with nanomaterials" should be taken into account. In addition, guidance documents on the toxicokinetics of nanomaterials and their detection in tissues are under development at OECD level. These are to be taken into account in the future. In addition, a specific guideline for nanoparticles is being developed within the Working Party on Manufactured Nanomaterials (WPMN)[58].

Results on toxicokinetics could help to explain the toxicological findings obtained with pigments, e.g. the occurrence of certain toxic effects could be associated with the formation of one or more metabolites or the accumulation of certain pigments or their degradation products in a specific target organ. In addition, it is possible to establish a connection to possibly existing studies with other forms of application on the basis of toxicokinetic information on a pigment administered intradermally.

[57] Engel E et al, (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(1):54-60. https://doi.org/10.1111/j.1600-0625.2009.00925.x

[58] Project 4.146: Development of new Test Guideline on toxicokinetics to accommodate testing of nano-particles (UK/NL)

3.1.3 *In vivo* testing of selected pigments

With regard to animal experiments, there are legal provisions that apply throughout Europe. Directive 2010/63/EU on the protection of animals used for scientific purposes sets the legal framework that must be followed. If, beyond the data presented in Chapter 2for pigments in tattoo inks, *in vivo* studies are considered necessary for a robust assessment, the evaluation of the European Commission with regard to the question of the (ethical) permissibility and indispensability of animal testing is therefore decisive for the safety assessment of the substances concerned. Since tattoo inks are not only used in Germany, there should be coordination at EU level on the uniform consideration of animal testing in connection with safety assessments of tattoo inks.

From a scientific point of view, most chronic endpoints cannot currently be adequately assessed without *in vivo* testing on animal models. For example, an assessment of chronic toxic effects on organs as well as carcinogenicity and reproductive toxicity is not possible. It is noted that tests on vertebrate animals may only be performed when all other data sources have been "exhausted" or are not considered adequate for the health evaluation of the tattoo pigment.

Where possible, a combination of *in vivo* tests should be used to minimise the number of animals used. Necessary in vivo tests shall be performed according to the relevant OECD test guidelines and GLP.

For *in vivo* studies*,* a suitable application of tattoo pigments must be selected. This must realistically represent the tattooing process. Such studies, on the other hand, must consider aspects such as applicability and bioavailability and ensure minimization of animal suffering. Since for most pigments a low oral bioavailability of the particles has to be assumed, their results can only be transferred to the situation after tattooing if a systemic distribution in the body through the bloodstream has been shown.

The endpoints eye irritation/damage, skin irritation/corrosion, phototoxicity, skin sensitisation, genotoxicity and photogenotoxicity can be assessed by appropriate *in vitro/in chemico* methods. However, some other toxicological effects (such as chronic toxicity and carcinogenicity) cannot be excluded at this stage, as OECD test guidelines are available for the application, but these have not yet been established for intradermal application. If subcutaneous or intravenous administration in animal studies is considered as an alternative route of administration, possible adverse effects have to be taken into account. In contrast to soluble substances, particulate pigments may lead to effects caused by bolus application, which may result in vascular occlusion, for example. This in addition to possible substance specific toxicity.

After the basic clarification of the handling of animal experiments in connection with safety tests of tattoo inks, open questions on the above-mentioned possible toxicological effects could be investigated, if necessary, in an animal model on pigs using selected representative pigments. The study could be conducted based on TG 417. Data on toxicokinetics should also be obtained as part of the study (see 3.1.2).

In the case that *in vivo* testing becomes necessary and would be considered legal, an animal model should be chosen which allows an observation period of at least two years and whose skin properties are similar to that of humans. The best comparability to the subsequent use

of tattoo pigments has the intradermal application. The comparatively thin skin in rodents presents a challenge for intradermal application that may not be practical under experimental conditions. For this reason, intradermal application in rodents is not considered operable. Intradermal studies on pigs, whose skin is more comparable to human skin than rodent skin, are conceivable and are already used as models for other dermally applied products. The pig could be used as part of a representative study to address questions of systemic distribution, metabolic processes and internal exposure. Such a representative study should provide the basis for deciding on the suitability of pigments for tattooing and on the need for further studies. In particular, aspects such as organ accumulation and long-term effects can be studied more realistically on pigs and allow better extrapolation to humans. Such a study can clarify the *in vivo* bioavailability of pigments and their metabolites. It can also assess whether different pigments differ with respect to these parameters. Pigment and metabolite concentrations in internal organs such as liver, spleen, kidneys, lymph nodes, heart, brain and gonads provide information on distribution and accumulation. They can also be compared with those from other studies in order to achieve a quantitative assessment of pigment distribution after intradermal application in relation to other routes of application.

In addition to the monitored clinical parameters, the recovery of the test substance represents an important component for the determination of pigment distribution, its accumulation and excretion. Furthermore, the detection of the pigments and their degradation products in the tissue could provide clues to the mechanisms of the observed effects. The pigments selected for the experiment could be prepared with a suitable label for their subsequent detection and quantification in animal organs. The properties of the pigments used should be similar to the pigments available on the market. The data obtained will be used for the development of a QSAR model of intradermal exposure of pigments.

3.2 Exposure

For the determination of the exposure as part of the risk assessment, different information regarding the product (tattoo ink), the application by the tattoo artist and the tattooed individual is used. Concerning the pigments contained in the tattoo ink, the pigment concentrations are required as a minimum for the exposure estimation (see description below). More refined assessments also require information on the composition, including possible impurities, and on the physico-chemical properties of the pigments. Information on the corresponding tattoo ink is beneficial. The data collection methods required for this have already been described above.

Description of the information currently available for exposure assessment with its uncertainties:

In the context of ECHA's restriction proposal for substances in tattoo inks and permanent make-up[59], an estimation of human exposure was carried out with the cooperation of BfR. The exposure scenario is based on individual tattooing sessions in which a maximum skin area of 300 cm^2 is tattooed per session and day. The tattooed skin area was determined in a survey of Danish tattoo artists conducted for the purpose of the restriction project. In this respect, the restriction dossier assumed an interval between tattooing sessions of approximately four weeks. The amount of tattooing agent applied per cm^2 was based on a study by

[59] ECHA (2017) ANNEX XV Restriction Report, Proposal for a Restriction: Substances in tattoo inks and permanent make up https://echa.europa.eu/documents/10162/6f739150-39db-7e2c-d07d-caf8fb81d153

Engel et al.[60] in which the amount of "Pigment Red 22" was quantified. This pigment was applied *ex vivo* to pig skin and human skin by both professional tattoo artists and scientists. For a tattooing agent containing 25 % pigment Red 22 by mass, the amount of pigment per skin area in a range of 0.60-9.42 mg/cm^2. Based on the 75th percentile of these values, corresponding to an amount of 3.59 mg of pigment per cm^2 of skin area for an ink with a mass fraction of 25 %, a total injected amount of tattooing agent of 14.36 mg/cm^2 was used in the restriction dossier for the exposure estimate and quantitative risk assessment. This proposal has been followed by the ECHA Risk Assessment Committee.

There have been only few more recent studies dealing with the amount of pigment deposited per tattooed skin area since 2016. In most publications after 2016, the above-mentioned study by Engel et al. from 2008 is cited and the mean value of 2.53 mg/cm^2 described therein for the amount of pigment per skin area is mentioned or used for further considerations[61]. This mean value results from all tests in the above-mentioned study (with 10 or 25 % pigment) and was not used for the above-mentioned restriction dossier in favour of the more conservative value of 3.59 mg/cm^2, but is in the comparable range.

Arbache et al. 2019 investigated the amount of drug that can be delivered *ex vivo* into human skin using a tattoo machine. This included tattooing 10 skin biopsies from abdominoplasty surgeries with "Electric Ink" brand black tattoo ink (density = 1.272 g/ml) and then quantifying the amount of ink remaining in the skin[62]. The amount of ink in a range of 0.291-2.371 mg/cm^2 (standard deviation = 0.601 mg/cm^2), and the mean value corresponded to 1.175 mg ink/cm^2 skin area. The pigment content of the ink was not reported in the study. However, assuming a typical pigment concentration of between 20 % and 45 %[63] based on the mean value of 1.175 mg ink/cm^2 skin area, this results in a range of 0.235-0.529 mg pigment/cm^2 skin area. For an ink with 25 % pigment content a value of 0.294 mg/cm^2 is resulted.

Butterfield et al. investigated the functionality of intradermally applied UV nanosensors in the form of tattoos both in pig skin *ex vivo* and *in vivo* in human skin. For this purpose, among others, a 10 % photochromic tattoo ink with nano-encapsulated UV sensors was prepared

[60] Engel E et al, (2008). "Modern tattoos cause high concentrations of hazardous pigments in skin." Contact Dermatitis 58(4): 228-233.
[61] Bäumler, W. (2016). "The possible health consequences of tattoos. "Deutsches Ärzteblatt International 113(40): 663-665,
Laux, P. et al, (2016). "A medical-toxicological view of tattooing." The Lancet 387(10016): 395-402,
Schreiver, I. and A. Luch (2016). "At the dark end of the rainbow: data gaps in tattoo toxicology." Arch Toxicol 90(7): 1763-1765,
Sepehri, M. et al, (2017). "Tattoo Pigments Are Observed in the Kupffer Cells of the Liver Indicating Blood-Borne Distribution of Tattoo Ink." Dermatology 233(1): 86-93,
Bocca, B. et al, (2018). "Hexavalent chromium in tattoo inks: Dermal exposure and systemic risk." Contact Dermatitis 79(4): 218-225,
Belikov, A. V. et al, (2019). "Experimental modeling of the physical process of laser tattoo removal." Quantum Electronics 49(1): 52-58,
Bäumler, W. (2020). "Chemical hazard of tattoo colorants." Presse Medicale 49(4),
Giulbudagian, M. et al, (2020). "Safety of tattoos and permanent make-up: a regulatory view." Archives of Toxicology 94(2): 357-369,
Herring, H. et al., "TatS: a novel in vitro tattooed human skin model for improved pigment toxicology research." (7): 2423-2434.
[62] Arbache, S. et al, (2019). "How much medication is delivered in a novel drug delivery technique that uses a tattoo machine?". International Journal of Dermatology 58(6): 750-755.
[63] JRC, 2015. Safety of tattoos and permanent make-up - State of play and trends in tattoo practices, Report on Work Package 2, s.l.: European Commission Joint Research Centre.

and tattooed[64] as a "solar freckle". Based on Cu et al.[65], it is assumed that 3 % of the amount of ink used by the tattoo machine[66] is injected during tattooing. Thus, a maximum amount of 3 mg of ink, or 0.3 mg of nanocapsules, is obtained in a "solar freckle" with a radius of 1 mm or 3.14 mm^2 of skin area. This corresponds to an ink amount of 95.54 mg/cm^2 or a nanocapsule amount of 9.554 mg/cm^2 for an ink with a mass fraction of 10 % nanocapsulated UV sensors. Alternatively, the mean pigment amount of approximately 2.5 mg/cm^2 in treated tattooed skin quantified by Engel et al. 2008 is also used by Butterfield et al. to calculate the final mass of photochromic nanocapsules per "solar freckle" (0.079 mg/cm^2). Both data on the final nanocapsule quantity can be equated in a figurative sense with the final pigment quantity in the skin.

The above information shows that there are still major uncertainties with regard to the amount of tattooing agent introduced and that the data situation must be regarded as insufficient. The exposure estimate from the REACH restriction dossier is conservative; it estimates a high amount of 1.077 mg pigment related to a skin area of 300 cm^2 or 3.59 mg pigment per cm^2 skin. In addition, the data on pigments in the marketed end product given in Chapter 2.1are also necessary for the assessment. Currently, the BfR is striving to conduct a short-term bioavailability study that, in addition to the systemic availability of soluble ingredients, will also quantitatively determine the area tattooed in one session and the applied colour per cm^2 in a human study. Initial results of this study are expected in the spring of 2022. With this information, exposure could be placed on a more accurate data basis in the future.

Further information on the subject of tattoos from the BfR website

FAQ on tattoo inks: https://www.bfr.bund.de/cm/349/faq-about-tattoo-inks.pdf

BfR Opinion: Risk assessment for Pigment Blue 15:3 and Pigment Green 7: https://www.bfr.bund.de/cm/349/tattoo-inks-risk-assessment-for-pigment-blue-15-3-and-pigment-green-7.pdf

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About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. The BfR advises the Federal Government and the States ('Laender') on questions of food,

[64] Butterfield, J. L. et al, (2020). "Solar freckles: long-term photochromic tattoos for intradermal ultraviolet radiometry." ACS Nano 14(10): 13619-13628.
[65] Cu, K. et al, (2020). "Delivery Strategies for Skin: Comparison of Nanoliter Jets, Needles and Topical Solutions." Ann Biomed Eng 48(7): 2028-2039.
[66] Average amount of tattooing agent in a 12-gauge 7RS tattoo needle after 1 s of immersion: 98 ± 16 mg over 5 trials (Butterfield et al., 2020).

chemical and product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.

This text version is a translation of the original German text which is the only legally binding version.