Danish Ministry of the Environment
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Recommendation from the Danish Environmental Protection Agency on the safety of Tattoo Ink

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Foreword

The recommendation presented in this document has been prepared by Sie Woldum Tordrup, Danish Technological Institute for the Danish Environmental Protection Agency (EPA) in the period from September 2013 to May 2014.

In the process of preparing this guideline manufacturers and users of tattoo ink as well as consultants deemed qualified to perform safety assessments of tattoo ink has commented on the presentation of the requirements.

Summary

The recommended requirements of tattoo ink

Tattoo ink should not endanger the health and safety of a person when applied and used as intended. In an effort to reduce the health risks associated with getting a tattoo, the Danish EPA has therefore prepared a set of recommendations concerning the safety of tattoo inks. The recommendation can be summarised as follows:

- **A safety assessment should be carried out for tattoo ink to ensure that it does not constitute a risk to human health when used for tattooing.**

  This means that the chemical content as well as other relevant properties of the tattoo ink should be assessed by a competent person in order to make sure that the ink can be considered safe to use for tattooing.

- **Tattoo ink should under no circumstances contain ingredients classified as carcinogenic, mutagenic or toxic for reproduction.**

  This means that no ingredient classified as carcinogenic, mutagenic or toxic for reproduction (CMR) should be added in the tattoo ink even if a safety assessment indicates that no risk to human health is to be expected due to the presence of a CMR substance.

- **Tattoo ink should under no circumstances contain the following substances above the specified threshold value:**
  - **Azo dyes which under degradation will release primary aromatic amines**
  - **Poly aromatic hydrocarbons (PAH) which can be found as impurities in, e.g., carbon black used in black inks**
  - **Lead**

  This means that the tattoo ink should not contain any of these substances above the threshold value set in this recommendation.

- **Tattoo ink should be labelled - giving the customer the possibility to check the content of the ink.**

  A list of ingredients should be available to the customer. The list should include any ingredient added in an amount above 1% of the ink. Regardless of the content, any ingredient, which is classified as an allergen, should also be listed on the label.

- **Tattoo ink should be sterile when unopened to reduce the risk of infections and other related complications following tattooing.**

  Getting a tattoo always involves some risk of infections. It is important that the work of the tattooist is as sterile as possible; and using a sterile ink will also help reduce the risk of infection.

- **Tattoo ink should be clearly marked with an expiry date and the products should only be sold and used within that date.**

  An expiry date for tattoo ink should be set according to the expected stability of the products with regard to, e.g., physical/chemical properties as well as other properties related to the safe use of the ink. This date should be clearly visible on the tattoo ink container in order to make it easy for the user as well as the customer to check before tattooing with the ink.

1. Background

In 2011, a survey of the 65 most used tattoo inks on the Danish market was conducted by the Danish Environmental Protection Agency (Danish EPA). The survey showed that many of the inks contained carcinogenic substances (Jacobsen et al, 2012). Based on the survey, the Danish EPA conducted a risk assessment showing that the concentration of the carcinogenic substances in some inks was so high that they probably would constitute an unacceptable health risk for those being tattooed with the inks (Danish EPA, n.d.).

In an effort to reduce the health risks associated with getting a tattoo, the Danish EPA has now prepared this recommendation concerning tattoo inks.

1.1 Scope of the recommendation

A tattoo ink is defined as any substance or mixture used for tattooing. In the context of the recommendation, the definition of tattooing covers both the introduction of tattoo ink into the dermis and under the horn layer (*stratum corneum*) of the epidermis. This means that also ink for permanent make-up is included in the scope of the recommendation.

1.2 Objective of the recommendation

The objective of this document is to list and describe the technical elements of the requirements for tattoo inks and permanent make-up, which are currently recommended by the Danish EPA.

Besides the description of the technical requirements recommended for tattoo ink detailed guidelines for safety assessment of tattoo ink has been prepared and is included as an appendix to this recommendation. Further, this guideline gives suggestions on how to obtain the information required for each section of the safety assessment and illustrates - by using specific examples - how to use it in order to document the safety of tattoo ink

2. Technical requirements recommended for tattoo ink

2.1 Safety assessment

Tattoo ink should not endanger the health and safety of a person when applied and used as intended. To ensure safe use of tattoo ink a safety assessment should be carried out. The safety assessment should be carried out in accordance with the guidelines given in Appendix 1, ensuring that a specific chemical composition of a tattoo ink does not adversely affect human health when used for tattooing.

It is recommended that a safety report should be compiled based on the assessment, summarizing the results of the assessment. The conclusion of the assessment should be clearly stated. If the product is safe, it is recommended to use the following wording “the tattoo ink does not constitute a risk to human health when used for tattooing”. If a safe use cannot be documented, the tattoo ink should not be used for tattooing and compiling a report is not relevant.

2.2 CMR substances in tattoo inks

A tattoo ink is a substance or mixture used for tattooing. A tattoo ink typically comprises several ingredients such as pigments, solvents, stabilisers, wetting agents and thickening agent. An ingredient of tattoo ink is any substance added to tattoo inks, whether it is added as a substance or as part of a mixture. Impurities within the raw materials are not deemed to be ingredients. The same goes for technical auxiliary substances used for manufacturing tattoo ink that are not part of the final product.

In order to minimize the risk associated with getting a tattoo, it is not recommended to import, sell or use tattoo inks, which contain ingredients classified as carcinogenic, mutagenic or toxic to reproduction (CMR) in Categories 1A or 1B in accordance with the European regulation on classification, labelling and packaging (the CLP regulation, Regulation (EC) No. 1272/2008), or those which are to be classified as carcinogenic, mutagenic or toxic to reproduction in Categories 1 or 2, in accordance with the Order on classification, packaging, labelling, sales and storage of substances and mixtures.

2.2.1 Regulation in REACH of CMR-substances in consumer products

Tattoo inks can be seen as consumer products since they are injected into the skin of consumers (REACH, Regulation (EC) no. 1907/2006).

According to REACH (Regulation (EC) No. 1907/2006) the concentration of CMR substances in any ingredient applied in a consumer product must be below the limits given in the CLP regulation for classification (Regulation (EC) No. 1272/2008).This means that for an ingredient containing a substance classified as carcinogenic or mutagenic in categories 1A or 1B where no other information is available the maximum concentration allowed in the ingredient is 0.1% according to the CLP regulation (Regulation (EC) No. 1272/2008). For a substance classified as toxic to reproduction in

category 1A or 1B where no other information is available the maximum concentration allowed in the ingredient is 0.3% according to CLP regulation (Regulation (EC) No. 1272/2008).
In the guidelines for safety assessment of tattoo ink, section 4.1 of Appendix 1 of this recommendation, the CLP regulation is described in further details including links to relevant homepages and databases.

If a CMR substance is present as an impurity below the classification limits described above the substance should be assessed in the safety assessment of the tattoo ink.

Example 1: Content
A content of 0.2% cadmium oxide (CAS No. 1306-19-0) is stated on the SDS of an ingredient for tattoo ink. Cadmium oxide is classified as Carc. 1B with H350: May cause cancer (The harmonised classification can be accessed via the Classification and Labelling Inventory at www.ECHA.com). For cadmium the limit value is a maximum 0.1% before classification is required. A content of 0.2% therefore means that classification of the ingredient is required and that it is not allowed in consumer products.

Conclusion: This ingredient is not allowed in tattoo ink.

2.3 Primary aromatic amines, PAH and lead in tattoo inks

Some substances are considered to be of particular concern with regards to tattoo ink; primary aromatic amines, polyaromatic hydrocarbons (PAH) and lead. In order to minimize the risk associated with getting a tattoo, it is recommend not to import, sell or use tattoo inks:

- which contain azo dyes which, by reductive cleavage of one or more azo groups, may release one or more of the primary aromatic amines listed in Table 1, Nos. 1-9 that exceed the threshold values stated;
- which contain substances — listed in Table 1, Nos. 10-12 — which are over the threshold values stated therein.

Tattoo ink should not contain azo dyes, which by reductive cleavage of one or more azo groups may release any primary aromatic amine listed in Table 1, numbers 1-9, above the threshold values given. Eight of the nine primary aromatic amines are also included in the Council of Europe's Resolution on requirements and criteria for the safety of tattoos and permanent make-up, ResAp (2008)1 (Council of Europe, 2008), however, one additional primary aromatic amine (Aniline) has been included by the Danish EPA in this recommendation. The content of Aniline in tattoo inks on the Danish market was confirmed in the survey made by the Danish EPA (Jacobsen et al, 2012) and in some inks the substance constituted an unacceptable risk to human health (Danish EPA, n.d.).

Other substances considered to be of particular concern are polyaromatic hydrocarbons (PAH) and lead. PAHs are known for their carcinogenic properties and lead is considered a neurotoxic metal even at low concentrations. Tattoo ink should therefore not contain the PAH and lead as listed in Table 1, numbers 10-12, in a concentration above the threshold values given.

For the substances no. 1-12 in Table 1, the threshold values are much lower than the minimum concentration requirements for the listing as a CMR substance in category 1A and 1B in an EU safety datasheet (SDS). Therefore information on content of these substances of particular concern must be retrieved either from the manufacturer or by performing an analysis of the content in the product.

Analytical methods developed for the purpose of chemical analysis of these substances in tattoo ink are referenced to in Chapter 4 of the survey on tattoo inks conducted in 2011 by the Danish EPA

(Jacobsen et al 2012). Relevant analytical methods are also given in ResAP (2008)1, standards for toys (EN 71-7:2002) and/or textiles (EN 14362-1). The methodologies might need minor adjustments in order to be suitable for tattoo inks.

No.	CAS No.	Name	Limit value (ppm)
1	95-69-2	4-chlor-o-toluidine	10
2	99-55-8	5-nitro-o-toluidine	10
3	106-47-8	4-chloraniline	10
4	615-05-4	4-methoxy-m-phenylenediamine	10
5	91-94-1	3.3'-dichlorobenzidine	10
6	95-53-4	o-toluidine	10
7	95-80-7	4-methyl-m-phenylenediamine	10
8	90-04-0	o-anisidine	10
9	62-53-3	Aniline	5
10	50-32-8	Benzo(a)pyrene	0.2
11	192-97-2, 56-55-3, 218-01-9, 205-99-2, 205-82-3, 297-08-9, 53-70-3	The total amount of: benzo(e)pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene and dibenzo(a,h)anthracene	2
12	7439-92-1	Lead	10

TABLE 1: RECOMMENDED THRESHOLD VALUES FOR SUBSTANCES OF PARTICULAR CONCERN IN TATTOO INK

Example 2: Content
A content of 5.0 µg/g benzo(a)pyrene (CAS 50-32-8) is detected analytically in a tattoo ink using GC/MS analysis as described by Jacobsen et al (2012). Benzo(a)pyrene is also classified as Carc. 1B with H350: May cause cancer (The harmonised classification can be accessed via the Classification and Labelling Inventory at www.ECHA.com). Benzo(a)pyrene is, however, listed with a specific limit value of 0.2 ppm in Table 1. 5.0 µg/g benzo(a)pyrene corresponds to 5 ppm benzo(a)pyrene which is above the threshold value.

Conclusion: With a content of benzo(a)pyrene above the threshold value the tattoo ink is not recommended for tattooing.

2.4 Labelling

It is recommended that the following information should be stated on the tattoo ink container in indelible, legible and visible letters for the consumer or user to read:

- the manufacturer's company name and address, as well as the importer's company name and address, if this person is importing for resale.
- the container's nominal amount (nominal mass or nominal volume).
- the expiry date; the wording "May not be used after ..." should be placed before the expiry date, and the date should be clearly stated, either as 'month and year' or 'day, month and year'. If necessary, the conditions under which the shelf life can be maintained may be stated.
- the batch number of manufacture or reference in order to identify the tattoo ink.
- the list of ingredients. The list should begin with the word "Ingredients". The ingredients should be listed in descending order according to their weight at the time they were added to the ink. Ingredients with a concentration of less than 1% do not need to be listed, unless the substance is or should be classified as a skin sensitiser. The information should be stated using international nomenclature. 'International nomenclature' means substance names in the INCI (International Nomenclature of Cosmetic Ingredients), the EINECS (European Inventory of Existing Commercial chemical Substances) or the ELINCS (European List of Notified Chemical Substances). If the substance is not found on the aforementioned lists, then the ISO or IUPAC names can be used. Dyes can be stated using their Colour Index (CI) Constitution Numbers.

For products imported, sold or used on the Danish market, the labelling, in accordance with the above specifications should be in Danish or English.

If it is not practically possible to state the necessary information described above on the ink contained in the required manner, then the information should be stated on the label or similar note which should be affixed to the tattoo ink container.

Information on international nomenclature for ingredients can be found in the guidelines for safety assessment of tattoo ink, section 4.1 of Appendix 1 of this recommendation and further details on how to set an expiry date can be found in section 2.6.

2.5 Sterility

To reduce complications related to infections caused by microorganisms originating from the tattoo ink it is recommended that tattoo inks should be sterile in unopened condition. This could be accomplished by sterilization of the ink product following production. Sterilization of the tattoo ink may be carried out by using a standard method such as ionizing radiation sterilization, ISO 11137. Unopened tattoo ink should comply with sterility test in accordance with the European Pharmacopoeia, chapter 2.6.1. The sterility test is a biological challenge test where media containing the product is incubated with a certain amount and type of microorganisms and the growth is monitored under specified growth conditions. If no growth occurs, the product does not sustain microbial growth and complies with the tests.

2.6 Expiry date

An expiry date should be set according to the expected stability of the tattoo ink. The stability of the tattoo ink under normal storage conditions can be evaluated using specifically designed stability tests in order to ensure the product quality throughout the stated shelf life. Inspiration can be found in, e.g., "Guidelines on stability testing of cosmetic products" (COLIPA, 2004) although adaption of tests for the purpose of stability testing of tattoo ink may be necessary. The tests should assure physical integrity of the ink under appropriate conditions of storage and use, chemical stability, microbiological stability and should be performed in the container intended for sale to ensure

compatibility between the container and its content. Interaction of substances as well as the possible degradation of substances over time can lead to the formation of new chemical substances that could be of concern from a health perspective. Therefore the potential formation of new substances as well as their expected health effect must be considered (e.g., like the formation of primary aromatic amines upon cleavage of azo dyes). Identification and quantification of chemical substances during stability testing can be carried out by using analytical methods such as HPLC, GC, AAS or IR analysis, when relevant. Selection of parameters for evaluation of product quality (such as viscosity, change in colour, sedimentation and chemical composition) should be carried out according to experience and the stability should preferably be tested at one or more temperatures. The parameters should be monitored over time to ensure that the quality and safety of the product when used for tattooing is not compromised within the shelf life of the product.

This shelf life should be evaluated based on experience as well as conducted stability tests and an expiry date calculated for each batch of tattoo ink. The date should be clearly visible on the tattoo ink container in order to make it easy for both the user and customer to check before tattooing with the ink.

The expiry date should be stated on the tattoo ink label as "May not be used after ..." followed by the expiry date clearly stated, either as 'month and year' or 'day, month and year'. If necessary, the conditions under which the shelf life can be maintained may be stated, e.g., only in unopened container and/or stored at a specific temperature.

Appendix 1: Guidelines for safety assessment of tattoo ink

Tattoo ink should not endanger the health and safety of a person when applied and used as intended. To ensure safe use of tattoo ink a safety assessment should be carried out. It is recommended that the safety assessment is carried out in accordance with the requirements described in this appendix, ensuring that a specific chemical composition of a tattoo ink does not adversely affect human health when used for tattooing.

It is recommended that a safety report should be compiled based on the assessment, summarizing the results of the assessment. The conclusion of the assessment should be clearly stated. If the product is safe, it is recommended to use the following wording “the tattoo ink does not constitute a risk to human health when used for tattooing”. If a safe use cannot be documented, the tattoo ink should not be used for tattooing and compiling a report is not relevant.

A safety assessment of tattoo ink can follow existing guidelines for safety assessment such as described for cosmetics by the Scientific Committee on Consumer Safety (SCCS, 2012), for consumer products as described by Boyd and Larsen (2014) or in guidance documents published by the European chemicals agency (ECHA). This guide is built on the same principles. However issues not relevant for tattoos are omitted. Further, the available scientific data for tattoo ink is in some respects currently considered inadequate for some elements of the assessment following the existing guidelines, thus suggestions for a practical approach are provided here.

The safety assessment must be based on available and current knowledge and if new knowledge becomes available the safety report should be updated up to the expiry date of the tattoo ink. The report should include clear references to all sources of information used.

A safety assessment of tattoo ink should contain two subparts; A and B, see illustration in Figure 1.

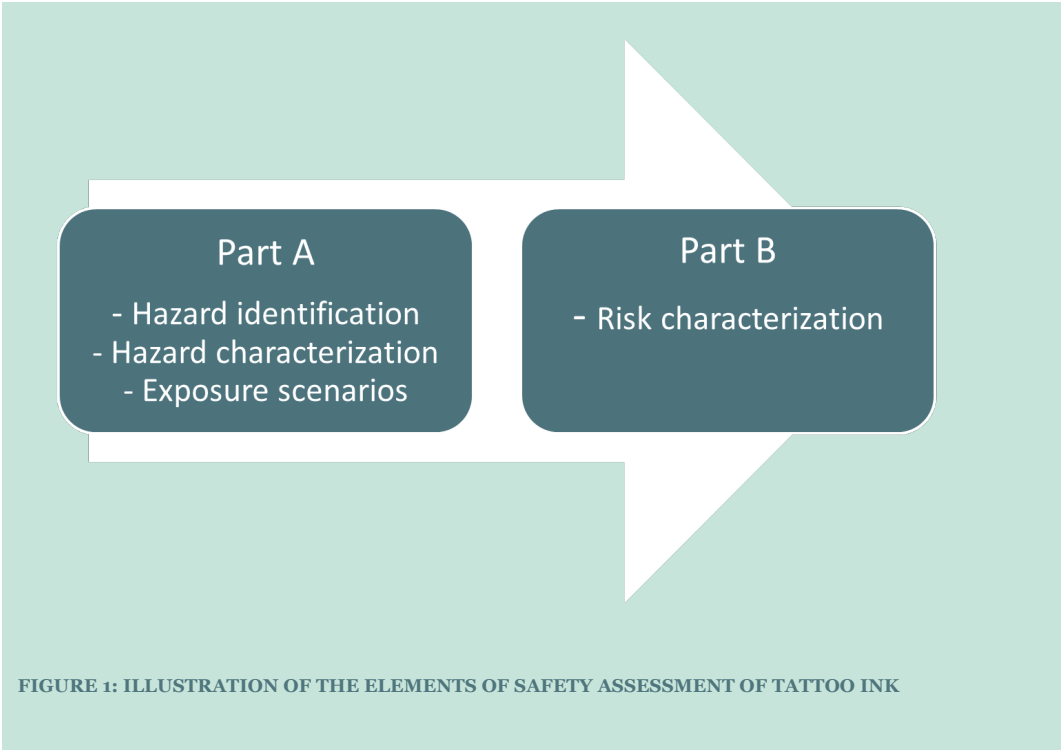

FIGURE 1: ILLUSTRATION OF THE ELEMENTS OF SAFETY ASSESSMENT OF TATTOO INK

Part A includes the compilation of all available data relevant for the safety of the tattoo ink and contains a hazard identification, hazard characterization and development of exposure scenarios.

Part B includes the assessment of the safety of the tattoo ink; the risk characterization, based on the data compiled in part A. The two parts will be explained in detail in the following paragraphs.

In order to further illustrate the practical approach described for the safety assessment a number of examples will also be given.

1. Available information

Manufacturers and importers of tattoo ink should base the safety assessment on all available information. It is not necessary to carry out a toxicological test in order to assure the safety of the inks. This further assures that the economic burden is kept at an acceptable level. It is acknowledged that the safety assessment might to some degree be compromised by this. The recommendation and suggested requirements for a safety assessment and a safety report should however assure that all available information is applied in the assessment of the tattoo inks. If no information is available or if the available information is of a poor quality, it should be clearly stated in the assessment report.

2. Sources of information

Information on the safety of the tattoo ink can be found through various sources. The safety datasheet (SDS) of the ingredients should be available from the manufacturer or importer of the substances or mixture of substances and should contain some of the information needed. Information might also be available from the supplier on request or may be found in the scientific literature. A number of databases containing references to toxicological information are also available. Experience gained with similar or other product categories (e.g., cosmetics or pharmaceuticals), available data on similar formulations, or computer models used to estimate, e.g.,

physico-chemical properties or toxicological effects of substances are also considered as valuable sources of information. Relevant sources of information will be referenced to in the following paragraphs and will be summarized in Tabel A, at the end of this appendix.

3. Confidential information

Some information needed for the safety assessment as performed according to this guideline might be considered confidential information by the supplier of the ingredients used to manufacture tattoo ink. In that case, one possible solution is to hire a third party to perform the safety assessment and give the information from the supplier directly to the third party with no involvement of the tattoo ink manufacturer.
Another solution is to legally restrict the use of any confidential information supplied by the manufacturer of ingredients to the tattoo ink manufacturer during the preparation of the safety evaluation through a confidential disclosure agreement (CDA).

4. Part A – Information on the safety of tattoo ink

The first part of the safety assessment covers the requirements for data on substances in the tattoo ink. Information that should be compiled in part A is:

- Chemical identity of the tattoo ink – section 4.1
- Physico-chemical properties and stability of the tattoo ink – section 4.2
- Impurities of the tattoo ink and information on the tattoo ink container – section 4.3
- Toxicological profile of ingredients including any impurities – section 4.4
- Exposure to the tattoo ink, its ingredients and impurities – section 4.5
- Other relevant information – section 4.6

The following sections will specify the listed requirements.

4.1 Chemical identity of the tattoo ink

For substances that are either a substance or a part of mixture in the tattoo ink the following should be stated, regardless of their concentration:

- *The chemical name, using international nomenclature. 'International nomenclature' means substance names in the INCI (International Nomenclature of Cosmetic Ingredients), the EINECS (the European Inventory of Existing Commercial chemical Substances) or the ELINCS (the European List of Notified Chemical Substances). If the substance is not found on the aforementioned lists, then the ISO or IUPAC names must be used. Dyes can be stated using their Colour Index (CI) Constitution Numbers.*
- *Chemical formula (where possible).*
- *INCI name, CAS, EINECS and ELINCS numbers (where possible).*
- *The intended use (i.e. the function of the chemical).*

All substances in the tattoo ink formulation should be listed, including any known impurities (see section 4.3 below for details).

For information on the chemical identity and properties of substances and mixtures the SDS is an important source of knowledge. Chemical name and chemical formula could be found on the SDS if this is available. According to the European Chemical Agency (ECHA) the “safety data sheets are the main tool for ensuring that suppliers communicate enough information along the supply chain to allow safe use of their substances and mixtures” (ECHA, n.d.A), and they should therefore contain much of the information required here.

A manufacturer or importer is in many cases obliged to procure an SDS following European regulation requirements.

An SDS is required if:

1) a substance (and from 1 June 2015 a mixture) meets the criteria for classification as hazardous according to the Classification, Labelling and Packaging (CLP) regulation,
2) a mixture meets the criteria for classification as dangerous[1],
3) a substance is persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) according to the criteria given in Annex XIII of REACH, or
4) a substance is included in the candidate list for eventual authorization according to Article 59 (1) of REACH for any other reasons (ECHA, n.d.A).

The substance name can be specified according to INCI, EINICS or ELINCS. In the case of cosmetic substances and ingredients, the correct substance name according to INCI can be found using the European Commission Database, CosIng (CosIng, n.d.), e.g., from the CAS or EINECS/ELINCS number. CAS number, EINECS or ELINCS number can be found through the European chemical Substances Information System (ESIS, n.d.). International Union of Pure and Applied Chemistry (IUPAC) generated substance names using a defined set of rules in order to systematically name chemical compounds based on their chemical structure (IUPAC, n.d.). For pigments and dyes, the Colour Index (CI) constitution number is often used for identification. The CI numbers are assigned by Colour Index International and their database can be accessed on-line (a subscription is required) (Colour Index International, 2012). CI numbers of pigments and dyes allowed in cosmetics can also be found in Annex IV of the Cosmetics Regulation (EC) No 1223/2009. One or more of these names are also available from the SDS, if available.

The chemical formula, if not given on the SDS, can be found in several public databases, such as ChemSpider (ChemSpider, n.d.) and PubChem (PubChem, n.d.) by using, e.g., the CAS number or one of the chemical names.

The intended use of the ingredients should also be stated. An ingredient may typically function as, e.g., a pigment, solvent, binder, preservative, surfactant or a thickening agent but other intended uses may be stated as appropriate.

Concentrations of the substances in the tattoo ink are required in order to evaluate the exposure.

4.2 Physico-chemical properties and stability of the tattoo ink

For substances which are either a single substance or a part of a mixture in the tattoo ink their physical and chemical properties should be listed.

For the tattoo ink, the stability under normal storage conditions as well as the shelf life when opened and unopened should be stated.

Physical and chemical properties are considered important for the assessment of safety since they may be closely related to certain toxicological properties. Physical properties may include: form (liquid, solid, powder etc.), particle size, density, colour and smell (sharp, sweet etc.), vapour pressure, melting point, boiling point, refractive index, flash point and viscosity. Chemical properties may include: pH, reactivity and solubility.

[1] Dangerous Preparations Directive 1999/45/EC (DPD) (until 1 June 2015)

The physical and chemical properties for substances and mixtures are typically given on the SDS or may be found in on-line chemical databases, e.g., SciFinder (subscription necessary) (SciFinder, n.d.) or ChemSpider (ChemSpider, n.d.). Properties may also be found on ECHAs homepage for registered substances (ECHA, 2013A).

The stability of the tattoo ink under normal storage conditions should be evaluated in order to ensure that the quality and safety of the product when used for tattooing is not compromised within the shelf life of the product. Interaction of substances as well as the possible degradation of substances over time can lead to the formation of new chemical substances that could be of concern from a health perspective. Therefore, the potential formation of new substances as well as their expected health effect should be considered (e.g., like the formation of primary aromatic amines upon cleavage of azo dyes). Identification and quantification of chemical substances during stability testing can be done using analytical methods such as HPLC, GC, AAS or IR analysis, when relevant. See also section 2.6 for further details on stability testing.

4.3 Impurities of the tattoo ink and information about the tattoo ink container

- List the purity of the substances and mixtures in the tattoo ink
- List impurities in the tattoo ink
- Assess relevant properties of the tattoo ink container; particularly its purity and stability

The purity of the substance or mixtures used in the tattoo ink is typically given by the manufacturer either on the SDS, through specifications or on a technical datasheet.

All known impurities of substances or mixtures used as ingredients in the manufacturing of tattoo ink must be identified as part of the chemical composition of the tattoo ink (See section 3), since they may be relevant for the safe use of tattoo ink.

The chemical identity of any impurity of a substance or mixture which is it-self classified and contributes to the overall classification of the substance or mixture must be stated on the SDS. Information on these impurities should therefore be available on the SDS (ECHA, 2011).

If an impurity is not classified and therefore not stated on the SDS, the manufacturer of ingredients may be a valuable source of information regarding impurities. Information on impurities can be supplied through the supply chain either directly through inquiry to a supplier or manufacturer, through specifications/technical data sheets for each ingredient or obtained indirectly based on knowledge of the process for manufacturing the ingredient (origin of substance, production process, synthesis route, extraction process, solvent used, etc.).

The type of impurities will depend on the substance or mixture of substances and might include, e.g., primary aromatic amines, polyaromatic hydrocarbons (PAHs), residual organic solvents, residual monomers for polymeric substances, softeners like phthalates, heavy metals etc.

Known impurities emerging during the production of the ink, which are not removed or impurities that will be generated during the expected shelf life of the tattoo ink should also be listed.

For impurities stemming directly from the container, information on the material might be available from the supplier on request, from the literature or could perhaps be further assessed by someone skilled in the area of material science.

4.4 Toxicological profile of ingredients including any impurities

4.4.1 Information on toxicity of substances

The toxicological profile for substances which are either a substance or a part of a mixture in the tattoo ink should be determined and to do so the following should be stated:

- information on whether they are carcinogenic, mutagenic, toxic to reproduction, corrosive, irritating to the skin or eyes, skin sensitizers, show signs of UV photo-induced toxicity, genotoxicity or systemic toxicity
- effects on the toxicological profile due to particle size, including nanomaterials and chemical and physical reactions
- any read-across shall be documented and justified
- the source of information shall be clearly identified

The health risk associated with the use of tattoo ink will be determined by the substances contained in the tattoo ink, possible interactions between substances and degradation where new substances of health concern are formed. Therefore, information on toxicological effects of the substances or mixture of substances contained in the tattoo ink must be compiled in order to assess the safety of the formulation. This process is typically referred to as the hazard identification.

The CLP regulation (Regulation (EC) No 1272/2008) ensures that hazards presented by chemicals are clearly communicated through standard statements and pictograms to workers and consumers in the European Union. The classification system is based on the United Nations' Globally Harmonised System (GHS). The CLP database accessible through ECHA's homepage (ECHA, 2013B) contains information on substances notified under Regulation (EC) No 1272/2008 and registered under Regulation (EC) No 1907/2006 (the REACH Regulation) by manufacturers and importers. It also contains the list of legally binding harmonized classifications (Annex VI to the CLP Regulation) as well as self-classifications reported by manufacturers and importers. The database states the hazard class(es) (whether they are carcinogenic, mutagenic, toxic to reproduction, corrosive, irritating to the skin or eyes, skin sensitizers, genotoxic or show systemic toxicity) for each substance and can be searched using the chemical name or CAS number. More information on the registered substances (10.655 unique substances by November 28th 2013) can be accessed through "Chemical Substances Search" on ECHA's homepage (ECHA, 2013A).

The toxicological effect of a chemical substance can vary from mild skin irritation to more severe effects such as development of allergies or even cancer. After the hazard identification a hazard characterization is needed. In the characterization the toxicological effects of all substances should be evaluated and the critical effect of each substance be determined. The critical effects are the toxicological effects considered as the most essential for the risk assessment. The critical effects will depend on the specific endpoints identified and must be considered on a case-by-case basis by a person skilled in the art. The severity with respect to human health of each of the identified effects is a factor in determining the critical effects. The expected exposure route for the product in question should also be considered with respect to the critical effects, e.g., if only endpoints for inhalation effects are identified for a substance used in tattoo inks they will most likely not be considered relevant.

The No Observed Adverse Effect Level (NOAEL) of a substance is a threshold value, below which no adverse effects can be expected. NOAEL is the highest concentration or level of exposure to a substance where there is no biologically or statistically significant increase in the frequency of observed adverse toxicological effects seen in the exposed population when compared to a control population. Toxicological tests can be performed on a number of different species and the relevance of the conclusion with respect to expected effects on exposure to humans should be discussed and evaluated.

A threshold value is not seen for all toxicological effects and the exposure of substances with this type of effect will be associated with a risk of adverse effects regardless of the concentration, such as for some carcinogenic substances. Instead of a NOAEL, a Bench Mark Dose ($BMDL_{10}$ or T_{25}) is often established and used in place of the NOAEL to evaluate the risk of adverse effects.

A good strategy for gathering information on health hazards for a specific chemical is to search for safe doses derived internationally by authoritative bodies, and compare doses to more recent data to consider whether modification is necessary. If you need to characterize the hazard of a chemical substance, for which no specialist assessments are available, you might consider consulting an experienced toxicological risk assessor.

Studies on toxicological effects of substances are typically found in the scientific literature and critical dose levels may be found based on data on experience in humans or more often based on data from experimental animal testing. Searches for relevant scientific literature on the toxicological effect of substances can be carried out on-line, e.g., at the toxicology data network (TOXNET, n.d.) or PubMed (PubMed, n.d.). Toxicological data and safety assessments of substances used in cosmetics can also be found in opinions published by scientific committees, e.g., the Scientific Committee on Consumer Safety (SCCS) or the former Scientific Committee on Cosmetic products and non-food products intended for consumers (SCCNFP) (European Commission, n.d.). Also The Cosmetic Ingredient Review (CIR) reviews and assesses the safety of individual chemical compounds for cosmetics and publishes them on their homepage as well as in peer-reviewed scientific literature (CIR, n.d.).
Data from studies performed according to internationally recognized test methods (such as OECD Guidelines (OECD, n.d.)) should be preferred. The assessment should ideally be based on studies performed with the exact substances used in the tattoo ink, e.g. substances with the same chemical and physical characteristic such as particle size distribution.

Guidance on the assessment of toxicological data can be found in a number of guidance documents available from ECHA (ECHA, n.d.B): Quantitative Structure-Activity Relationships (QSARs) and read-across can be used between structurally related chemicals if no available or reliable information is found for a specific substance in cases where knowledge on similar substances exists. Such modelling and read-across can give valuable information in cases where no information is available through animal or human studies. Modelling and the resulting data are, however, associated with some uncertainty and must be carried out and evaluated by a trained toxicologist and always used with caution. Software for QSAR modelling is also available from OECD (QSAR Toolbox, n.d.).

Determining the NOAEL value (or benchmark dose) for toxicological effects should be carried out by careful evaluation of all available data and should only be carried out by a person skilled in the art as should the identification of the critical effects. It should be noted that the critical effect could be a local as well as a systemic effect. Also, it should be recognized that the critical effect is not necessarily the most severe effect of the chemical substance. In general safety assessments should be carried out by careful evaluation of all available data and requires the judgment of professionals suitably qualified and experienced in toxicology and chemical risk assessment.

4.4.2 The chemical composition and the production of tattoo ink

The assessment of the toxicological profile of a mixture, e.g., such as tattoo ink, is usually based on the knowledge of the toxicological profile of each single substance in the mixture. Therefore, the concentration of each substance in the tattoo ink must be known to evaluate the overall expected toxicological effect of the tattoo ink. Changes in the chemical composition during the production process should be accounted for, if at all possible, e.g., evaporation of solvent added or degradation of substances as a result of processing.

4.4.3 Effects of substance properties on toxicological profile

Some properties of the substances in the tattoo ink may impact the toxicological profile of the mixture and need to be addressed. Particular consideration shall be given to parameters including, but not limited to:

- Particle size, specifically with attention to substances (e.g. pigments) that fall under the category nanomaterials, as defined by the European Commission (European Union, 2011).
- Chemical and physical reactions: Substances known to interact upon mixing or degrade under production or normal storage conditions.

4.5 Exposure to the tattoo ink, its ingredients and impurities

To evaluate safety of tattoo ink exposure scenarios must be developed. Since tattoo inks are introduced into the skin, a parameter such as absorption is expected to affect exposure differently from a situation when the chemical substance is applied on the skin as is the case for e.g. cosmetics. In order to assess the tattoo inks according to the recommended requirements some assumptions may be made regarding exposure. The assumptions currently considered necessary and acceptable will be explained below.

4.5.1 Normal and foreseeable use

The normal and foreseeable use of the tattoo ink, including the expected use thereof per cm^2, as well as precautions when mixing and diluting should be described.

The normal and foreseeable use of the tattoo ink is for introduction directly into the skin without dilution of the ink. One study indicates that a normal concentration of pigment used for tattooing may be in the range of 0.6-9.4 mg/cm^2 with an average of 2.5 mg/cm^2 (Engel et al, 2008). This concentration is based on pigment alone and compensation for the remaining components in the tattoo ink is therefore necessary. According to the previous survey done for the Danish EPA, the dry matter content (pigment) of a tattoo ink is typically 30-60% (Jacobsen et al, 2012). Other available data may be used if source of information is clearly stated.

Sometimes mixing or diluting colours is desired by tattooists in order to get the exact shade wanted by the customer. Mixing and dilution can have a negative effect on the stability and integrity (e.g. hydrolysis) of the tattoo ink. Any details regarding relevant precautions when mixing which might affect the safe use of the tattoo ink should be addressed. Thus, if possible, e.g., recommendations on solvents for dilution or specific inks identified and tested as compatible on mixing of different coloured inks.

4.5.2 Exposure of tattoo ink

Exposure to tattoo ink should be estimated in relation to:

- the surface area(s) of application. If no other information is available, an area of 30 x 30 cm shall be used (equivalent to a large tattoo)
- the duration and frequency of exposure. If no other information is available, it is assumed that the chemical substances are absorbed over a 6-week period
- potential exposure of particular surface areas of the body
- potential exposure of a specific population group

To aid in the calculation of exposure, a range of assumptions on exposure to tattoo ink are given in the above. The assumptions are based on the presently available knowledge and should be updated as new, more relevant and valid data emerges. Other assumption might also be made for specific products where significantly different exposures are to be expected, e.g. for permanent make-up.

The exposure of tattoo ink from a newly made tattoo may be calculated using the information on normal and foreseeable use, and if no other information is available a surface area of application of 30 x 30 cm (900 cm^2) and assuming an absorbance of chemical substances over 6 weeks.

Very few scientific studies are available on absorption of tattoo ink and therefore only a few substances have been examined. The assumption of an absorbance of the chemical substances at a constant rate over a 6 week period after the tattoo ink is introduced into the skin is based on the results of a study performed on mice (Engel et al, 2008). The particle size is expected to have an effect on absorption. Smaller particles are expected to be absorbed easier and faster into the system than larger particles of the same chemical substance (e.g., for titanium dioxide (Wu et al, 2009)). If substances used in the tattoo ink are of a significantly smaller particle size than the pigments tested in the study, then the uptake might be faster and the systemic exposure limited to a shorter time and therefore a higher daily dose.

Considerations regarding the area of skin expected to be tattooed under normal foreseeable use could also affect the absorption time, e.g., for tattoos under the arms or other areas where the skin is thin.

Using the above, an expected systemic exposure can be calculated, e.g., in amount of tattoo ink/kg bodyweight/day. A default human bodyweight (BW) of 60 (SCCS, 2012) or 70 (ECHA, 2012; EFSA, 2012) kg is suggested for this calculation.

4.5.3 Exposure of ingredients and impurities

For evaluation of exposure to substances and mixtures, including impurities present in tattoo inks the level of exposure to substances with identified critical toxicological effects should be calculated based on:

- Normal and foreseeable use
- Estimated exposure of tattoo ink
- Concentration of substance in the tattoo ink
- Normal and foreseeable absorption. If no other information is available, it is assumed that 100% of the absorbed substances and mixtures, and two-thirds of the suspended particles, are absorbed systemically
- Effects on exposure due to particle size

The next step is developing exposure scenarios further and calculating the exposure to substances with a critical toxicological effect. Again, impurities present in the tattoo ink should be included if relevant.

The exposure is calculated using the exposure of tattoo ink (mg ink per kg body weight per day) and:

- Multiplying with the concentrations of each substance in the tattoo ink for which a critical toxicological effect has been identified (e.g. in μg/g ink).
- If no other information is available, it may be assumed that 100% of the absorbed substances and mixtures and two-thirds of the suspended particles are absorbed systemically.

As mentioned earlier particle size can be expected to have some effect on absorption and should, if possible, be considered when setting parameters for absorption in the exposure scenarios.

The calculations will give a unique systemic exposure dosage (SED) for each substance included in the assessment which should be compare to relevant toxicological threshold values, e.g., NOAEL, in the risk characterization in part B.

Example A: Calculation of systemic exposure dosage (SED):

In Jacobsen et al (2012) a tattoo ink with a concentration of 5.3 μg/g benzo(a)pyrene was found. To illustrate the calculation, the systemic exposure (SED) of that ink will be calculated here using the following assumptions:

Body weight (BW) = 70 kg
Area=900 cm²
Period = 42 days
Ink use =2.5 mg/cm²

Furthermore, it is assumed that all of the substance is absorbed since no other information is available for the calculation.

$$SED = \frac{C_{substance} \cdot Ink \cdot Area \cdot Absorption}{BW \cdot Period} = \frac{0.0053\mu g/mg \cdot 2.5mg/cm^2 \cdot 900cm^2 \cdot 100\%}{70kg \cdot 42d} = 0.004\mu g/kg/d$$

Example B: Calculation of systemic exposure dosage (SED):

In Jacobsen et al (2012) the measured content of phthalocyanine blue ranged from 46.000-189.000 μg/g. One sample had a content of 112.000 μg/g. Calculation of a systemic exposure (SED) of that ink can be carried out using the following assumptions:

BW = 70 kg
Area =900 cm²
Period = 42 days
Ink use= 2.5 mg/cm²

Furthermore it is assumed that 2/3 of the substance is absorbed since this is considered suspended particles (low water solubility) and no other information is available for the calculation.

$$SED = \frac{C_{substance} \cdot Ink \cdot Area \cdot Absorption}{BW \cdot Period} = \frac{112\mu g/mg \cdot 2.5mg/cm^2 \cdot 900cm^2 \cdot 67\%}{70kg \cdot 42d} = 57.4\mu g/kg/d$$

4.5.4 Margin of Safety (MoS)

Calculation of margins of safety (MoS) based on a no observed adverse effects level (NOAEL) are made and discussed if possible.

The final step of the safety assessment is to make a risk characterization. Here the probability that a substance under assessment causes damage to human health and the level of risk, are evaluated. The purpose of a risk characterization is to provide a quantitative statement about the risk by comparing the estimated exposure to an appropriate threshold value, if such a value is identified during the safety assessment.

Calculation of MoS should be included in the safety assessment of tattoo ink if possible with respect to the available toxicological data. The MoS should be based on the lowest NOAEL and the expected systemic exposure. It is calculated as (SCCS, 2012):

$$MoS = \frac{NOAEL}{SED}$$

SED being the systemic exposure dosage calculated based on exposure scenarios. If relevant, MoS for substances is calculated and assessed further in Part B. A high MoS represents a low risk of adverse effects occurring as a consequence of the examined use situation. A detailed description of the calculation of MoS can be found in "the SCCS's notes of guidance for the testing of cosmetic substances and their safety evaluation" (SCCS, 2012).

Other terms than MoS are often used as a mean to quantify the risk in safety assessments. The term used by ECHA is the Risk Characterization Ratio (RCR) which is the ratio of exposure to the derived no effect level (DNEL). MoE – margin of exposure is another term used for risk quantification which is very similar to MoS. For non-threshold effects a risk level may be calculated as an alternative to the MoS (Boyd and Larsen, 2014).

Example C - Calculation of Margin of Safety (MoS)

For phthalocyanine blue, Jacobsen et al (2012) identified a NOAEL of 200 mg/kg/d. The critical effect was a reduced number of red blood cells which was observed after oral administration of the pigment by gavage. The NOAEL is based on a 28 day rat study. Using this NOAEL, the MoS can be calculated (see Example A for data input to the formula):

$$MoS = \frac{NOAEL}{SED} = \frac{200 mg/kg/d}{0.0574 mg/kg/d} = 3484$$

4.6 Other relevant information

Any other known information considered to be of significance with regard to the risk to human health when tattooing with the tattoo ink currently being assessed should be included, e.g., sterility of the tattoo ink, see section 2.5 of *Recommendation from the Danish Environmental Protection Agency on Tattoo Ink*.

5. Part B – Assessment of safety

5.1 Assessment of safety

The safe use of a tattoo ink should be scientifically justified through the safety assessment and the safe use should be documented using the information in Part A with a clear account for the relevance of the toxicological profiles and how the conclusion is reached.

The scientific justification for the conclusion of the assessment should be given in part B of the safety assessment. The justification of the safe use of the tattoo ink shall be based on the information in Part A and must describe how the compiled toxicological information is relevant for exposure when the ink is used for tattooing and how it is used.

The assessor shall take all the compiled information on substances and mixture of substances used in the tattoo ink into consideration when evaluating the safety of the tattoo ink. Substances and their toxicological profiles, their chemical and physical properties and the conditions under which they are used should be assessed.

If a MoS can be calculated based on the available information this value should be assessed to determine if it reflects a safe use of the tattoo ink. Several aspects are involved in the extrapolation of experimental data to the exposure situation in humans, such as the variability in the experimental data and from intra- and inter-species variation, the nature and severity of the effect, length of the study and the sensitivity of the human (sub-) population. The calculated MoS should therefore be discussed in order to justify that the MoS is adequate and represents a safe use of the tattoo ink.

MoS must account for safety factors in extrapolation of a toxicological effect seen e.g. in a group of test animals to an average human being, and subsequently from average human beings to sensitive subpopulations. Generally a MoS of 100 is considered acceptable for a conclusion of safe use (SCCS, 2012). But depending on the factors such as the quality of data and the severity of the criticaleffect a higher margin could be considered appropriate. Guidelines for safety factors (or assessment factors; Boyd and Larsen, 2014) for some common extrapolations can be found in ECHA guidance documents. An example of a choice of adequate safety factors as well as calculation and assessment of MoS is given in example D below.

Example D: Evaluation of MoS calculations:
Any calculated MoS should be assessed to determine if it is sufficient for demonstrating a safe use of the tattoo ink. Assessment factors depending on the quality of the used NOAEL value are here used to estimate an acceptable MoS.

The NOAEL used in example C for phthalocyanine blue was based on a 28 day rat study with oral administration. In order to estimate the long-term effect in humans several assessment factors will be considered. The product of these factors constitutes an acceptable MoS.

Factor 1: 4	due to differences between rats and humans (4 is the value given in REACH guidance documents for interspecies variation between rat and human, but a default value of 10 is often seen)
Factor 2: 10	due to human variability (intraspecies)
Factor 3: 6	to account for a 28 day study being used for assessing chronic effects.

The product of factors being: 4 x 10 x 6 = **240**

The product is in this case much lower than the calculated MoS in example C and, under the assumptions made, indicates a safe use of this particular ink with respect to phthalocyanine blue.

Other factors may be applied to account for poor quality of data, e.g., if a LOAEL is used in place of a NOAEL. For detailed description and examples see "*Guidance on information requirements and chemical safety assessment Chapter R.8 (ECHA, 2012).*

5.2 Assessment of stability and reactivity

The safety of the tattoo ink should be assessed with respect to potential reactions between substances and mixtures, including impurities in the tattoo ink, and the possible effects of stability.

The information, from part A of the safety assessment, should be reviewed. The potential reactions between substances and mixtures, including impurities in the tattoo ink, how the chemical and/or physical stability possibly affects the safety of use of the tattoo ink should be discussed, if relevant.

5.3 Conclusion of the assessment

The conclusion of the safety assessment of tattoo ink should be that the tattoo ink does not present a risk to human health.

The recommendation is that a tattoo ink should not be sold or used if its safety has not been assessed and a safety report has not been compiled, concluding that the tattoo ink does not constitute a risk to human health when used in tattooing.

NOTE: Although a safe use can be confirmed by carrying out the safety evaluation, some restrictions regarding use of chemicals of particular concern are recommended for tattoo ink, see section 2.2.

TABEL A: KEY SOURCES OF INFORMATION

Links	Description
www.echa.europe.eu	**ECHA**, The European Chemicals Agency, provides data for a number of chemical substances registered under REACH. The data includes, e.g., toxicological data, physical properties and chemical properties.
http://www.ncbi.nlm.nih.gov/pubmed	**PUBMED** is a database of references and abstracts on biomedical literature from MEDLINE, life science journals and online books. The database is maintained by the US National Library of Medicine (NLM) at the National Institutes of Health.
http://toxnet.nlm.nih.gov/.	**TOXLINE** (TOXicology information onLINE): Databases on toxicology, hazardous chemicals, environmental health, and toxic releases under The US National Library of Medicine (NLM). Also containing the **HSDB** (Hazardous Substances Data Bank)
http://www.cas.org/	**CAS,** Chemical Abstracts Service, is a division of the American Chemical Society. It is the world's authority for chemical information and the CAS registry contains information on, e.g., property data more than 74 million unique organic and inorganic chemical substances.
http://www.oecd.org/	**OECD**, The Organisation for Economic Co-operation and Development publishes guidelines for the testing of chemicals. The guidelines are a collection of the most relevant internationally agreed testing methods used by governments, industry and independent laboratories to assess the safety of chemical products.
http://esis.jrc.ec.europa.eu/	**ESIS**, European chemical Substances Information System, is a complex, heterogeneous information system which provides information on chemicals. It includes information on substances in EINICS (European Inventory of Existing Commercial chemical Substances) and ELINCS (European List of Notified Chemical Substances).
http://ec.europa.eu/health/scientific_committees/index_en.htm	The European Commission relies on a number of scientific committees when preparing its policy and proposals related to consumer safety, public health and the environment. The **Scientific Committees** work independently and provide the Commission with sound scientific advice. From time to time, the Scientific Committees will publish opinions on substances containing, e.g., review on toxicological information on the substance.
http://www.colour-index.com/	**Colour Index** International is a database on colorants published on-line by the Society of Dyers and Colourists and the American Association of Textile Chemists and Colourists. Serves as an authoritative international reference work on colorant nomenclature (CI numbers), physical form, constitution and application.
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32009R1223:EN:NOT	**EU regulation on cosmetic products**, REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30 November 2009 on cosmetic products. ANNEX IV lists COLORANTS ALLOWED IN COSMETIC PRODUCTS and corresponding CI index numbers, while Annex V lists preservatives allowed in cosmetic products.
http://www.who.int/ipcs/en/	World Health Organisation- International Program on Chemical Safety- (**WHO/IPCS**) work to establish the scientific basis for the sound management of chemicals, and to strengthen national capabilities and capacities for chemical safety. Tools for safety assessment and other literature is published.

http://www.atsdr.cdc.gov/	Agency for Toxic Substances and Disease Registry (**ATSDR**).
http://www.cir-safety.org/	The **Cosmetic Ingredient Review** (CIR) reviews and assesses the safety of individual chemical compounds for cosmetics and publishes the results in peer-reviewed scientific literature.

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Vocabulary

BMDL10:	Bench Mark Dose, the lower 95% confidence limit on the benchmark dose associated with an adverse toxicological effect in 10% of the tested population
BW:	Bodyweight
CI-number:	Colour Index Constitution Numbers
CLP:	Classification, labelling and packaging
CMR:	Carcinogenic, mutagenic and toxic to reproduction
DNEL:	Derived No Effect Level
ECHA:	European Chemicals Agency
EINECS:	the European Inventory of Existing Commercial chemical Substances
ELINCS:	the European List of Notified Chemical Substances
INCI:	International Nomenclature of Cosmetic Ingredients
Mixture:	A mixture or solution composed of two or more substances
MoS:	Margin of Safety
NOAEL:	No Observed Adverse Effect Level
QSAR:	Quantitative Structure-Activity Relationship, used for modeling of toxicological effect of a substance using knowledge of toxicological effect of a structurally similar chemical.
RCR:	Risk Characterization Ratio
SDS:	Safety Data Sheet
SCCS:	Scientific Committee on Consumer Safety
Substance:	A chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.
T25:	Dose or concentration, at which the adverse toxicological effect occurs in 25% of the tested population

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Recommendation from the Danish Environmental Protection Agency on the safety of Tattoo Ink

A tattoo should not endanger the health and safety of a person when applied and used as intended. In an effort to reduce the health risks associated with getting a tattoo, the Danish EPA has therefor prepared recommendations concerning the safety of tattoo inks including guidelines for safety assessment of the tattoo ink.

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